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Inula tuzgoluensis (Asteraceae), a new species from Central Anatolia, Turkey

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Abstract: The new species *Inula tuzgoluensis* M.Öztürk & O.Çetin (Asteraceae) is described from Central Turkey. The species grows on salty marshes, in the Cihanbeyli District of Konya Province. *Inula tuzgoluensis*, an endemic confined to Central Anatolia, is related to *I. aucheriana* DC. The new species is distinct from the closely allied *I. aucheriana* by habitus, indumentums, leaves, flowers, pollen grains, and achenes. Photos of the new species are provided. Special attention is also given to its ecology and conservation status. In addition, pollen characteristics and achene surface features are examined by scanning electron microscopy. Phylogenetic relationships between closely related *Inula* species and *Phagnalon* Cass. were examined with molecular methods performed in the regions of the intersimple sequence repeats. DNA was isolated from the collected samples using modified CTAB protocols. The data were analysed with the PAUP package. Standardised data were used to generate the dendrogram that revealed the phylogenetic relationships of the taxa. The chromosome number for *Inula tuzgoluensis* was n = x = 9, it and was counted for the first time here.

Key words: Compositae, *Inula*, scanning electron microscopy, palynology, intersimple sequence repeat, Turkey

1. Introduction

Asteraceae comprises approximately 23,000 species in 1535 genera (Judd et al., 2007). The tribe *Inuleae* is mainly a Eurasian and East and South Africa tribe, but some genera (e.g., *Pluchea* Cass.) have a worldwide distribution. This tribe includes about 66 genera and 687 species (Anderberg & Eldenas, 2007). The genus *Inula* L. (s.l) Asteraceae belongs to the tribe *Inuleae* of the family Compositae and is mainly distributed in Europe, Africa, and Asia (Lack, 2007). The family Asteraceae has about 1186 species in 140 genera that are present in Turkey. Almost 446 endemic species occur all over Turkey (Erik & Tarıkahya, 2004; Duran et al., 2009; Makbul et al., 2012).

Grierson (1975) also accepted the genus *Inula*, in the broader sense, while revising the genus for *Flora of Turkey*. He recognised 26 species within the genus *Inula*. Recently, 2 taxa were added to *Flora of Turkey*. These taxa are *Inula sechmenii* Hartvig & Strid and *I. spiraeifolia* L. (Davis et al., 1988; Özhatay & Kültür, 2006). Overall, *Inula* includes 33 subspecies and varieties, and 8 of them are endemic for Turkey; the endemism rate of the genus *Inula* is 28.5% in Turkey (Güner et al., 2012)

The genus *Inula* (s.l) was recognised by Linnaeus (1753, 1754). It was previously treated by various researchers (De Candolle, 1836; Boissier, 1875; Clarke, 1876; Hooker, 1881; Blatter, 1927; Gorshkova, 1959; Kitamura, 1960, 1964; Rechinger, 1980; Anderberg, 1991; Kumar, 1995). However, most of these treatments are either outdated or do not cover the entire aspects of the genus *Inula*. The concept of this genus according to most of the above-mentioned authors was rather broad and heterogeneous. This has resulted in an assemblage of several unrelated taxa under the broad canopy of *Inula*, thus causing much taxonomic confusion. *Inula* is heterogeneous and not monophyletic, as is now circumscribed (Anderberg & Eldenas, 2007).

Many karyological and cytological studies have been performed in the Asteraceae (Valles et al., 2005; Chehregani & Mehanfar, 2008). The most common basic chromosome number in the Asteraceae is x = 9, although x = 8 has also been reported by some researchers (Valles et al., 2005). Nevertheless, it seems that chromosome numbers are currently known for less than 40% of the species of the family, and new studies are still necessary to improve our knowledge of these plants (Volkova & Boyko, 1986; Valles et al., 2005; Chehregani & Mehanfar, 2008).

In general, 2500–3000 species of halophytes are found in the world. Nearly 700 species are distributed in the Mediterranean climatic zone (Güvensen et al., 2006). *Flora of Turkey* has some information about requirements of nearly all the taxa distributed in Turkey. Additionally, there are studies on the halophytes in Turkey, in particular including the publications of Beyce (1960), Birand (1960), Gehu and Uslu (1987), Güvensen and Öztürk (2003),

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Yurdakulol and Ercoşkun (1990), Yurdakulol et al. (1996), and Zeybek (1969, 1976). Turkey has a number of interior salt lakes and salt marshes, the largest of which is Lake Tuz (Turkish: Tuz Gölü), which is a centre of halophytic vegetation (Zohary, 1973).

2. Materials and methods

2.1. Specimen collection

During a field trip, some specimens belonging to the genus *Inula* were collected in Central Anatolia, in Konya Province. After careful examination, it was concluded that they constituted a new species belonging to the genus *Inula*. By studying the specific descriptions of *Inula* provided by Grierson (1975), Davis et al. (1988), Özhatay et al. (1999, 2009, 2011), and Özhatay and Kültür (2006), the new species was compared with specimens in the herbaria P, G, HUB, GAZI, ANK, AEF, and KNYA. It was found that this specimen was new to science. The averages of 15 measurements from different specimens were used. The specimens of *Inula tuzgoluensis* were examined and compared with specimens of the related species *Inula aucheriana* DC. in Turkey. Diagnostic morphological characters are discussed and arranged in a key with the *I. aucheriana* taxon. The authors of plant names were checked according to Brummitt and Powell (1992). The investigated representative specimens of *Inula aucheriana* and *I. tuzgoluensis* from different localities are cited in the Appendix.

2.2. Palynology

Polleniferous materials of *Inula aucheriana* and *I. tuzgoluensis* were obtained from herbarium specimens, and 20 pollen grains per specimen were studied. Palynological investigations were conducted with a scanning electron microscope. For scanning electron microscopy (SEM) investigations, achenes and pollen grains were directly mounted on the prepared stubs and coated with gold for SEM studies. Photographs were taken with a Zeiss LS-10 after coating with a Polaron SC7620 sputter coater for SEM studies. The terminologies of Erdtman (1952), Faegri and Iversen (1975), and Walker and Doyle (1975) were used for SEM aspects.

2.3. Cytogenetics

Karyological observations were made on metaphase cells of root tips obtained from germinating seeds. Root tips were pretreated at 4 °C for 16 h in α-monobromonaphthalene, washed, and fixed in Carnoy's solution (3:1, absolute ethanol:glacial acetic acid) overnight. The root tips were hydrolysed for 10 min in 1 N HCl at room temperature, and washed then stained in 2% acetoorcein for 2 h. Chromosome counts were conducted using Bs200Pro image analysis software (Martin et al., 2009; Dirmenci et al., 2010; Hamzaoğlu et al., 2010; Martin et al., 2011).

2.4. DNA isolation

Nuclear DNA was isolated from both herbarium and fresh leaf materials using the CTAB method (Sambrook et al., 1989). Dried silica gel leaf samples belonging to 7 *Inula* and outgroup *Phagnalon graecum* specimens from different localities were used in molecular analysis. DNAs were isolated with CTAB, and concentrations were determined by NanoDrop. Sample DNAs were diluted to 25 ng/μL. Stock DNAs were kept at –86 °C.

2.5. ISSR amplifications

Intersimple sequence repeat (ISSR) primers were amplified in a PCR thermal cycler. The characteristics of the primers used are given in Table 1. Each PCR reaction contained 25 μL comprising 2.5 μL of PCR buffer (10 mM TRIS/50

Table 1. List of the ISSR primers used for amplifications.

Primer	Nucleotide sequences	T_m (°C)	GC ratio (%)	Length (bp)	Number of bands
ISSR 1	5′- GAG AGA GAG AGA GAG AGA C- 3′	34.2	52.6	19	10
ISSR 2	5′- GAG AGA GAG AGA GAG AGA G- 3′	56.7	52.6	19	21
ISSR 7	5′- CAG CAC ACA CAC ACA CAC A- 3′	56.7	52.6	19	13
ISSR 8	5′- CGT CAC ACA CAC ACA CAC A- 3′	56.7	52.6	19	11
ISSR 5	5′- ACA CAC ACA CAC ACA CCG- 3′	56	55.6	18	8
F3	5′- AGA GAG AGA GAG AGA GCG- 3′	56	55.6	18	9
F2	5′- CTC GTG TGT GTG TGT GTG T- 3′	56.7	52.6	19	6
F4	5′- AGA GAG AGA GAG AGA GTG- 3′	53.7	50	18	14
F5	5′- AGA GAG AGA GAG AGA G- 3′	49.2	50	16	13
F6	5′- CCA CCA CCA CCA CCA- 3′	53.3	66.7	15	8

mM KCl buffer, pH 8.0), 3 µL of 25 mM $MgCl_2$, 0.5 µL of each primer, 0.5 µL of dNTP mix, 0.4 µL of Taq DNA polymerase, 4 µL of each DNA, and 14.1 µL of distilled water. After a predenaturation step of 3 min at 94 °C, amplification reactions were cycled 40 times at 94 °C for 1 min, at annealing temperature (Table 1) for 1 min, and at 72 °C for 1 min; a final extension was allowed for 10 min at 72 °C in an Eppendorf Mastercycler gradient thermocycler. Upon completion of the reaction, 15-µL aliquots of the PCR products were mixed with 3 µL of loading dye (50% glycerol, 0.25% bromophenol blue, and 0.15% xylene cyanol), then loaded onto a 2% agarose, 1X Tris-borate-EDTA gel and electrophoresed at 4 V/cm. Amplified fragments were visualised under a UV transilluminator and photographed using a gel documentation system (Vilbert Lourmat, Infinity model).

2.6. Data collection and cluster analysis

Amplified fragments from ISSR primers were visualised under UV transilluminator and photographed using a gel documentation system (Vilbert Lourmat, Infinity model). All the fragments amplified were treated as dominant genetic markers. Each DNA band generated was visually scored as an independent character or locus ('1' for presence and '0' for absence). Every gel was scored in triplicate (independent scoring), and only the consistently scored fragments were used for analyses. The data set was initially analysed using PAUP (Swofford, 1999). Analyses of standardised ISSR data were conducted using PAUP (Swofford, 1999).

2.7. Cluster analysis

In total, 113 ISSR data were determined from the 7 taxa of the genus *Inula* with *Phagnalon* sp. In both cluster analyses of the samples, the unweighted pair-group method with arithmetic mean (UPGMA) procedure was followed (Rohlf, 1992). ISSR data were obtained from 8 samples, and a total of 113 polymorphic bands were counted in a 113 × 8 matrix. The similarity coefficient method was used. Genetic distances were calculated with the simple matching coefficient. In order to determine the ability of ISSR data to display the interrelationships among the samples, analysis was conducted using PAUP.

3. Results

Inula tuzgoluensis M.Öztürk & O.Çetin **sp. nov.** (Figures 1 and 2).

Type: Turkey **C4** Konya: Cihanbeyli, between Gölyazı-Lake Tuz, 920 m, salty steppe, 13.07.2010, 38°33.57′N, 033°18.19′E, *M.Öztürk* 1248 & *A.Duran* (Holotype: KNYA, isotypes: GAZI, ANK, HUB, E).

Diagnosis: *Inula tuzgoluensis* is related to *I. aucheriana*. It mainly differs from *I. aucheriana* because it has middle and upper stem glabrous (not entirely white pilose hairs); cauline leaves clearly decurrent, glabrous (not decurrent,

Figure 1. General habitus view of a- *Inula aucheriana* and b- *Inula tuzgoluensis.*

white pilose hairs); outer phyllaries ±oblong, glabrous (not outer linear-lanceolate, usually reflexed, pilose); inner phyllaries narrowly lanceolate (not linear); achenes 2–2.4 mm, adpressed pubescent (not 1.6–1.8 mm, glabrous or only sparsely pubescent at apex), ray flowers' limb 2–3 mm wide (not 1.5–1.8 mm), pappus 5.5–6 mm (not 4–4.5 mm); 35–42 bristles (not 15–20).

Description: Perennial herb, rhizomatous or somewhat thick woody blackish rootstock. Stem erect, 15–50 cm tall, sometimes with a few sterile shoots, woolly with straight hairs at base, branching above, 1–4 mm diameter at base; lower stem sparsely simple hairs or ±glabrous; middle and upper part of stem glabrous. Leaves mostly basal, narrowly oblanceolate to obovate, semifleshy, entire. Basal and lower cauline leaves narrowly oblanceolate to obovate, gradually attenuating towards the base, 4–10 × 0.7–1.6 cm (incl. petiole), apex ±obtuse, glabrous, margins sparsely

Figure 2. *Inula aucheriana*: a- capitula, c- cauline leaves. *I. tuzgoluensis*: b- capitula, d- cauline leaves.

ciliate, with main midrib conspicuous; cauline leaves clearly decurrent; middle and upper cauline leaves narrowly obovate to oblong, decreasing size towards inflorescence, sessile or subsessile, glabrous or sometimes margins of middle cauline leaves minutely ciliate. Inflorescence laxly subcorymbiform panicles. Involucre 1–1.7 cm in diameter, semiglobular; capitula radiant, 3–32. Phyllaries imbricate, 4–5 seriate; outer phyllaries small, ±2 mm, ±oblong, glabrous; median phyllaries 3–4 mm, oblong to lanceolate, margin minutely ciliate; inner phyllaries 7–10 mm, narrowly lanceolate, margin ciliate, acuminate at apex. Flowers yellow; ray florets 13–19, 12–13 mm long, tube 3.5–4.5 × 0.6–1.1 mm long and ligules 7.5–8 × 2–3 mm long. Disc florets tubular, 5-lobed, 5.5–7 mm long, lobes ca. 0.7 mm long; style with 2 lobes, lobes 1–1.25 mm long. Anthers ca. 4 mm long. Achenes 2–2.4 mm long, cylindrical, densely adpressed-pubescent; pappus uniseriate, with 35–42 bristles, 5–5.6 mm long, scabrous, whitish, slightly connate. Flowering and fruiting in July–August.

Cypsela and pollen morphology: Pollen morphologies were examined with light microscopy and SEM. The pollen of *Inula tuzgoluensis* has radial symmetry, isopolar. Polar axis (P) is 35.38 µm, equatorial axis (E) is 36.20 µm. P/E ratio is 1.02 µm. The shape of the pollen grain is prolate-spheroidal. The aperture is tricolporate, and the colpi is 28 µm long. The exine is 1.61 µm. Spine is 3.17 µm long and 1.72 µm wide. The pollen of *I. aucheriana* has radial symmetry, isopolar. Polar axis (P) is 24.97 µm, equatorial

axis (E) is 26.27 µm. P/E ratio is 1.05 µm. The shape of the pollen grain is prolate-spheroidal. The aperture is tricolporate, and the colpi is 20 µm long. The exine is 0.9 µm. The spine is 2.88 µm long and 2.79 µm wide (Figures 3 and 4).

Achenes of *I. tuzgoluensis* are 2–2.4 mm long, cylindrical, densely adpressed-pubescent; pappus uniseriate, with 35–42 bristles, 5–5.6 mm long, scabrous, whitish, and slightly connate at base. Achenes of *I. aucheriana* are 1.6–1.8 mm long, cylindrical, glabrous or only sparsely pubescent at apex; pappus uniseriate, with 15–20 bristles, 4–4.5 mm long, scabrous, whitish, and free at base. Ridges are prominent on lateral sides of the pericarp, cell lines are placed in the inter costae (Figures 3 and 4).

Etymology: The specific epithet refers to the only known locality of the new species, Lake Tuz.

Distribution and ecology: *Inula tuzgoluensis* is an endemic species for Turkey, and Irano-Turanian element. It grows on salty steppe mainly in Central Anatolia. One of the shallowest lakes of Anatolia, Lake Tuz comes from a geologically tectonic origin. The Lake Tuz basin is an A-type watery area, based on international criteria in terms of conservation of biological variety. The species grows in salty steppe with *Verbascum pyroliforme* (Boiss. & Heldr.) O.Kuntze, *Silene salsuginea* Hub.-Mor., *Frankenia hirsuta* L., *Leymus cappadocicus* (Boiss. & Bal.) Melderis, *Panderia pilosa* Fisch. & C.A.Mey., *Camphorosma monspeliaca* L., *Achillea wilhemsi* C.Koch., *Limonium iconicum* (Boiss. & Heldr.) O.Kuntze, *L. anatolicum* Hedge, *Astragalus ovalis* Boiss. & Balansa, *Anthemis fumariifolia* Boiss., *Scorzonera hieraciifolia* Hayek, *Aeluropus littoralis* (Gouan) Parl., *Taraxacum farinosum* Hausskn. & Bornm., *T. mirabile* Wagenitz, *Onosma halophilum* Boiss. & Heldr., *Salvia halophila* Hedge, *Asparagus lycaonicus* P.H.Davis, and *Centaurea tuzgoluensis* Aytaç & H.Duman.

IUCN red list category: Lake Tuz is the second largest lake in Turkey and one of the largest salt lakes in the world. The habitat at Lake Tuz (Konya Province)

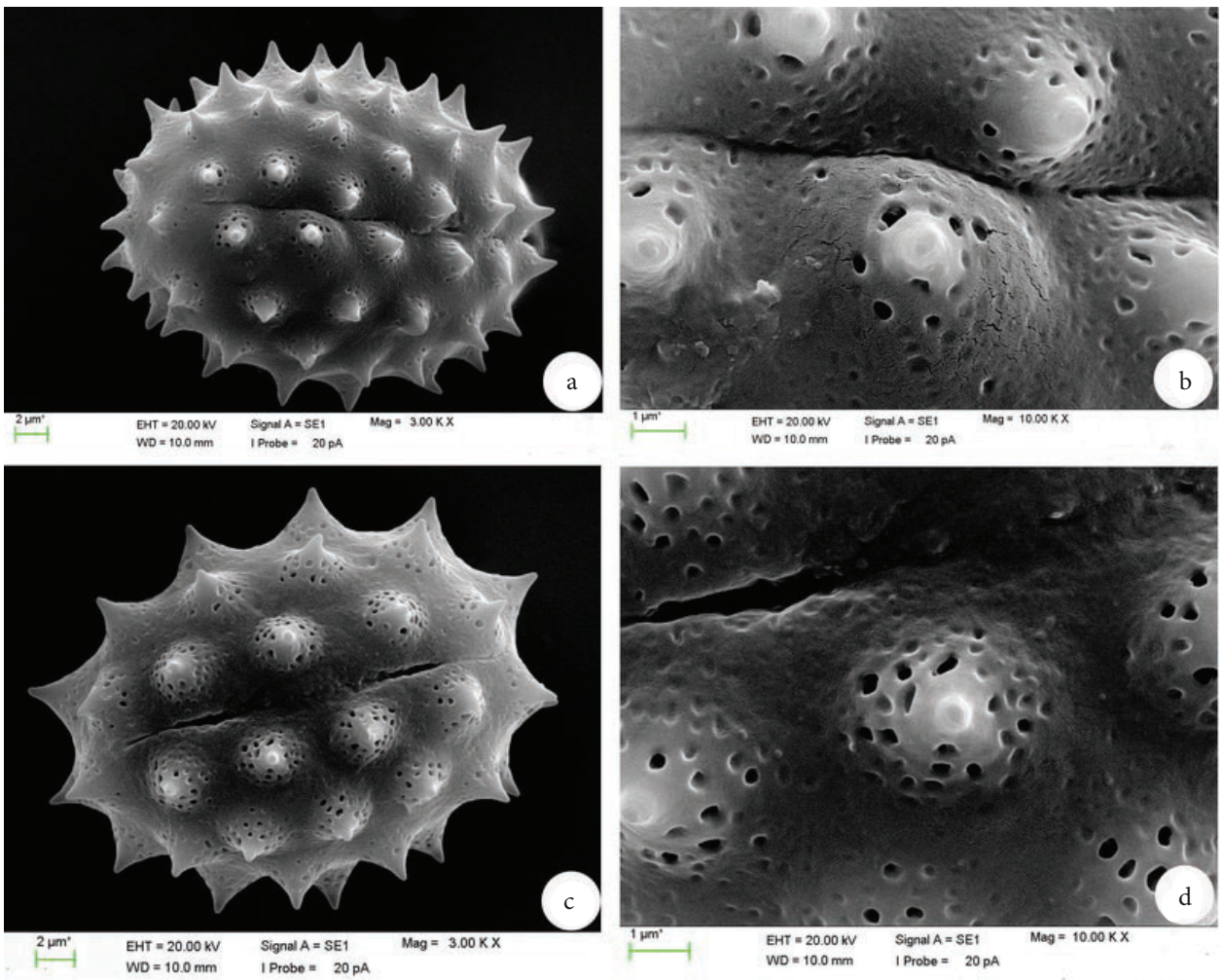

Figure 3. SEM photo of *Inula aucheriana*: a- general shape, b- details of surface. *Inula tuzgoluensis*: c- general shape, d- details of surface.

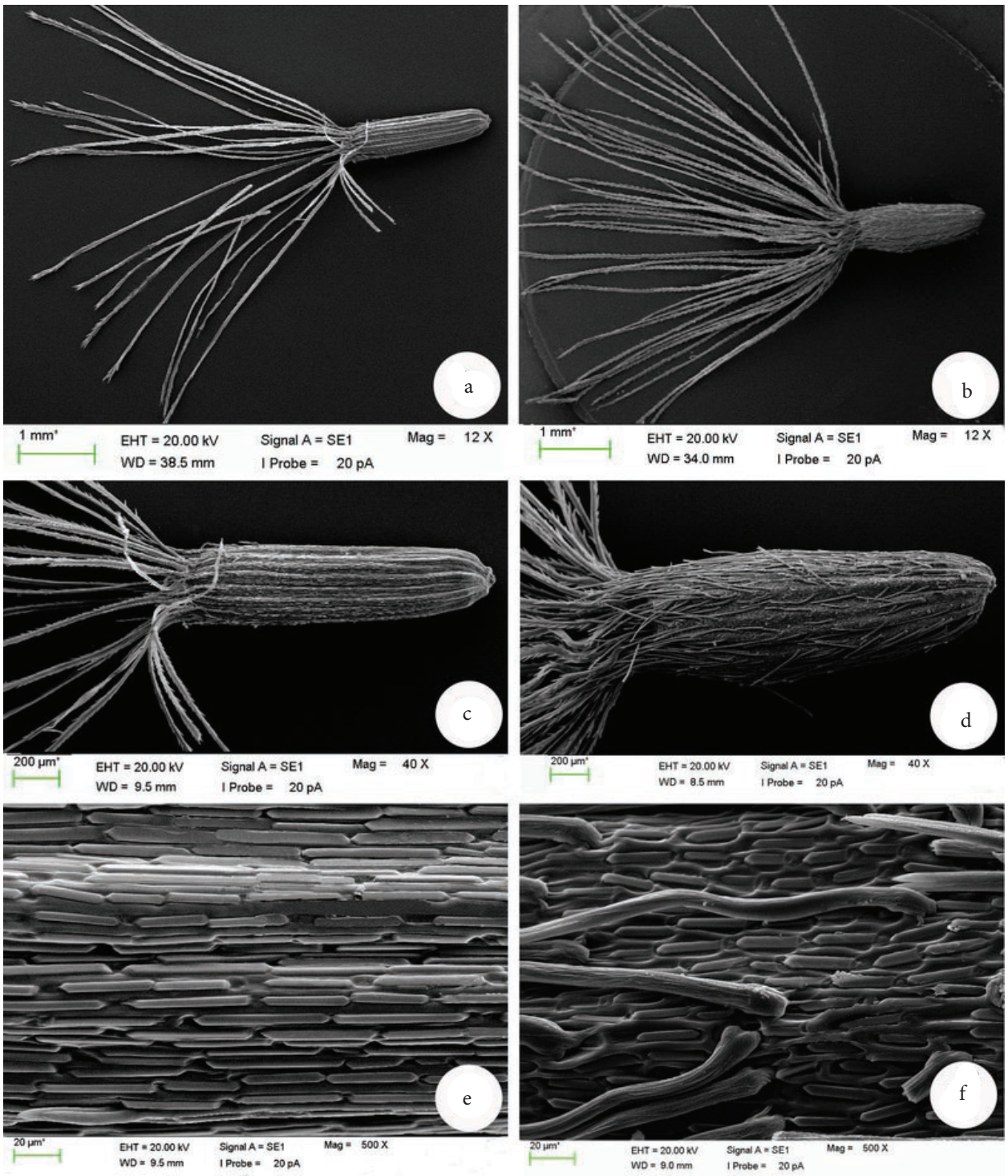

Figure 4. SEM images of *Inula aucheriana* and *I. tuzgoluensis*. *I. aucheriana*: a- cypsela general shape, c- achene indumentums, e- fruit wall details. *I. tuzgoluensis*: b- cypsela general shape, d- achene indumentums, f- fruit wall details.

and its surrounding area is one of the most important wetlands in Turkey and was declared the 'Lake Tuz Special Environmental Protection Area' by the Republic of Turkey Ministry of Environment and Forest in 2000 (Doğan et al., 2011). Lake Tuz is fed by rainfall, groundwater, and surface streams. Because the ecological balance of the ecosystem has been ignored, groundwater levels decrease with each passing year, and resources are weakening and drying.

Inula tuzgoluensis is known in only one locality, and this area is smaller than 2.5 km^2 (Criterion B1). Animal herds are watered in this area. Because of overgrazing, the habitat of this species is under threat, and destruction of the species is leading to the reduction in the number of plants (Criterion A). Mature individual members of the population constitute approximately 235 specimens (Criterion C2). Therefore, it should be considered critically endangered (CR) according to the IUCN Red List Criteria (IUCN Standards and Petitions Subcommittee, 2010).

4. Discussion

In the south and south-western parts of Lake Tuz, there are exceptional areas that include salty and fresh-water marshes. There are 54 local endemic plant species that grow in especially high concentrations of salt around Lake Tuz and its surroundings. Recently, 13 taxa were added to the *Flora of Turkey*. These taxa are *Halanthium kulpianium* (K.Koch) Bunge (Freigtag et al., 1999); *Astragalus demirizii* R.Kramer & Podlech (Podlech, 1999); *Dianthus aydogdui* Menemen & Hamzaoğlu (Menemen & Hamzaoğlu, 2000); *Taraxacum tuzgoluense* Yıld. & Doğru-Koca (Yıldırımlı & Koca, 2005); *Centaurea tuzgoluensis* Aytaç & H.Duman (Vural et al., 2006); *Limonium adilguneri* Yıld. & Doğru-Koca (Yıldırımlı & Doğru-Koca, 2006); *Senecio salsuginea* H.Duman & Vural (Vural et al., 2006); *Acantholimon halophilum* Bokhari var. *coloratum* Dogan & Akaydın (Dogan & Akaydın, 2007); *Linum ertugrulii* Tugay, Bağcı & Uysal (Tugay et al., 2010); *Scorzonera tuzgoluensis* A.Duran, B.Dogan & S.Makbul (Dogan et al., 2011); *Frankenia salsuginea* Adıgüzel & Aytaç (Vural et al., 2012); and *Iberis halophila* Vural & H.Duman (Vural et al., 2012).

During the evaluation of local endemic plants in Lake Tuz and its environs, 17 plants were determined to be critically endangered (CR), 7 plants endangered (EN), 16 plants vulnerable (VU), 7 plants of least concern (LC), and 6 plants near threatened (NT). One species is in the data deficient (DD) category according to IUCN categories and criteria (IUCN Standards and Petitions Subcommittee, 2010).

4.1. Key to related *Inula* species

1. Middle and upper part of stem glabrous; cauline leaves clearly decurrent; achenes 2–2.4 mm long adpressed pubescent, pappus 5.5–6 mm long, with 35–42 bristles ……..…….. ***I. tuzgoluensis***
1. Stem entirely pilose hairs; cauline leaves not decurrent; achenes 1.6–1.8 mm long, glabrous or only sparsely pubescent at apex, pappus 4–4.5 mm long, with 15–20 bristles ……...……...……..….. ***I. aucheriana***

Inula tuzgoluensis is related to *I. aucheriana*, which is endemic in Turkey. It mainly differs from *I. aucheriana* by stem erect, middle and upper stem glabrous (not ascending, entirely sparsely covered with white spreading pilose hairs); cauline leaves clearly decurrent, glabrous (not decurrent, sparsely white spreading pilose hairs); margins of inner phyllaries ciliate (not membranous, densely glandular); achenes 2–2.4 mm long, densely long adpressed pubescent (not 1.6–1.8 mm long, glabrous or only sparsely pubescent at apex), pappus 5.5–6 mm (not 4–4.5 mm); with 35–42 bristles (not 15–20); pollen polar axis (P) 35.38 µm, equatorial axis (E) 36.20 µm [not polar axis (P) 24.97 µm, equatorial axis (E) 26.27 µm], spine 3.17 × 1.72 µm (not 2.88 × 2.79 µm). A comparison of *I. tuzgoluensis* and *I. aucheriana* is given in Table 2. When

Table 2. Comparisons between the diagnostic characters of *Inula tuzgoluensis* and *I. aucheriana*.

Characters	*Inula tuzgoluensis*	*Inula aucheriana*
Stem	erect	ascending
Stem indumentums	woolly with straight hairs at base, lower stem sparsely simple hairs or glabrous, upper and middle part of stem glabrous	becoming dense and lanate at base, entirely covered with white spreading pilose hairs
Lower leaves	narrowly oblanceolate to obovate, gradually attenuating towards the base, margins sparsely ciliate	ovate-lanceolate, base attenuate, margin sparsely pilose
Upper leaves	clearly decurrent, narrowly obovate to oblong, glabrous	not decurrent, oblong, amplexicaule and somewhat auriculate, sparsely white spreading pilose hairs
Phyllaries	outer small, ±oblong, ±2 mm, glabrous; median oblong to lanceolate, 3–4 mm, margin minutely ciliate; inner narrowly lanceolate, margin ciliate	outer and median linear-lanceolate, 3–7 mm, usually squarrose tips, minutely glandular and white pilose; inner membranous, ±linear, margin glabrous, densely glandular
Ray flowers	13–19, 12–13 mm long, tube 3.5–4.5 × 0.5–0.6 mm long and ligules 7.5–8 × 2–3 mm long	20–45, 5–8 mm long, tube 2–3 × 0.2–0.4 mm long and ligules 3–5 × 1.5–1.8 mm long
Disc flowers	5.5–7 mm	5–5.5 mm
Achenes	2–2.4 mm long, densely adpressed pubescent	1.6–1.8 mm long, glabrous or only sparsely pubescent at apex
Pappus	5.5–6 mm long, with 35–42 bristles and slightly connate at base	4–4.5 mm long, with 15–20 bristles and free at base

the new species is compared with the other *Inula* species in *Flora of Turkey*, *Flora of Europe*, and *Flora Iranica*, it is similar to species *I. aucheriana* (Figures 3 and 4). Most of the cypselae characters are quite stable characteristics. As is evident from the different generic and specific keys, almost all of the taxa can be delimited on the basis of cypselae characters. Therefore, the micromorphological characters of the cypselae have proven very rewarding in these taxa.

The *Inula tuzgoluensis* chromosome number, 2n = 18, was determined in this study for the first time. The chromosome numbers were determined as 2n = 18 for *I. crithmoides* Spreng. and *I. aucheriana* DC. (Pavone et al., 1981; Chehragani & Hajisadeghian, 2009); 2n = 16 for *Inula britannica* L., *I. bifrons* L., *I. candida* (L.) Cass., *I. helvetica* Weber, *I. hirta* L., *I. montana* L., *I. salicina* L., *I. spiraeifolia*, and *I. sechmenii* (Skalińska et al., 1959; Brullo et al., 1977; Kuzmanov & Georgieva, 1983; De Montmollin, 1984; Strid, 1987; Opova & Sekovski, 1989; Kokubugata & Koyama, 1999; Chepinoga et al., 2009); and 2n = 32 for *I. britannica*, *I. conyza* DC., and *I. oculus-christi* L. (Kuzmanov & Nikolova, 1977; Mizianty et al., 1981; Magulaev, 1982; Dmitrieva, 1987; Chepinoga et al., 2009) in previous studies. It seems that polyploidy and variation in chromosome number is common in Asteraceae, and there is triploidy of some members.

The most common basic number in the Asteraceae is x = 9, although x = 8 has also been reported by some researchers (Carr et al., 1999; Valles et al., 2005). In this study, in *Inula tuzgoluensis* and *Inula aucheriana* the chromosome number was n = x = 9. Here, the *Inula tuzgoluensis* chromosome numbers are given for the first time to the scientific world (Figure 5).

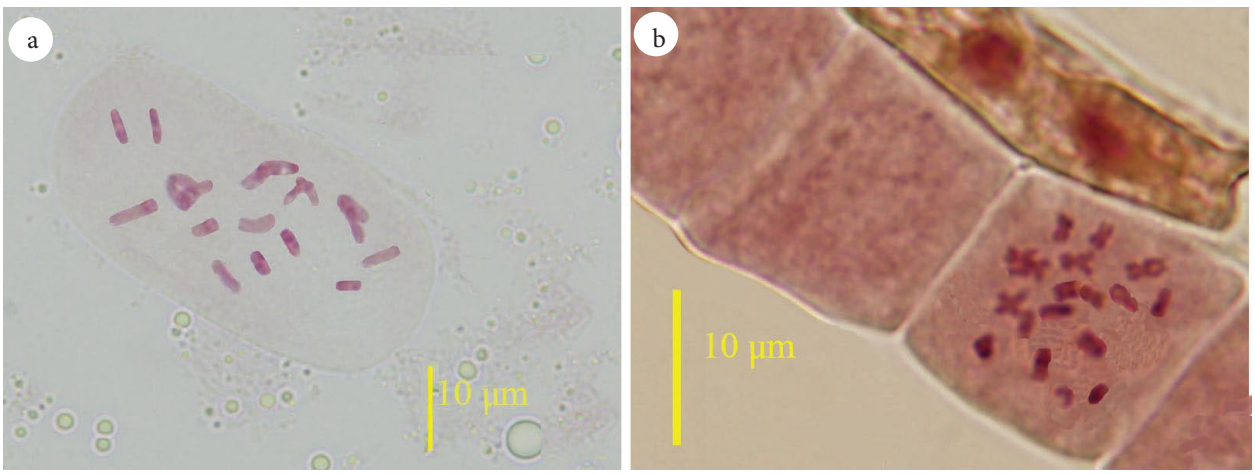

Figure 5. Somatic metaphase 2n = 18 in a- *Inula tuzgoluensis* and b- *I. aucheriana*.

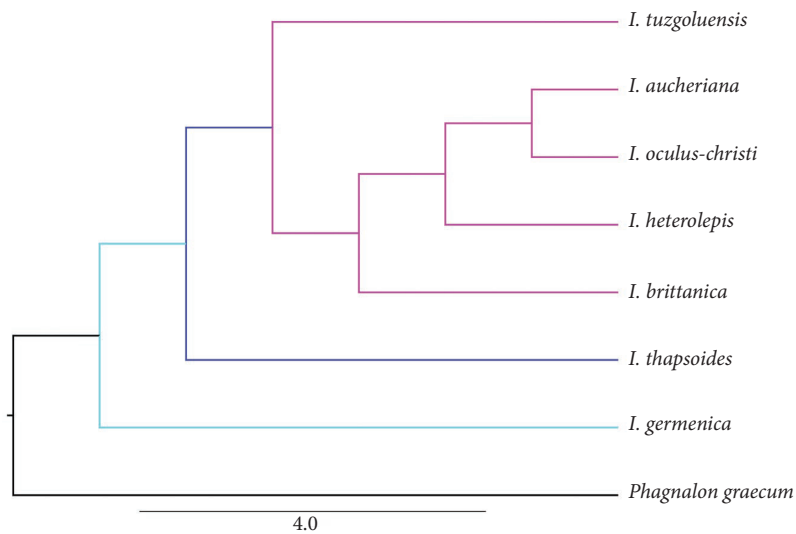

Figure 6. The UPGMA tree showing genetic relationship of the *Inula* species using with PAUP.

In the phylogenetic analysis, 7 *Inula* taxa were evaluated with the outgroup *Phagnalon*. From an initial screening of 10 ISSR primers, 6 primers revealed high levels of polymorphisms. These primers generated 113 highly polymorphic fragments that were consistently amplified in repeated experiments. The GC percentages of the selected primers were within the range of 34.2%–56.7% (4 of them being 56.7%). Genetic distances were calculated as 0.46–0.99, with the SM coefficient ranging from the evaluation of the ISSR data. These similarities are supported by the UPGMA tree.

The cophenetic correlation of the distance matrix and tree matrix was 0.4, indicating the dendrogram to the distance matrix (Rohlf, 1992). Two major groups and 7 clades can be distinguished: (I) *I. aucheriana* and *I. oculus-christi*, (II) a major group with *I. heterolepis* Boiss., (III) *I. britannica* L. and *I. conyza*, (IV) *Inula tuzgoluensis*, (V) *I. thapsoides* Spreng., (VI) *I. germanica* L., and (VII) *Phagnalon* as the outgroup. The taxonomic relationships of the *Inula* and *Phagnalon* taxa are shown in the UPGMA tree comprising all operational taxonomic units in the present work (Figure 6).

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Appendix

Representative specimens examined:

(*molecular samples) - ***Inula tuzgoluensis*** (paratypes): Turkey. C4 Konya: Cihanbeyli, between Gölyazı and Lake Tuz, after Dumanağıl place, 923 m, 25.09.2011, *A.Duran* 9313 & *B.Doğan*, *E.Martin*, Ö.Çetin (KNYA); ibid., 9. km, 922 m, 12.06.2006, *A.Duran* 7180 (*KNYA). - ***Inula aucheriana***: Turkey. C4 Konya: Cihanbeyli, between Gölyazi and Lake Tuz, 13. km, 915 m, salty steppe, 13.07.2010, *M.Öztürk* 1245 & *A.Duran* (*KNYA); Konya: Cihanbeyli, between Gölyazı and Lake Tuz, 13. km, 923 m, 25.09.2011, salty steppe, *A.Duran* 9316 & *B.Doğan*, *E.Martin*, Ö.Çetin (KNYA); Konya: Cihanbeyli, environments of Bolluk Lake, 1000 m, 13.08.1993, *M.Koyuncu* 10652 (AEF; ANK); Konya: Cihanbeyli, back side of the Alkim factory, salty fields, 1010 m, 11.07.1992, *M.Koyuncu* 9581 & *H.Güvenç* (AEF); Konya: Kaşınhanı, *Huber-Morathii* 5091; A4 Çankırı: 10 km to Çankırı, 27.08.1995, 650 m, *N.Adıgüzel* 2497 & *Z.Aytaç* (GAZI); B5 Aksaray: Sultanhanı, Eşmekaya, 950 m, 22.9.1993, *M.Vural* 7073 & *H.Duman*, *N.Adıgüzel*, *F.Karavelioğlu* (GAZI); B3 Afyon: Çay, surroundings of Eber Lake, meadows, 960 m, 10.08.1992, *A.A.Dönmez* 2958 & *N.Emin* (HUB, GAZI); Cappadocia ad Euphraterm, *Aucher* 3091 (P photo!, P 02707088; G-DC photo!); B5 Aksaray: Sultanhanı, Eşmekaya, 950 m, 22.9.1993, sides of the drainage canal, *M.Vural* 1993 & *H.Duman*, *N.Adıgüzel*, *F.Karamanoğulları* (GAZI). - ***I. germenica***: A1 Edirne: Enez, Gala Lake, surroundings of Avcının çeşmesi, 2 m, 9.7.2010, clearings of maquis, *A.Duran* 9069 (*KNYA). - ***I. heterolepis***: C3 Denizli: Honaz, Menteş village, surroundings of Alçı madeni ocağı, 924 m, 17.7.2008, *A.Duran* 8063 & *M.Öztürk, Ö.Çetin* (*KNYA).; C1 Muğla: Eski Kale road, Yılanlı mountain, 1360 m, 23.07.2006, calcareous slopes, *A.Duran 7307* (*KNYA). - ***I. thapsoides***: A5 Yozgat: Aydıncık, surroundings of Dereçiftlik-Çalıntaş, 6.10.1998, 1300 m, clearings of forest, *A.Duran* 4425 (*KNYA). - ***I. brittanica*** Edirne: Enez, Gala Lake, surroundings of Avcının çeşmesi, 2 m, 9.7.2010, clearings of maquis, *A.Duran* 9068 (*KNYA). - ***I. oculis-christi***: A5 Yozgat: Aydıncık, Kazankaya valley, 750 m, 28.06.2000, *A.Duran* 5444 (*KNYA). – ***Phagnalon graecum:*** C1 Muğla: Marmaris, back of Günücek Park, roadside, 1–50 m, 14.7.2012, Ö.Çetin 1080 & *B.Doğan, M.Çelik* (*KNYA).

References

Anderberg A (1991). Taxonomy and phylogeny of the tribe *Inuleae* (Asteraceae). *Plant Systematics and Evolution* 176: 75–123.

Anderberg A & Eldenäs P (2007). Tribe *Inuleae* Cass. In: Kubitzki K. (ed.) *The Families and Genera of Vascular Plants*, Vol. 8. Berlin: Springer, pp. 374–390.

Beyce O (1960). Indicator plants of aridity and water table studies in Alifaki. *Agriculture Research Institute* (Turkey) 26: 17–53.

Birand H (1960). Erste Ergebnisse der Vegetations-Untersuchungen in der zentralanatolischen Steppe; 1-Halophytengesellschaften des Tuz Gölü. *Botanische Jahrbücher* 79: 255–296.

Blatter E (1927). *Beautiful Flowers of Kashmir*, Vol. 1. London: John Bale.

Boissier E (1875). *Flora Orientalis*, Vol. 3. Geneva and Basel: H. Georg.

Brullo S, Pavone P, Terrasi MC & Zizza A (1977). Numeri cromosomici per la flora Italiana: 299–314. *Informatore Botanico Italiano* 9: 57–71.

Brummitt RK & Powell CE (1992). *Authors of Plant Names*. Kew: Royal Botanic Gardens.

Carr GD, King RM, Powell AM & Robinson (1999). Chromosome numbers in Compositae. *American Journal of Botany* 86: 1003–1013.

Chehregani A & Hajisadeghian S (2009). New chromosome counts in some species of Asteraceae from Iran. *Nordic Journal of Botany* 27: 247–250.

Chehregani A & Mehanfar N (2008). New chromosome counts in the tribe Anthemideae (Asteraceae) from Iran. *Cytologia* 73: 189–196.

Chepinoga VV, Gnutikov AA, Enushchenko IV & Rosbakh SA (2009). In: IAPT/IOPB chromosome data 8. *Taxon* 58: 1281–1282.

Clarke CB (1876). *Compositae Indicae*. Calcutta: Thacker.

Davis PH, Mill RR & Tan K (1988). *Inula* L. In: Davis PH, Mill RR & Tan K (eds.) *Flora of Turkey and the East Aegean Islands* (Suppl. 1), Vol. 10. Edinburgh: Edinburgh University Press, p. 159.

De Candolle AP (1836). *Inula* L. In: De Candolle A (ed.) *Prodromus Systematis Naturalis Regni Vegetabilis*. Vol. 5. Paris: Fortin, Masson et Sociorum, pp. 343–476.

De Montmollin B (1984). Etude cytotaxonomique de la flore de la Cre`te. II. Nombres chromosomiques. *Botinica Helvetica* 94: 261–267.

Dirmenci T, Dündar E, Deniz G, Arabacı T, Martin E & Jamzad Z (2010). Morphological, karyological and phylogenetic evaluation of *Cyclotrichium*: a piece in the tribe *Mentheae* puzzle. *Turkish Journal of Botany* 34: 159–170.

Dmitrieva SA (1987). Kariologicheskaja kharakteristika nekotorykh predstaviteley sem. slozhnocvetnykh (Asteraceae Dumort.) flory Belorussii. *Botanika* (Minsk) 28: 23–33.

Doğan B, Duran A & Makbul S (2011). *Scorzonera tuzgoluensis* sp. nov. (Asteraceae), a new halophytic species from central Anatolia, Turkey. *Nordic Journal of Botany* 29: 20–25.

Doğan M & Akaydın G (2007). Synopsis of Turkish *Acantholimon* Boiss. (Plumbaginaceae). *Botanical Journal of the Linnean Society* 154: 397–419.

Duran A, Öztürk M & Doğan B (2009). A new species of the genus *Psephellus* (Asteraceae) from North-East Anatolia, Turkey. *Ozean Journal of Applied Sciences* 2: 103–111.

Erdtman G (1952). *Pollen Morphology and Plant Taxonomy Angiosperm, An Introduction to Palynology*, Vol. 1. Stockholm: Almqvist and Wiksell.

Erik S & Tarıkahya S (2004). Türkiye florası üzerine. *Kebikeç* 17: 117–137 (in Turkish).

Faegri K & Iversen J (1975). *Text Book of Pollen Analysis*. New York: Hafner Press.

Freitag H, Vural M & Adıgüzel N (1999). A remarkable new *Salsola* and some new record of records of Chenopodiaceae from Central Anatolia, Turkey. *Willdenowia* 29: 123–139.

Gehu JM & Uslu T (1987). Donnés sur la Végétation Littorale de la Turquie du Nord-Ouest. Phytocoenologia 17: 449–505.

Gorshkova SG (1959). *Inula* L. In: Komorov VL & Shishkin BK (eds.) *Flora of the USSR*, Vol. 25. Bishen Singh Mahendra Pal Singh & Koeltz Sci. Books: Dehradun, pp. 464–513.

Grierson AJC (1975). *Inula* L. In: Davis PH (ed.) *Flora of Turkey and the East Aegean Islands,* Vol. 5. Edinburgh: Edinburgh University Press, pp. 54–73.

Güner A, Aslan S, Ekim T, Vural M & Babaç MT (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. İstanbul: Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayını (in Turkish).

Güvensen A, Görk G & Öztürk M (2006). An overview of the halophytes in Turkey. In: Khan MA, Böer B, Kust GS & Barth HJ (eds.) *Sabkha Ecosystems*, Vol. 2, West and Central Asia. Dordrecht: Springer, pp. 9–30.

Güvensen A & Öztürk M (2003). Halophytic plant diversity of South Aegean coastal zone in Turkey. *Pakistan Journal of Botany* 35: 853–864.

Hamzaoğlu E, Aksoy A, Martin E, Pınar NM & Çölgeçen H (2010). A new record for the flora of Turkey: *Scorzonera ketzkhovelii* Grossh. (Asteraceae). *Turkish Journal of Botany* 34: 57–61.

Hooker JD (1881). *The Flora of British India*, Vol. 3. Ashford: JD Hooker.

IUCN Standards and Petitions Subcommittee (2010). Guidelines for Using the IUCN Red List Categories and Criteria. Version 8.1., March 2010. Available at http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf [accessed 19 February 2013].

Judd WS, Campbell CS, Kellogg EA, Stevens PF & Donoghue MJ (2007). *Plant Systematics: A Phylogenetic Approach*, 2nd ed. Sunderland, Massachusetts: Sinauer Associates, Inc.

Kitamura S (1960). *Flora of Afghanistan. Results of the Kyoto University Scientific Expedition to the Karakorum and Hindukush 1955*, Vol. 2. Kyoto: Kyoto University.

Kitamura S (1964). *Plants of West Pakistan and Afghanistan*. Kyoto: Committee of the Kyoto University Scientific Expedition to the Karakorum and Hindukush, Kyoto University.

Kokubugata G & Koyama H (1999). A cytotaxonomical study of two species of *Inula* (Asteraceae) by fluorescence in situ hybridization using ribosomal DNA probe. *Acta Phytotaxonomica et Geobotanica* 50: 27–33.

Kuzmanov B & Georgieva S (1983). IOPB chromosome number reports 81. *Taxon* 32: 665.

Kuzmanov B & Nikolova V (1977). IOPB chromosome number reports 58. Taxon 26: 557–565.

Kumar S (1995). Asteraceae (Inuleae-Vernonieae). In: Hajra PK, Rao RR, Singh DK & Uniyal BP (eds.) *Flora of India*, Vol. 13. Calcutta: Botanical Survey of India, pp. 11–32.

Lack HW (2007). Tribe Cichorieae Lam. & DC. In: Kubitzki K (ed.) *The Families and Genera of Vascular Plants*, Vol. 8, Flowering Plants: Eudicots, Asterales. Berlin: Springer, pp. 180–199.

Linnaeus C (1753). *Species Plantarum*, 1st ed. London: The Ray Society.

Linnaeus C (1754). *Genera Plantarum*, 5th ed. New York: H. R. Engelmann.

Magulaev AY (1982). The number of chromosomes of the species of Asteraceae, Caryophyllaceae and Plantaginaceae of the North Caucasus. *Biologicheskie Nauki (Minsk)* 11: 74–79.

Makbul S, Coskunçelebi K, Gültepe M, Okur S & Güzel ME (2012). *Scorzonera ahmet-duranii* sp. nov. (Asteraceae) from southwest Anatolia, and its phylogenetic position. *Nordic Journal of Botany* 30: 2–11.

Martin E, Çetin Ö, Akçiçek E & Dirmenci T (2011). New chromosome counts of genus Stachys (Lamiaceae) from Turkey. *Turkish Journal of Botany* 35: 671–680.

Martin E, Dinç M & Duran A (2009). Karyomorphological study of eight *Centaurea* L. taxa (Asteraceae) from Turkey. *Turkish Journal of Botany* 33: 97–104.

Menemen Y & Hamzaoğlu E (2000). A new species of *Dianthus* (Caryophyllaceae) from Salt Lake, Central Anatolia, Turkey. *Annales Botanici Fennici* 37: 285–287.

Mizianty M, Frey L & Mirek Z (1981). Contribution to the knowledge in the chromosome numbers of Polish vascular plants. *Fragmenta Floistica et Geobotanica* 27: 19–29.

Opova M & Sekovski Ž (1989). Chromosome atlas of some Macedonian angiosperms. *Godishen Zbornik Biologija Prirodno-Matematichki Fakultet na Univerzitetot Kiril i Metodij* 39–40: 353–365.

Özhatay N & Kültür Ş (2006). Check-list of additional taxa to the supplement flora of Turkey III. *Turkish Journal of Botany* 30: 281–316.

Özhatay N, Kültür Ş & Aksoy N (1999). Check-list of additional taxa to the supplement flora of Turkey II. *Turkish Journal of Botany* 23: 151–170.

Özhatay N, Kültür Ş & Aslan S (2009). Check-list of additional taxa to the supplement flora of Turkey IV. *Turkish Journal of Botany* 33: 191–226.

Özhatay NF, Kültür Ş & Gürdal NB (2011). Check-list of additional taxa to the supplement flora of Turkey V. *Turkish Journal of Botany* 35: 589–694.

Pavone P, Terrasi CM, Zizza A (1981). In chromosome number reports LXXII. *Taxon* 30: 695–696.

Podlech D (1999). New *Astragali* and *Oxytropis* from North Africa and Asia, including some new combinations and remarks on some species. *Sendtnera* 6: 135–174.

Rechinger KH (1980). *Inula* L. In: Rechinger KH (ed.), *Flora Iranica*, Compositae 4- *Inuleae*, Vol. 145. Graz: Akad. Druck-u Verlagsanst, pp. 77–96.

Rohlf FJ (1992). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.0. Stony Brook, New York: State University of New York at Stony Brook.

Sambrook J, Fritschi EF & Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Skalińska M, Czapik R, Piotrowicz M (1959). Further studies in chromosome numbers of Polish Angiosperms (Dicotyledons). *Acta Societatis Boticorum Poloniae* 28: 487–529.

Strid A (1987). Chromosome numbers of Turkish mountain plants. *Notes Royal Botanical Garden Edinburgh* 44: 351–356.

Swofford DL (1999). PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 40b10. Sunderland, Massachusetts: Sinauer Associates.

Tugay O, Bağcı Y & Uysal T (2010). *Linum ertugrulii* (Linaceae), a new species from central Anatolia, Turkey. *Annales Botanici Fennici* 47: 135–138.

Valles J, Garnatje T, Garcia S, Sanz M & Korobkov AA (2005). Chromosome numbers in the tribes Anthemideae and Inuleae (Asteraceae), Kazakhstan. *Botanical Journal of Linnean Society* 148: 77–85.

Volkova SA & Boyko EV (1986). Chromosome numbers in some species of Asteraceae from the southern part of the Soviet far east. *Botanicheskii Zhurnal* 71: 16–93.

Vural M, Duman H, Aytaç Z & Adıgüzel N (2006). *Saponaria karapinarensis*, *Senecio salsuginea* and *Centaurea tuzgoluensis*, three new species from central Anatolia, Turkey. *Belgian Journal of Botany* 139: 252–260.

Vural M, Duman H, Aytaç Z & Adıgüzel N (2012). A new genus and three new species from Central Anatolia. *Turkish Journal of Botany* 36: 427–433.

Walker JW & Doyle JA (1975). The bases of angiosperm phylogeny: palynology. *Annals of the Missouri Botanical Garden* 62: 664–723.

Yıldırımlı Ş & Doğru-Koca A (2005). A new species and variety of *Taraxacum*, *T. tuzgoluensis* and *T. tuzgoluensis* var. *eskilensis* (Asteraceae) from Tuz Gölü, Turkey. *Ot Sistematik Botanik Dergisi* 12: 1–12.

Yıldırımlı Ş & Doğru-Koca A (2006). A new species *Limonium adilguneri* Yıld. & Doğru-Koca (Plumbaginaceae) from edge of Tuz Gölü, inner Anatolia Turkey. *Ot Sistematik Botanik Dergisi* 13: 11–16.

Yurdakulol E & Ercoşkun T (1990). Ecological and syntaxonomical studies on the vegetation of arid lands in central Anatolia. *Turkish Journal of Botany* 14: 109–123.

Yurdakulol E, Öncel I, Demirörs I, Yıldız A & Keleş Y (1996). Ecological and syntaxonomical investigation of salt marshes vegetation in the vicinity of Burdur and Acıgöl (Denizli-Turkey). *Ecologia Meditterrenea* 22: 51–61.

Zeybek N (1969). *Coastal halophytes of the Aegean Sea*. VI. Turkish Nat. Biology Congress, İzmir, pp. 3–7.

Zeybek N, Sheikh KH & Öztürk MA (1976). *Coastal Halophytes of the Aegean Region*. CENTO Symposium, Adana, pp. 148–153.

Zohary M (1973). *Geobotanical Foundations of the Middle East*, Vol. 1. Stuttgart: Gustav Fischer Verlag.