

Morphological and molecular data reveal a new species of *Jurinea* (Asteraceae) from Western Black Sea Region, Turkey

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Abstract. The authors describe a new species for science: *Jurinea efea* N. Aksoy. It is endemic to Northwestern Anatolia, Turkey. Morphologically, the new species is most similar to *J. pontica*. Molecular and morphological analyses have been used to distinguish the two *Jurinea* taxa. The species has been first identified morphologically and then studied by ISSR markers. Genetic similarities have been compared using ISSR band profiles and two main clusters were observed. Combined data have revealed that *J. efea* was different from the related samples of *J. pontica*. Furthermore, the morphological similarities to *J. pontica* and the diagnostic morphological characters are discussed. The ecology and distribution of *J. efea* are also presented. The leaf surface characteristics and achene surface of *J. efea* and *J. pontica* are examined by SEM.

Key words: *Compositae*, ISSR, *Jurinea*, new species, SEM, taxonomy, Turkey

Introduction

The genus *Jurinea* Cass. comprises about 200 species across the world. Native distribution of *Jurinea* covers Central Asia, Iran, Turkey, and the Mediterranean region. *Jurinea* falls into the monophyletic subtribe *Car-duinae* (Susanna & al. 2006). The numbers of species in the neighbouring countries are as follows: 152 in the former USSR (Iljin 1962), 37 in Iran (Rechinger & Wagenitz 1979) and 17 in Europe (Kožuharov 1976). Turkey is not only amongst the richest countries in *Jurinea* diversity but *Jurinea* endemism in Turkey accounts for about 42.1%. It is represented by 18 species in the Mediterranean and Irano-Turanian phytogeographic regions of Turkey (Danin & Davis 1975; Doğan & al. 2007, 2010a, b). Eight of these species are endemic to Turkey (Danin & Davis 1975; Doğan & al. 2009, 2010c, 2014). Furthermore, on the basis of mo-

lecular data Turkey is the main centre of diversity for genus *Jurinea* (Doğan & al. 2010a).

In the present study, two *Jurinea* species, which are difficult to characterise by morphological traits alone, were collected from the natural flora of Turkey. A reliable and highly informative system for DNA fingerprinting ISSR-PCR was used, in order to reveal the relationships between the two *Jurinea* species.

Material and methods

The new species was collected by the senior author (NA) in July of 2014, from Yiğilca (Düzce) in the Western Black Sea Region of Turkey, during floristic and vegetation fieldwork on a Biodiversity Project in Düzce Province. In July 2015, the research area was visited again and more specimens with flowers and fruits were

collected. In total, 15 herbarium specimens (25 individuals) of the presumably new species were collected from two adjacent localities and deposited in the herbaria of DUOF. These specimens were checked with the herbaria of DUOF, GAZI, KEW, ISTO, and ISTE, as well as with the *Flora of Turkey* (Danin & Davis 1975; Davis & al. 1988; Güner & al. 2000), *Flora Europaea* (Kožuharov 1976), *Flora of the USSR* (Iljin 1962), *Flora Iranica* (Rechinger & Wagenitz 1979), a revision of the Turkish *Jurinea* (Doğan & al. 2007), and the latest checklist of the *Flora of Turkey* (Doğan 2012). After a comparison with the material of morphologically similar taxa, the authors have decided that the specimens represented a species new to science. A substitute name was provided and a detailed description and illustration were submitted. The authors of plant names were cited after Brummitt and Powell (1992).

The new species was collected from an open rock area of *Fagus orientalis-Quercus petraea* forest in Yiğilca – Düzce province, 1000–1200 m a.s.l., in 2014–2015, and deposited at DUOF Herbarium. The location of the new species in the Düzce province falls within A3 grid square (Davis 1965). The threat category of the new species was evaluated according to IUCN Criteria (IUCN 2001).

Specimen collection. Dried plant leaf samples belonging to six *Jurinea* specimens were taken from the GAZI and DUOF Herbaria. Their localities according to the Herbaria are as follows: *Jurinea pontica* (GAZI); 1 – *M. Vural 4183* (Ankara-Ayaşbeli), 2 – *S. Aslan 1571-b* (Ankara-Mamak), 3 – *M. Vural 5071*, *U. Kol and N. Adıgüzel* (Nevşehir-Avanos), 4 – *M. Sağıroğlu 1405* (Çankırı-Ilgaz), 5 – *E.G. Çakır 1517* (Ankara-Nallıhan) and *Jurinea efeae* sp. nov. (DUOF), 6 – *N. Aksoy 7926* (Düzce-Yiğilca).

Extraction of DNA. DNA was isolated from dried leaves of the herbarium samples by the method of Doyle & Doyle (1987), by the modified CTAB (cetyltrimethylammonium bromide) extraction method. Dried leaf tissues (0.03 g) were ground by mortar and pestle under liquid nitrogen. Homogenisation of the plant material was accomplished with 1500 µL of preheated CTAB extraction buffer (100 mM Tris [pH 8.0], 20 mM EDTA [pH 8.0], 1.4 M NaCl, 0.2 % (p/v) β-mercaptoethanol, 2 % [p/v] CTAB, 1 % [v/w] PVP) and incubated at 60°C for two hrs. A volume of chloroform: isoamyl alcohol mix (24:1) was added

and centrifuged at 10 000 rpm for 10 min. DNA was precipitated in the presence of isopropanol by centrifugation at 13.000 rpm for 30 min after incubation at –20°C for ≥1 hr. The DNA pellet was dissolved in 100 µl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]). After addition of 1 µL of RNase (10 mg/ml) and incubation at 37°C for 30 min, DNA was precipitated with 3 mM ammonium acetate and 96 % EtOH. The DNA pellet was washed with 70 % EtOH, then dried and dissolved in 100 µl TE Buffer. The DNA samples were quantified by the spectrophotometric method (NanoDrop ND-1000 spectrophotometer). The quality of DNA samples was evaluated by electrophoresis on 1 % agarose gel, according to the method of Sambrook & al. (1989).

PCR amplification and DNA analysis. Eight ISSR markers were used for amplification. To optimise the reaction conditions, different PCR parameters were tested, including MgCl₂ concentration, DNA concentration, primer concentration and number of cycles. Amplification conditions were a 5 min initial denaturation step (94°C), followed by 35 cycles of 1 min (94°C), 1 min (specific annealing temperature) and 1 min (72°C). The reactions were completed by a final extension step of 10 min (72°C). Amplification was carried out in an ABI Thermocycler. The optimum annealing temperature was determined for each primer.

The PCR products were run on a 1.5 % agarose gel against 100 bp ladder (MBI Fermentas) and stained with ethidium bromide for visualisation. Three technical replications were performed for each sample (Table 1).

Table 1. The list of ISSR markers.

Primer	Primer Seq	bp	Tann	Tm
ISSR1	ACCACCACCACCACCACCg	19	61	63,1
ISSR2	gAgAgAgAgAgAgAgAC	19	56	56,7
ISSR3	AgAgAgAgAgAgAgAgC	19	56	56,7
ISSR4	ACACACACACACACACg	19	56	56,7
ISSR5	ACACACACACACACCg	18	55,5	56
ISSR6	ACACACACACACACCC	18	55,5	54,8
ISSR7	CAGCACACACACACACA	19	56	56,7
ISSR8	CgTCACACACACACACA	19	56	56,7

SEM Study. Achenes and leaves were mounted on stubs by double-sided adhesive tape. Each sample was coated with a 100 Å thick layer of gold in a Polaron SC7620 rotating and tilting vacuum-coating apparatus for 60 seconds and scanned by JEOL

5600 LV scanning electron microscope (SEM) at 20 kV accelerating voltage. Afterwards, the achenes and leaves were examined with a light microscope and SEM and were measured to obtain the morphological findings.

Results and discussion

Jurinea efea N. Aksoy sp. nov. (Figs. 1 and 2).

Diagnosis. *Jurinea efea* is close to *J. pontica* Hausskn. & Freyn ex Hausskn., but it can be distinguished from it as follows: a perennial herb with a woody rootstock

(no such rootstock in *J. pontica*), up to 60–85 cm (20–90 cm in *J. pontica*), with arachnoid-tomentose, numerous thickened below capitula, and generally 4–20 one-headed branches in the upper part. Basal leaves rosulate, pinnatipartite, 8.5–17.0 × 3.37–4.82 cm and withering at flowering-time (basal leaves pinnatisect, 3–15 × 2–6 cm in *J. pontica*). Lower and median cauline leaves; sessile or 1.5–3.4 cm asymmetrically decurrent, stem wings 1.4–4.8 mm wide, narrowly lanceolate to subulate, up to half of the stem, 8.2–11.8 × 1.2–1.8 cm (lower and upper cauline leaves undecurrent and stem unwinged in *J. pontica*). Capitula are usually numerous (4–20), borne on 40 cm branchlets.

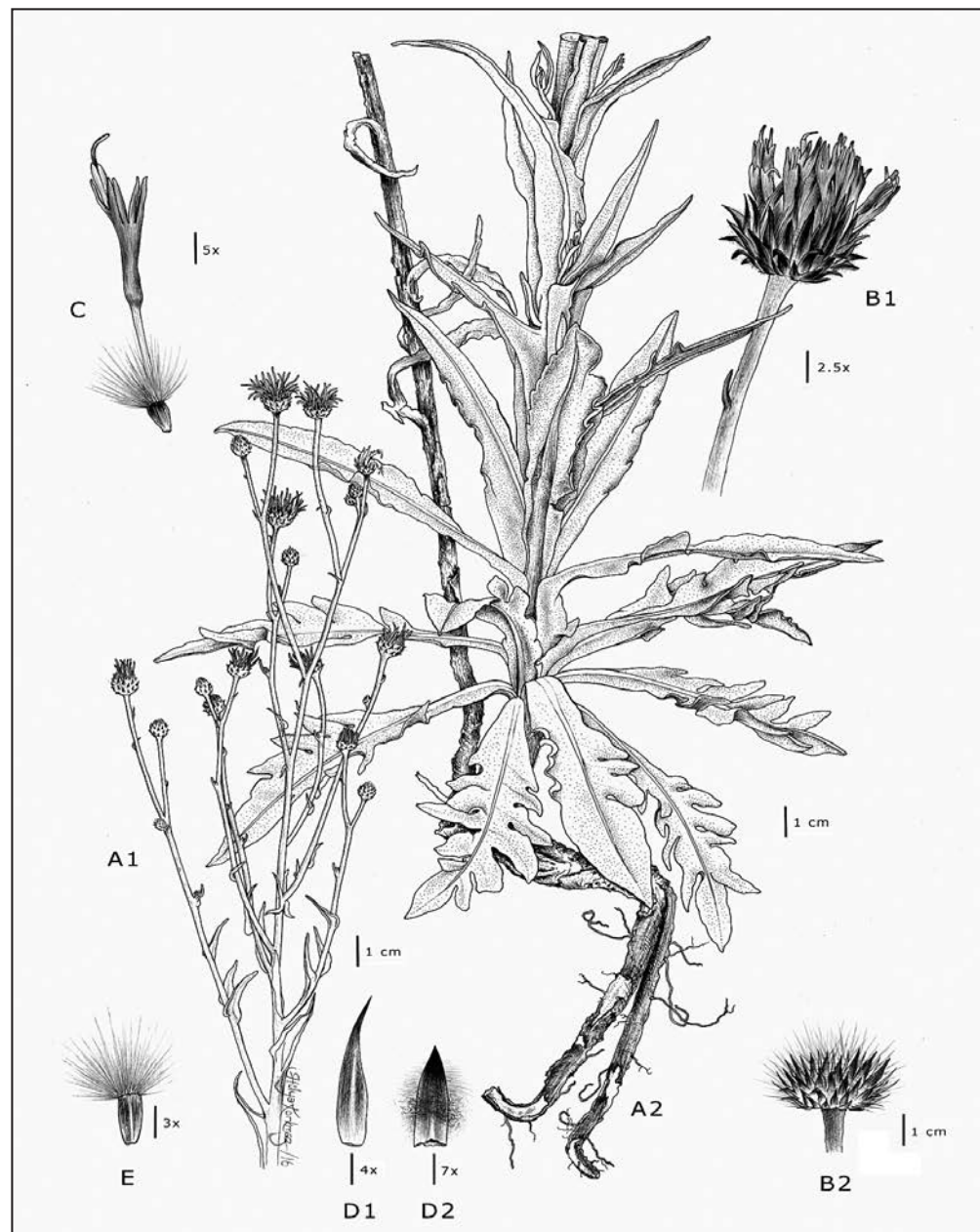


Fig. 1. *J. efea* sp. nova (from the holotype) – A1, A2. Habitus – B1. Capitulum with flowers – B2. Capitulum with seeds – C. Flower – D1. Median phyllaries – D2. Outer phyllaries – E. Achene (A-H: N. Aksoy 7926 (DUOF 7020)).

Involucre subglobose, 6.06–10.47 × 9.42–12.36 mm; phyllaries 4–5-seriate (6–7-seriate in *J. pontica*), imbricate, outer phyllaries lanceolate 2.64–4.15 × 0.85–1.1 mm, (2–4 × 1 mm in *J. pontica*). Achenes obpyramidal, 3.8–5.5 mm long, beak at apex (tetragonal, 3–4 mm long, without beak at apex in *J. pontica*).

Type. Turkey, A3 Düzce, Yiğilca, on in the road between Yoğunpelit and Yaylatepe villages, 1175 m, in a *Fagus-Quercus* mixed forest and in open limestone rock areas, on southeastern slopes, 40°50'03"N, 031°39'37"E, 19.06.2016, N. Aksoy 7926 (Holotype: DUOF 7020!; Isotypes: GAZI!, ISTO!).

Description. A perennial herb with a woody rootstock. Stem erect, arachnoid-tomentose all over, (50) 60–85 cm long and 4–9 mm in diameter at base, thickened below the capitula, generally, with 4–20 one-headed branches in the upper part. Basal leaves rosulate, pinnatifid, 8.5–17.0 × 3.4–4.8 cm, white-woolly beneath, arachnoid-tomentose on top, revolute margin repandate in shape; lateral segments irregular, 3–6 pairs, opposite to alternate, variable, narrowly lanceolate to falcate, entire, 10.20–30.7 × 4.17–4.13 mm, undivided, apex acute; terminal lobe similar to lateral ones, 22.17–47.91 × 8.33–12.50 mm. Basal leaves wither at flowering time; lower and median cauline leaves sessile, or 1.5–3.4 cm asymmetrically decurrent (stem wings 1.4–4.8 mm wide), narrowly lanceolate to subulate, up to half of the stem, 8.2–11.8 × 1.2–1.8 cm, white-woolly beneath, arachnoid-tomentose on top, revolute margin; upper cauline leaves sessile or 0.3–1.5 cm asymmetrically decurrent (stem wings 0.1–4.8 mm wide), tendrillous, subulate to acicular, 1.01–2.79 × 1.2–1.6 cm, revolute margin, apex acute. Capitula usually numerous (4–20), borne on 3–40 cm branchlets. 9.58–22.06 × 6.13–14.85 mm; involucre subglobose, 6.06–10.47 ×

9.42–12.36 mm; phyllaries 4–5-seriate, imbricate, outer phyllaries lanceolate, 2.64–4.15 × 0.85–1.1 mm, arachnoid, herbaceous, erect or with slightly reflexed erect tips, black at apex, inner phyllaries lanceolate, 0.91–4.94 × 0.47–0.84 mm, glabrous, purplish-red at apex and margin, spinescent and reflexed. Paleas yellowish, linear, 0.5–1.5 mm long. Corolla purple-reddish, 5.85–9.86 mm, 5-lobed, lobes 2–3.5 mm long, tubes 3.81–6.96 mm long; anthers 6.4–9.3 mm long, styles 6.55–9.25 mm long, with two branches. Achenes obpyramidal, longitudinally ridged, ridges edentulate, 3.8–5.5 mm long, dull yellow-brown, beak at apex, pappus persistent, barbellate, dirty-white, 2.5–8 mm long. Flowering from June to July, fruiting from mid-July to the end of August.

Distribution and ecology. *Jurinea efea* is endemic to the Euro-Siberian Floristic Region and Northwestern Anatolia (Yiğilca-Düzce) and (Yenice-Karabük). It is an Euxine element (Fig. 2). It flowers and fruits in June and July. *Jurinea efea* is distributed in the open limestone rocky areas of a *Fagus orientalis-Quercus petraea* forest, on disturbed ground, Yiğilca in Düzce province, 1080–1200 m a.s.l. It grows together with *Taxus baccata* L., *Quercus petraea* Liebl. ssp. *iberica* (Steven ex Bieb.) Krassiln., *Quercus cerris* L. var. *cerris*, *Carpinus betulus* L., *Corylus avellana* L., *Chamaecytisus hirsutus* (L.) Link, *Erica arborea* L., *Cistus creticus* L., *Rosa canina* L., *Genista lydia* Boiss. var. *lydia*, *Lathyrus undulatus* Boiss., *Hypericum calycinum* L., *Eupatorium cannabinum* L., *Tanacetum parthenium* (L.) Sch. Bip., *Teucrium chamaedrys* L. and *Campanula lyrata* L.

Conservation status. *Jurinea efea* is not widespread in the Düzce and Karabük provinces and is known only from two localities: around Yeşilyayla village in Yiğilca (Düzce) and on the banks of river Çitdere valley in Yenice (Karabük). Apparently, it is known only from the present localities and its estimated area of occupancy is less than 10 km² (criterion B). The population is unhealthy, with less than fifty mature individuals (criterion D). Furthermore, such extremely restricted area implies a high risk of extinction owing

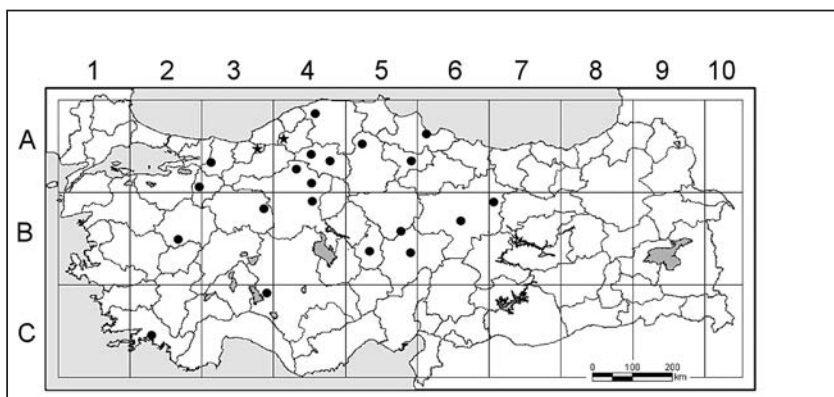


Fig. 2. Geographical distribution of *J. efea* sp. nov. (★) and *J. pontica* (●) in Turkey.

to grazing, and its natural habitat is destroyed by road and dam constructions. Therefore, it should be classified as Critically Endangered (CR) B1ab(iii)+2ab(iii); D on the basis of the criteria of the IUCN Red List Categories (IUCN 2001).

Etymology. This new species is dedicated to Prof. Dr. Asuman Efe, who passed away in 2010, a well-known Turkish dendrologist who performed research on *Liquidambar* L., *Rhamnus* L. and other woody and herbaceous species in Turkey and had contributed much to the knowledge of Turkish plants.

ISSR profiles. ISSR markers seem to be quite effective in differentiating the two taxa in this study, Figs 3 and 4.

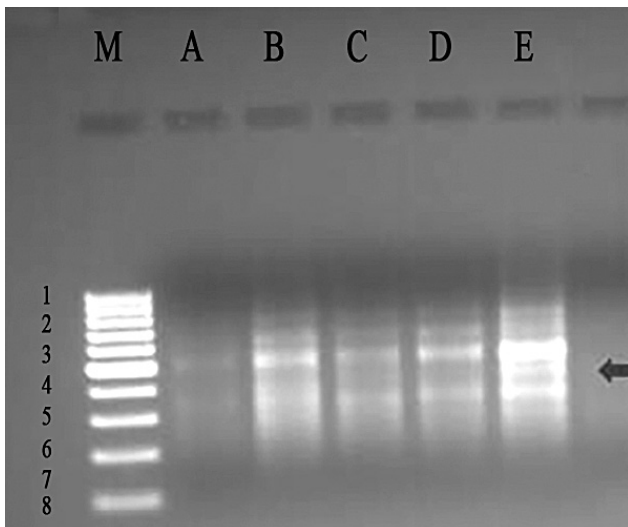


Fig. 4. Band profiles of five different DNA samples generated by ISSR1 marker M: 100 bp ladder (MBI Fermentas), (A-D. *J. pontica* (GAZI) and E. *J. efea* sp. nov. sp. nov. (from the holotype, (DUOF))).

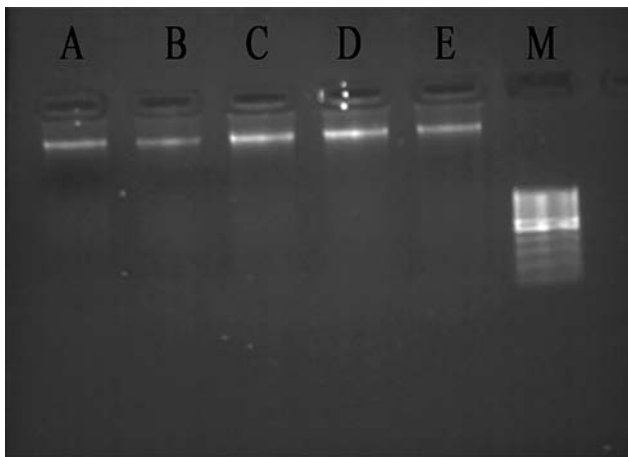


Fig. 3. Extracted genomic DNA samples from different herbarium samples. M: 100 bp ladder (MBI Fermentas), (A-D. *J. pontica* (GAZI) and E. *J. efea* sp. nov. sp. nov. (from the holotype, (DUOF))).

Taxonomic affinities

This new species is relatively close to *Jurinea pontica* but differs from it by the characters listed in Table 2. Furthermore, micro morphological structures of the leaf surfaces and achene belonging to the species of *Jurinea efea* sp. nov. and *J. pontica* were examined in this study, Table 3, Figs 5 and 7.

Table 2. Morphological comparison of *Jurinea efea* sp. nov. with *J. pontica*.

Characters	<i>Jurinea efea</i>	<i>Jurinea pontica</i>
Stem	(50)60–85 cm	20–90(–125) cm
basal leaves	8.5–17.0 × 3,37–4.82 cm	3–15 × 2–6 cm
Cauline leaves decurent	1.5–3.4 cm 1.4–4.8 mm	1.5–7 × 0.2–1 cm –
Capitula	0.95–2.2 × 0.61–1.48 cm	2.1–4 × 2–3 cm
Involucre	0.6–1,47 × 0.94–1.23 cm	1.5–3 × 1.4–2 cm
Outer phyllaries	2.64–4.15 × 0.85–1.1 mm	2–4 × 1–1.5 mm
Inner phyllaries	0.91–4.94 × 0.47–0.84 mm	9–15 × 1–2 mm
Paleas	0.5–1.5 mm	2–4 mm
Corolla	5.85–9.86 mm	5–7 mm
Papus	3.8–5.5 mm	6–8 mm
Achene	3.8–5.5 mm	3–4 mm
Habitat	open lime stone rock area of <i>Fagus orientalis</i> – <i>Quercus petraea</i> forest	woods, steppe, fields and slopes

Table 3. Some characters of *Jurinea efea* sp. nov. and *J. pontica*.

Characters	<i>Jurinea efea</i>	<i>Jurinea pontica</i>
Upper leaf surface	Subsessile oil glands and non-glandular trichomes	Subsessile oil glands, short non-glandular trichomes and curving non-glandular trichomes
Lower leaf surface	Curving non-glandular trichomes (villous)	Curving non-glandular trichomes (villous)
Achene	Obpyramidal	Tetragonal
Achene surface	Rough, rectangular cells with deep gaps	Smooth, long hexagonal cells
Achene hairs	Glabrous	Minute conic non- glandular hairs
Pappus	Longer than achene, scaberulous	Longer than achene, scabrous

The upper leaf surfaces of *Jurinea efea* have sessile oil glands and non-glandular trichomes, whereas *Jurinea pontica* has sessile oil glands, short non-glandular trichomes and curving non-glandular trichomes. However, the lower leaf surface of *Jurinea efea* and *Jurinea pontica* have curving non-glandular trichomes, Table 3 and Fig. 5.

Taxonomic treatments use names for particular trichome appearances, but a SEM study offers greater precision (Metcalf & Chalk 1950; Fahn 1988; Malesci & Servettaz 1991; Navarro & El Oualidi 2000; Eshratifar & al. 2011). Curving non-glandular trichomes are defined in *Krameria grayi* (Krameriaceae) by Sherwin Carlquist similarly to *Jurinea efea* and

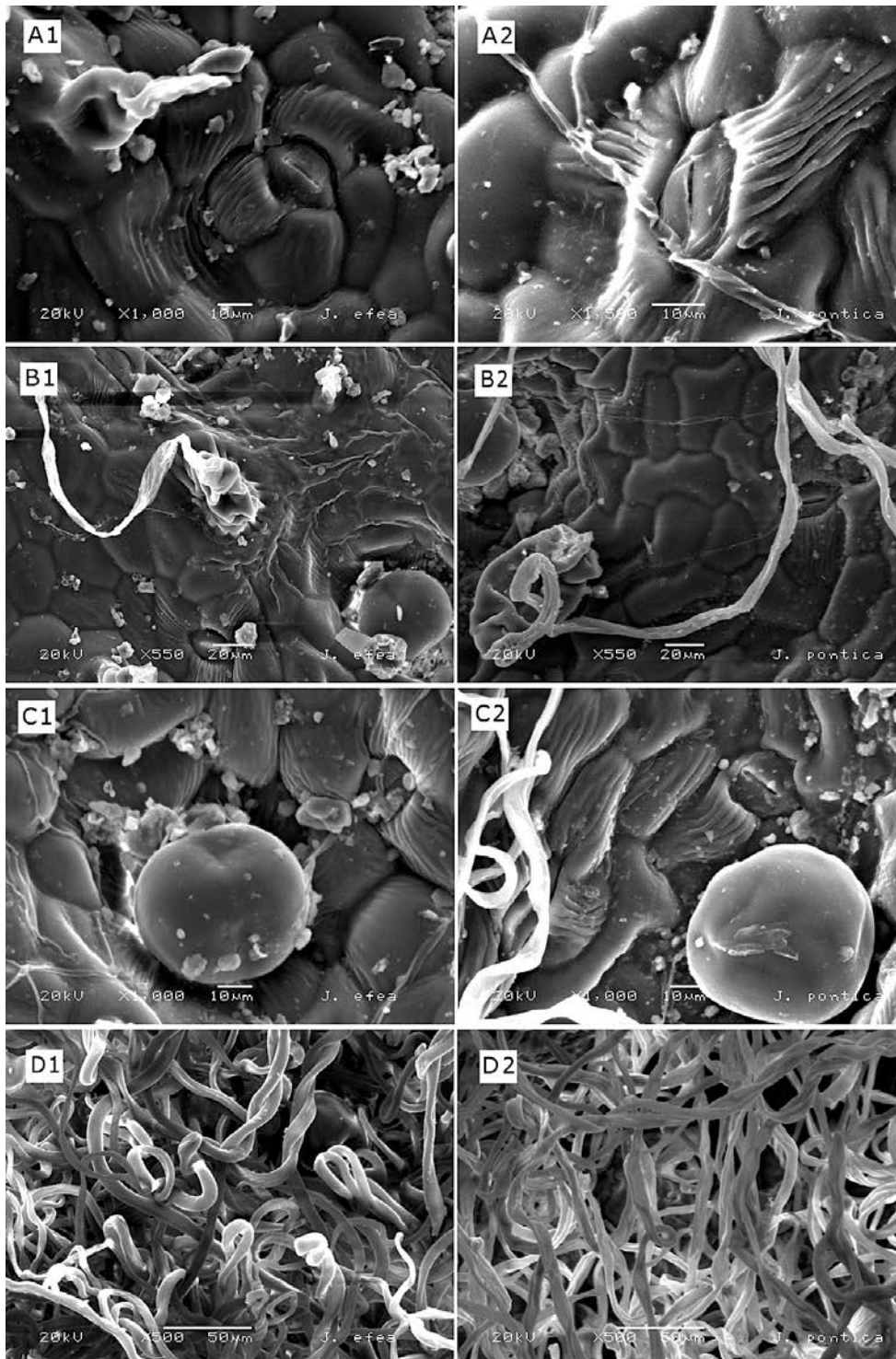


Fig. 5. SEM micrographs of leaves. Stoma: A1 – *J. efea*, A2 – *J. pontica*; Upper leaves surface: B1 – *J. efea*; B2 – *J. pontica*; Glandular hair (upper leaves surface): C1 – *J. efea* C2 – *J. pontica*; Lower leaves surface: D1 – *J. efea* D2 – *J. pontica* (*J. efea* from the holotype N. Aksoy 7926, DUOF 7020, *J. pontica* 5 from E.G. Çakır 1517, GAZI).

Jurinea pontica leaves (<http://www.sherwincarlquist.com>). On the other hand, Rustaiyan & Taherkhani (2013) have determined the composition of the essential oil of *J. leptoloba*. This finding suggests that there are oil glands in some *Jurinea* species.

Characteristics of the seed surfaces are very important for distinguishing the species (Dinç & al. 2008; Eshratifar & al. 2011) and are commonly used in systematic and evolutionary studies (Barthlott 1981, Marin & al. 1994). Achene surfaces of *Jurinea efea* consist of glabrous rectangular cells with deep gaps. Achene surfaces of *Jurinea pontica* are formed by long hexagonal cells, with minute conic non-glandular hairs. The pappus is longer than the achene in both *Jurinea efea* and *Jurinea pontica*. However, the pappus of *Jurinea efea* is scaberulous, while in *Jurinea pontica* it is scabrous, Table 3, Figs 6 and 7.

Specimens examined

***Jurinea efea* (paratypes).** Turkey – A3 Düzce, Yiğilca, at the entrance to Yaylatepe village, 1080 m, a.s.l., in a *Fagus orientalis*-*Pinus nigra* mixed forest and open limestone rock areas, on southeastern slopes, 40°59'37"N 031°40'14"E, 19 vi 2016, N. Aksoy 7927, DUOF 7021. A4 Karabük, Yenice, at the entrance to the Şeker Canyon, 220-300 m, in a *Fagus-Quercus* mixed forest and open limestone rock areas,

41°11'54"N 32°22'00"E, 10 vi 2012, N. Aksoy 7339, DUOF 7022. Turkey-Prov. Zonguldak, Balıkısık (near Yenice), 150 m., on rocky limestone slopes, fls. purple, perennial, 22 vii 1962, Davis, Coode and Yaltırık, D. 37969, Det. Danin and Davis, Davis 1972 sub *J. pontica* subsp. *integrifolia*, RBG EDIN!. Zonguldak, Şimşir Dere gorge behind Yenice, 300–400 m., on limestone rocks, fls. purple, 22 vii 1962. Davis, Coode and Yaltırık, D. 37923, RBG EDIN! (sub *J. pontica* subsp. *integrifolia*). When Danin studied the material of *J. pontica* from Northern Anatolia, he found that it is somewhat different from the type and annotated these specimens as “subsp. *integrifolia*” in the RBGE Herbarium, but he never published that name. This subspecies differs from *J. pontica* in its erect stem, arachnoid-tomentose, thickened below capitula, generally with 3–12 one-headed branches in the upper part, sessile lower and median cauline leaves with a asymmetrically decurrently. On the basis of these characters, it is closely related to the new species, *J. efea*.

***J. pontica*.** B4 Ankara, Ayaşbeli, Akkaya hill, 1250 m, 23 vi 1986, M. Vural 4183!. B4 Ankara, Mamak, valley of Kıbrıs village, in rocky areas, 1000-1100 m, 01 vi 2004, S. Aslan 1571-b!. B5 Nevşehir, Avanos, on volcanic tuff, at the hedge of fruit orchards, 1150 m,

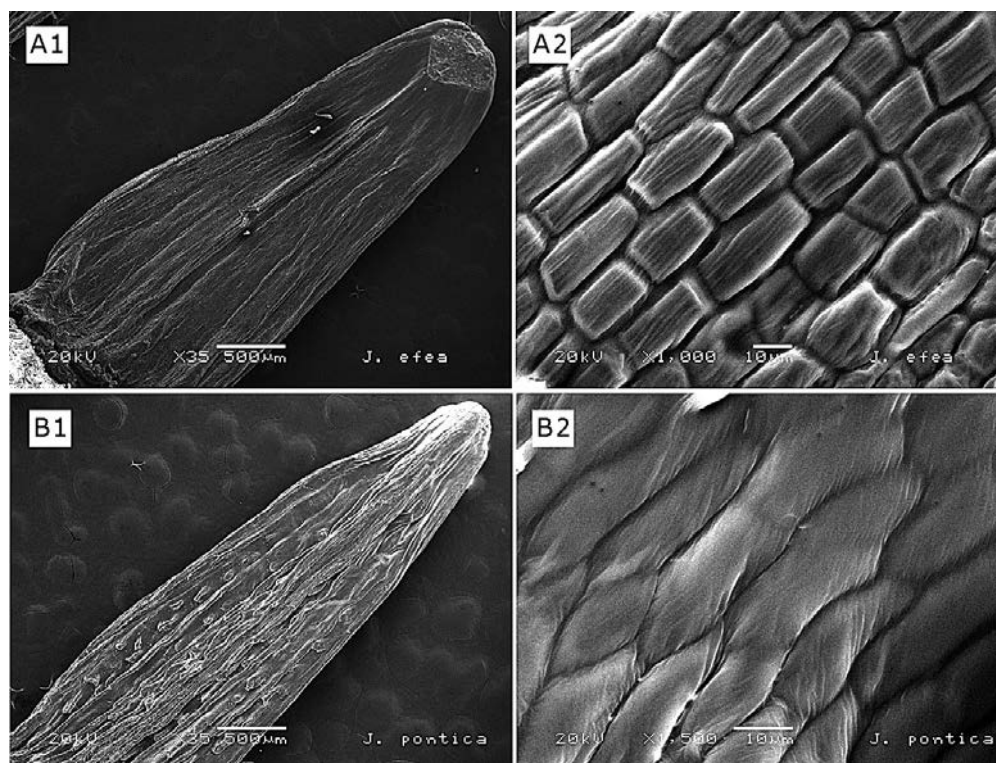


Fig. 6. SEM-micrographs of achenes. *J. efea*: **A1.** General shape: **A2.** Details of surface (from the holotype N. Aksoy 7926, DUOF 7020) and *J. pontica* – **B1.** General shape: **B2.** Details of surface (from E.G. Çakır 1517,GAZI).

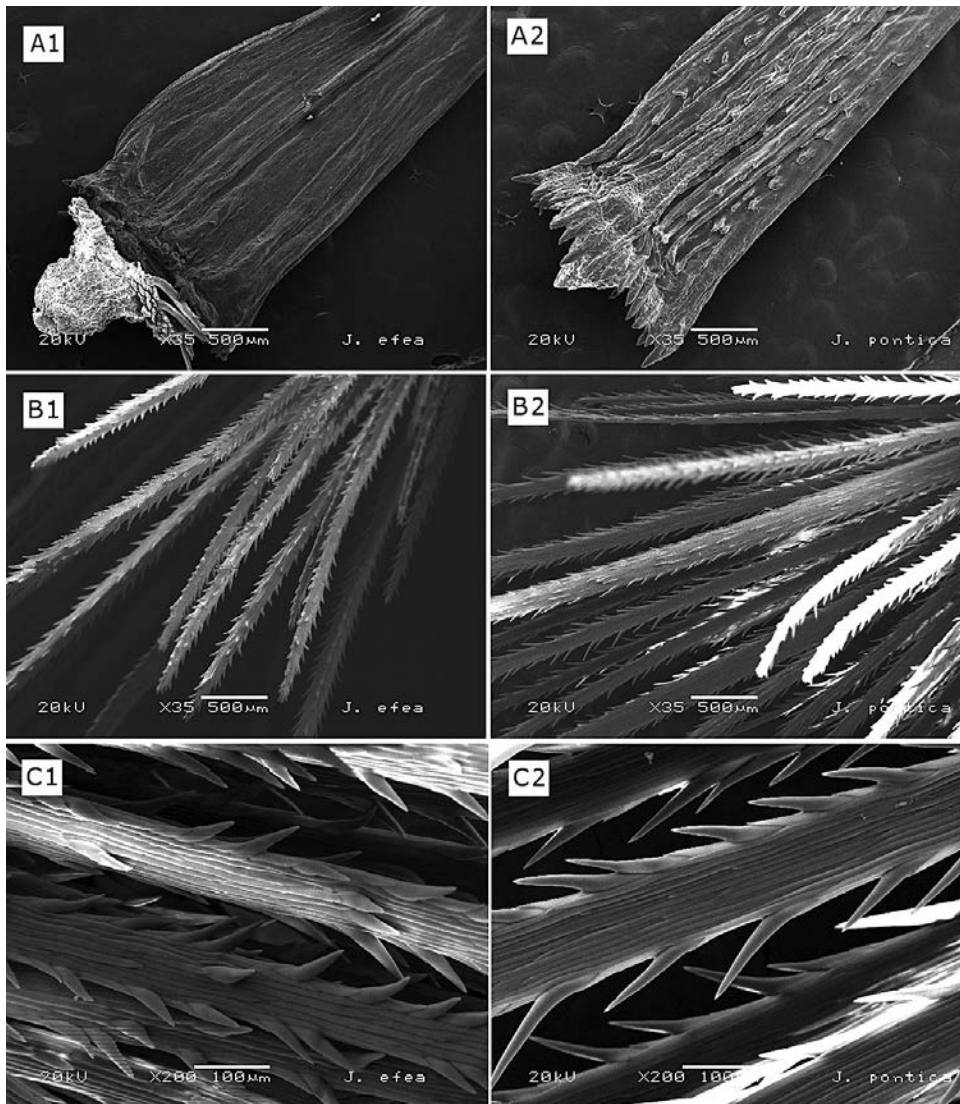


Fig. 7. SEM micrographs of achenes and papus. Beak of achene: **A1** – *J. efea*; **A2** – *J. pontica*. General shape of papus: **B1** – *J. efea*; **B2** – *J. pontica*. Details of papus surface: **C1** – *J. efea*; **C2** – *J. pontica* (*J. efea* from the holotype N. Aksoy 7926, DUOF 7020, *J. pontica* from E.G. Çakır 1517, GAZI).

23 vi 1989, M. Vural 5071, U. Kol and N. Adıgüzel!. A4 Çankırı, on the road to Ilgaz, Karlık Hill, 850-950 m Gyps Hills, 23 vi 1986, M. Sağıroğlu 1405!. A3 Ankara, Nallıhan, between Uluhan and Sarılar, on Horse Plateau, 1000 m, in an open area of *Pinus nigra* forest, 06 vi 2010, E.G. Çakır 1517!. Paphlagonia austr.: Ad oppidum Çankırı, in vinetis derelictis vallis Çakmaklı dere, 800 m., 28-30 vi 1929 J. and F. Bornmüller 14307, RBG KEW!. Ankara, Beynam, 300-400 m. above Ankara, in *Quercus pubescens* macchie, 05 vii 1947, P.H. Davis 13086, Det. A. Danin, RBG KEW!. Anatolia: ad Angora Galatiae, Jehri Dagh. 1892, J. Bornmüller, RBG KEW!. Ankara, Hacıkadın, step, 14 vi 1944, Baki Kasaplıgil 393, Det. A. Danin, RBG KEW!. Niğde/Kayseri: between Nevşehir and Ürgüp, 1200-1300 m., fl. purple, 22 vi 1952, Davis 19137, Dodds and Çetik, Det. A. Danin, RBG KEW!.

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