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Original Scientific Paper

A new species of *Centaurea* L. subgen. *Cyanus* Mill. (Asteraceae) from Turkey

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ABSTRACT:
Here we describe *Centaurea hekimhanensis*, a new species found in Hekimhan (Turkey, Malatya Province). This *Centaurea* grows on the stony-gravel mountain slopes of Yamadağ and is morphologically similar to *C. reuteriana*, *C. bourgaei*, and *C. pichleri* in terms of their flowering lateral stem at the base of the rosette, but differs regarding the shape of the stem and rosette leaves, and the size of the rosette leaves and median appendages; the achene ornamentation is irregularly sulcate. The chromosome number of the new species is *2n* = 20.

Keywords:
achene, Compositae, Centaureinae, *Cyanus*, karyology

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INTRODUCTION

The *Cyanus* Miller group from the Asteraceae family was first described as a genus (Miller 1754), and later reduced to a section within the genus *Centaurea* L., a position that was accepted by some taxonomists (de Candolle 1838; Bentham 1873; Boissier 1875; Hoffmann 1893; Stefanov & Gheorghiev 1931; Dittrich 1968; Wagenitz 1975). However, it is also recognized by certain other taxonomists as a subgenus of *Centaurea* based on molecular evidence (Susanna & Garcia-Jacas 2009; Boršić *et al*. 2011; Hilpold *et al*. 2014a, b). Garcia-Jacas *et al*. (2001) and Wagenitz (2006) conducted molecular studies on Centaureinae and determined that the *Cyanus* group is actually sister to the genus *Centaurea* s.s. However, *Cyanus* is also considered by some botanists as a separate genus based on morphological evidence (Soják 1972; Greuter 2003, 2008; Hellwig 2004; Wagenitz 2006; Kaya & Bancheva 2009; Olšavská *et al*. 2009, 2011, 2013; Stoyanov 2016; Negaresh 2018).

Subgenus *Cyanus* is distributed throughout central and southern Europe, North Africa, Anatolia, and the Caucasus, with some species appearing as far east as Iran and Afghanistan, and it currently includes 38 species (Hellwig 2004; Kaya & Bancheva 2009; Boršić *et al*. 2011; Olšavská *et al*. 2013; Stoyanov 2016; Kaya & Ozel 2017; Kaya *et al*. 2018; Negaresh 2018; Şırın *et al*. 2019b, 2020).

The florets of the species from subgenus *Cyanus* are blue or purplish blue (with only a few exceptions of cream or pale pink flowered taxa), which are extremely unusual colors for the subtribe Centaureinae (Boršić *et al*. 2011). While decurrent appendages and the absence of a mucro are characteristic of *Cyanus*, their involucrum and leaf features are effective in species distinction (Wagenitz 1975).

In Turkey, *Cyanus* was reclassified as a subgenus (Ertuğrul *et al*. 2018) according to molecular studies (Boršić *et al*. 2011; Hilpold *et al*. 2014a, b) and so we have opted for *Cyanus* as a subgenus. The present study focuses on the detailed karyological, morphological and micromorphological features used to characterize a new species in *Centaurea* subgenus *Cyanus* (Wagenitz 1975; Şırın *et al*. 2019b, 2020).

MATERIALS AND METHODS

Flowering and fruiting specimens of *C. hekimhanensis* Şirin & Yıldırım were collected from Malatya (Yamadağı) by the second author in 19 July 2015. The material from the new species was compared with the herbarium collections of *Centaurea* in the ANK, GAZI, ISTF, ISTE,

and KNYA herbaria [acronyms follow those of Thiers (2018)].

In the first step of our work, we examined the morphology of 60 samples belonging to *C. hekimhanensis* and its relatives (*C. bourgaei* Boiss., *C. pichleri* Boiss., *C. reuteriana* Boiss. var. *phrygia* Bornm. and *C. reuteriana* Boiss. var. *reuteriana*). Thus, the stem, leaves, involucre, appendages, achenes and pappus were measured. The floral and involucral features were sampled from the terminal capitula. The measurements were carried out using dried herbarium materials. The size recordings relate to herbarium material with capitula, and not those deformed by pressing. The appendage border was included in the measurements, but never in the involucral bracts. The morphology of the specimens was examined under a binocular loupe.

Prior to scanning electron microscopy (SEM) analysis, the achenes of both species were dehydrated in an alcohol series (70%, 80%, 96%, and 100%) to clean them, and then coated with gold. They were examined under a ZEISS EVO LS-10 model SEM (Carl Zeiss NTS GmbH, Oberkochen, Germany) at high-vacuum mode to observe their surface ornamentations at magnifications of 18×, 35×, 1000×, and 2000×. The terminology of the achene characteristics follows Stearn (1995) and Koul *et al*. (2000).

Mature achenes were selected and periodically germinated for chromosomal analyses. Chromosome counts were conducted during the somatic metaphase using the squash technique. Primer root meristems were used to obtain the metaphase plates. The samples were pretreated with 0.002 M 8-hydroxyquinoline for 8 h at 40°C and then fixed with Carnoy's solution for 24 h at 4°C. Prior to dyeing, the material was hydrolyzed with 5N HCl for 30 min at room temperature and stained with 1% acetone-orcein. The mountings were made permanent according to the Bowen (1956) method. At least five metaphases were examined and the best image was photographed at a magnification of 100× using an Olympus DP-72 digital camera attached to an Olympus BX53 microscope (both from Olympus Scientific Solutions, Shinjuku, Tokyo, Japan).

The chromosomal nomenclature was carried out according to Levan *et al*. (1964). The karyotype asymmetry was calculated with respect to the average centromeric index, the shortest/longest pairwise rate, and the intrachromosomal asymmetry (A_1) and interchromosomal asymmetry (A_2) indices. The chromosome length change variation (CV_{CL}) and karyotype asymmetry index were calculated according to the method proposed by Paszko (2006), and the mean centromeric asymmetry (M_{CA}) according to the method of Peruzzi & Eroğlu (2013). The karyograms and idiograms of the taxa were conducted using a Kameram™ digital camera (Micro System Computer Aided Microscope Systems Co. Ltd. Maslak/İstanbul).

RESULTS AND DISCUSSION

Diagnosis of the new species. ***Centaurea hekimhanensis*** Şirin & Yıldırım, *sp. nov*. (Figs. 1, 2).

Appendage and leaf features are important characteristics in distinguishing the species in the subgenus *Cyanus*. The differentiation of *Centaurea hekimhanensis* in terms of these features shows that it is a new species.

Centaurea hekimhanensis morphologically resembles *C. reuteriana* var. *phrygia* and *C. reuteriana* var. *reuteriana* and it differs mainly in terms of its linear and acute (not lanceolate and obtuse) stem leaves, always undivided rosette leaves, lanceolate or linear-lanceolate, obtuse (not usually lyrate with broadly lanceolate to oval or suborbicular terminal segment, rarely undivided, rounded), median appendages with 8-9 cilia (not with 6-7 cilia), 8-11 × 4-5 mm (not 12-12.5 × 2-3 mm), peripheral florets 21-23 mm (not 16-19 mm) long, rose-purple flowers (creamy white in *C. reuteriana* var. *phrygia*), the outer rows of pappus bristles 1.2-1.7 mm (not 0.5-0.6 mm), and the inner rows of pappus bristles 0.6-0.8 mm (not 0.2-0.3 mm). Conversely, *C. hekimhanensis* is morphologically similar to *C. reuteriana* var. *phrygia* and *C. reuteriana* var. *reuteriana* with regard to rosette leaves, involucre length, radiant capitula, decurrent appendages, and achene measurements (Table 1; Fig. 2).

Additionally, according to the micromorphology of the achene, ornamentation was irregularly sulcate (not fine sulcate), the cells and cell walls were not distinct (distinct in *C. reuteriana* var. *reuteriana*), and the hilum was sparsely hairy (not densely hairy).

Type locality. TURKEY, Malatya, Southwestern slopes of the Yamadağı, stony-gravely areas, 2545 m, 19.VI.2015, *H. Yıldırım 3974* (holo-, KNYA; iso-, ANK).

Description. Perennial herbs, 6-16.5 cm tall, root not thickened and stolon present. Flowering stem lateral at the base of a rosette, floccose-tomentose, decumbent rarely ascending, unbranched, 0.5-1 mm diameter at the base. Cauline leaves decurrent, sessile, linear or lanceolate, with tomentose hairs, obtuse, with prominent veins, stem leaves linear, sessile, acute, 1.3-2.9 × 0.1-0.2 cm, basal leaves decurrent, sessile, linear or lanceolate, with tomentose hairs, obtuse, 1.2-2.8 × 0.1-0.2 cm, rosette leaves always undivided, sessile, lanceolate or linear-lanceolate, obtuse 2.5-5.3 × 0.1-0.6 cm. Capitula solitary at the ends of the stems, involucre cylindrical, 14-17 × 9-12 mm. Phyllaries usually with 4-5 series. Appendages a narrow brown or blackish brown border, membranous and glabrous, laceration absent, weakly decurrent, with brown border and 8-9 silvery cilia, cilia 1-2.5 mm, outer appendages ovate, 5-5.5 × 3-4.5 mm, median appendages ovate-orbicular, 8-11 × 4-5 mm (included cilia), inner appendages linear-lanceolate, 12-13 × 1.5-2 mm; outer phyllaries ovate, 2-3 × 1-1.5 mm, median phyllaries lanceolate, 6-8 × 1.6-2 mm, inner phyllaries linear-lanceolate, 8.5-9.5 × 1-2 mm. Florets rose-purple, peripheral florets slightly radiant, 20-23 mm long, with 5

Fig. 1. *Centaurea hekimhanensis* (A – Capitula, B – Original habitat), *C. reuteriana* var. *reuteriana* (C – Capitula, D – Original habitat) and *C. reuteriana* var. *phrygia* (E – Capitula, F – Original habitat).

Fig. 2. Whole plants of *C. hekimhanensis* (A) and *C. reuteriana* var. *reuteriana* (B)

acute and linear-filiform lobes, 8-10 mm, central florets hermaphrodite, 12-14 mm long, lobes 5-6 mm, anther tubes rose-purple, style usually exceeds anther tube, corolla usually longer than anther tube or of the same size, corolla usually smaller than style. Achenes 3.4-3.9 × 1.5-1.8 mm, oblong-ovoid, brown, rounded at base, hilum lateral-basal and densely hairy. Pappus double, scabrous, brown, 1.2-1.7 mm, the bristles of the inner rows shorter, 0.6-0.8 mm.

Micromorphology of the achene. Achene compressed and not pitted, ornamentation irregularly sulcate, cells and cell walls not distinct, hilum lateral basal and sparsely hairy (Fig. 3).

Phenology. *C. hekimhanensis* flowers from June to July and mature fruits are produced from July to August.

Etymology. Named after Hekimhan (in the Malatya Province) where it was discovered.

The proposed Turkish name for the new species. Hekimhan Gökbaşı, see also Menemen *et al.* (2016).

Ecology. The new species is only found on the Southwestern slopes of the Yamadağı and occurs in stony-gravely areas at c. 2600 m elevation. Within this area, the new species is associated with plants such as: *Acantholimon capitatum* Sosn. subsp. *sivasicum* Doğan & Duman, *Alchemilla lithophila* Juz., *Allium armenum* Boiss. & Kotschy, *Astragalus plumosus* Willd., *Bunium pestolozzae* Boiss., *Campanula tridentata* Schreb., *Cerastium perfoliatum* L., *Cicer incisum* (Willd.) K.Maly, *Cirsium macrobotrys* (K.Koch) Boiss., *Crepis pulchra* L. subsp. *pulchra*, *Eremogone gypsophiloides* (L.) Fenzl, *Erysimum pulchellum* (Willd.) J.Gay, *Euphorbia petrophila* C.A.Mey., *Gagea villosa* M.Bieb. var. *villosa*, *Heldreichia bupleurifolia* Boiss., *Melissa longifolia* (L.) L. subsp. *longifolia*, *Minuartia juniperina* (L.) Maire & Petitm., *Myosotis lithospermifolia* Hornem, *Onosma stenoloba* Hausskn. ex Riedl, *Phlomis armeniaca* Willd., *Rinderia caespitosa* (A.DC.) Bunge, *Rumex crispus* L., *Salvia multicaulis* Vahl., *Senecio pseudoorientalis* Schischk, *Silene spergulifolia* (Desf.) M.Bieb., *Tanacetum vulgare* L., *Tulipa armena* Boiss. var. *armena*, *Verbascum splendidum* Boiss. and *Veronica oxycarpa* Boiss.

Distribution. *C. hekimhanensis* is a local endemic species that is only known from its type locality (Fig. 4). It is an Iran-Turanian element that is rarely found in the field. Due to grazing in the local area, populations of the species are at extremely high risk of extinction if effective protection measures are not sufficiently established.

Karyology. The chromosome number of *C. hekimhanensis* is $2n = 20$ (Fig. 5A). The shortest chromosome length is 1.09 µm, while the longest is 1.76 µm, and the total chromosome length is 13.49 µm. The karyotype formula of this species consists of 20 metacentric pairs. The karyotype of this taxon is classified as 4A according to the symmetry

Table 1. A morphological, micromorphological and karyological comparison of *Centaurea hekimhanensis* Şirin & Yıldırım, sp. nov. and the other taxa.

→Taxa ↓Characters	*C. hekimhanensis*	*C. reuteriana*	*C. bourgaei*	*C. pichleri*
Macromorphological				
Stem leaves	Linear, acute	Lanceolate, obtuse	Pinnatilobate or entire, obtuse	Lanceolate to narrowly lanceolate, obtuse
Rosette leaves	Always undivided, lanceolate or linear–lanceolate, obtuse	Usually lyrate with broadly lanceolate to oval or suborbicular terminal segment, rarely undivided, rounded	Lyrate with lanceolate terminal segment and 3(–4) pairs of lateral lobes	Lanceolate to broadly lanceolate and entire or with large lanceolate terminal segment and 1–2 (–3) pairs of short lateral lobes or teeth
Rosette leaves measures (mm)	25–53×1–6	42–125×12–21	2.5–8.5×0.9–2.4	1.6–15.5×0.2–2.2
Involucre length (mm)	14–17×9–12	13–18×10–13	15–17×10–13	11–20×7–13
Median appendages measures (mm)	8–11×4–5	12–13×2–3	9–11×2.5–3.5	10–14×3.5–5
Flowers	Rose–purple	Rose to violet–purple (var. *reuteriana*) or creamy white (var. *phrygia*)	Violet to rose–purple or yellowish white	Marginal blue, central violet–purple
Marginal flowers (mm)	20–23	16–19	20–23	13–30
Achene (mm)	3.4–3.9×1.5–1.8	3.5–4.5×1.5–2	4.5–5×2.5–2.9	3.5–5.1×18.5–2.1
Inner pappus (mm)	0.6–0.8	0.2–0.3	0.6–0.8	0.6–1.5
Outer pappus (mm)	1.2–1.7	0.5–0.6	1.4–1.5	1.4–3
Micromorphological				
Achene surface ornamentation	Irregularly sulcate	Fine sulcate*	Irregularly sulcate*	Irregularly sulcate*
Achene cells and cell walls	Not distinct	Distinct*	Distinct*	Not distinct*
Hilum hairs	Scattered	Dense*	Scattered*	Scattered*
Karyological				
CV_{CL}	13.86	18.83**	19.03**	12.60**
M_{CA}	8.14	9.23**	9.92**	9.72**
Satellite	Absent	Present**	Absent	Present

*According to Şırın *et al.* (2017)
**According to Şırın *et al.* (2019a)

classes of Stebbins (1971). The A_1, A_2, CV_{CL}, and M_{CA} indices are 0.15, 0.13, 13.86, and 8.14, respectively. The karyogram (Fig. 5B) and ideogram (Fig. 5C) are also given.

The chromosome number of *C. reuteriana* var. *reuteriana* is also *2n* = 20 (Fig. 5D). The shortest chromosome length is 1.29 µm, while the longest is 2.52 µm, and the total chromosome length is 19.48 µm. The karyotype formula of this species consists of 20 metacentric pairs. Satellites are seen on the short arms of the second and seventh chromosomes. The karyotype of this taxon is classified as 4A according to the symmetry classes of Stebbins (1971). The CV_{CL} and M_{CA} indices are 18.83 and 9.23, respectively. The karyogram (Fig. 5E) and ideogram (Fig. 5F) are also provided.

There are two groups in the perennial species of *Cyanus*, distinguished by the main difference indicated by Wagenitz (1975), namely the flowering stem lateral at the base of the rosette or terminal out of the central rosette. *Centaurea hekimhanensis* is positioned in the first group and comparisons were made in light of this information.

Centaurea hekimhanensis is similar to *C. reuteriana*, *C. bourgaei*, and *C. pichleri* in terms of their flowering stem lateral at the base of the rosette, but it differs with regard to the shape of the stem and rosette leaves, and the size of the rosette leaves and median appendages. Micromorphological characters are found to be useful in the systematics of the family Compositae (Dittrich 1985; Zhang 2013; Akçın & Akçın 2014; Karaismailoğlu 2015; Şırın *et al.*

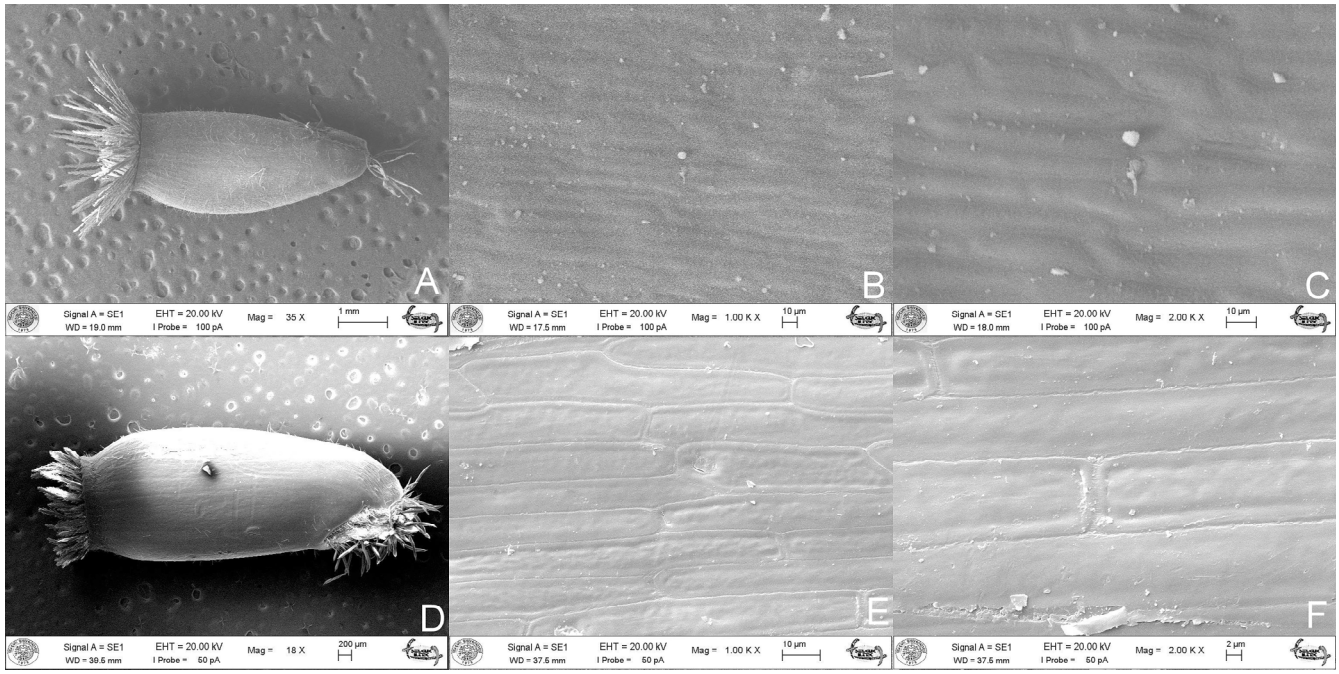

Fig. 3. Scanning electron micrographs of the achenes (general view, and details at 1000× and 2000× magnification, respectively): *C. hekimhanensis* (a–c), *C. reuteriana* var. *reuteriana* (d–f).

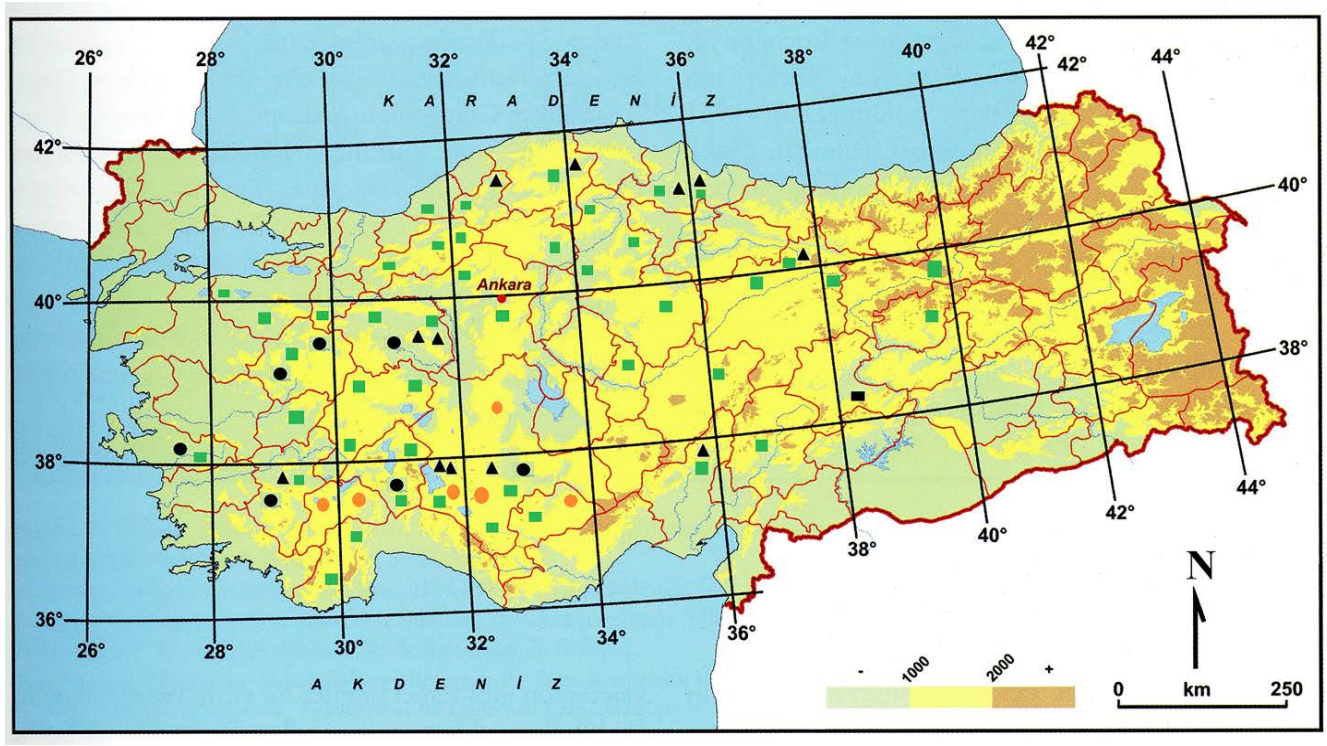

Fig. 4. Distribution maps of *C. hekimhanensis* (■), *C. reuteriana* var. *reuteriana* (●), *C. reuteriana* var. *phrygia* (▲), *C. bourgaei* (●) and *C. pichleri* (■) in Turkey

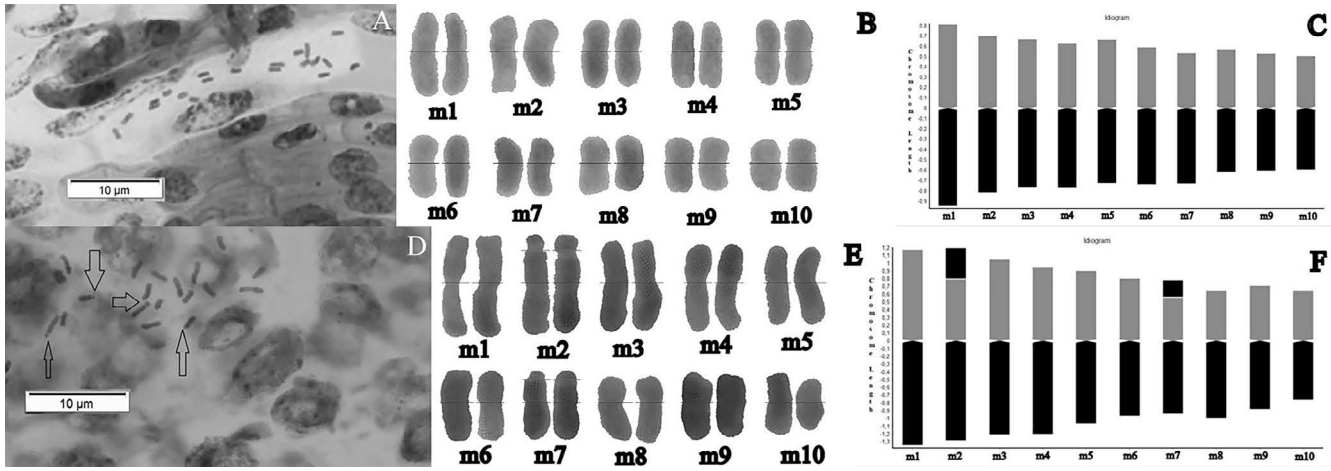

Fig. 5. Metaphase plates, karyotypes and idiograms of *C. hekimhanensis* (A–C) and *C. reuteriana* var. *reuteriana* (D–F) (the arrows indicate the satellite, m: metacentric)

2017; Ozcan & Akinci 2019; Bona 2020). In Centaureinae, karyology is found to be important in the systematic characterizations of several genera, which is verified using the connections between karyological, morphological, and molecular data (Wagenitz & Hellwig 1996; Hellwig 2004; Şirin *et al*. 2019a). Both micromorphological and karyological results contributed to the distinction of *Centaurea hekimhanensis*.

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Additional examined specimens

C. reuteriana var. *reuteriana*: [B1] İzmir: Karaburun, Summit of the Akdağ, 1210 m, 27.V.1995, *K. Alpınar & Ht. Hart* (ISTE 67968!); [B2] Kütahya: Murat Dağı above Banaz, 2000-2100 m, *Coode & Jones 2521* (KNYA!); Gediz, Murat Dağıabove Gölyeri, 1800 m, 09.VI.1977, *A. Çırpıcı* (ISTF 30505 A!); Gediz, Murat Dağıabove Kesik Söğüt, 2000 m, 15.VI.1982, *A. Çırpıcı* (ISTF 35246 A!); Uşak: Murat Dağı, Gürlekbelow Öküzkaya, stony slopes, 1850 m, 18.VI.1978, *A. Çarpıcı* (ISTF!); [B3] Afyon: Sultandağı, Büyükyayla, stony slopes, 1850 m, 21.V.2015, *E. Şirin 554a & M. Şirin* (KNYA!); Maymun Dağı, Northwest of the Demirciköy, steppe, 1000 m, 08.V.1984, *Z. Aytaç 1147* (GAZI!); Sultandağları, Büyük Yayla, 1860 m, 02.VI.1974, *A. Baytop et al.* (ISTE 29172!); [C2] Muğla: Köyceğiz, Sandras Dağı, North of the Gökçeova Lake, open *Pinus nigra* forest, 1750 m, 29.VI.2015, *E. Şirin 574 & M. Şirin* (KNYA!); Köyceğiz, Ağla Köyü-Gölova Gölü, Sandras Dağı, North of lake, lakeside, open *P. nigra* forest, 1763 m, 17.V.2014, *K. Ertuğrul 4745 & H. Dural* (KNYA!); Sandras Dağı, Ağla, Ekşialan, 1500 m, 10.VI.1978, *E. Özhatay* (ISTO 20241!); Sandras Dağı, Beşparmak Tepesi, 1900 m, 12.VII.1978, *E. Özhatay* (ISTO 20286!); Sandras Dağı, Beşparmak Tepesi, 1950 m, 13.VII.1979, *E. Özhatay 1540* (ISTO 23877!); Sandras Dağı, Çiçek Baba Tepesi, 2200 m, 26.VII.1977, *E. Özhatay 324* (ISTO 19464!); Sandras Dağı, Southwest slopes of the Çövenli Yayla, 1400 m, 08.VI.1978, *E. Özhatay 1719* (ISTO 20084!); Sandras Dağı, between Serçe Gediği-Çiçekbaba Tepesi, 1900 m, 19.VI.1980, *N. Özhatay et al.* (ISTE 44837!); Sandras Dağı, 1900 m, 10.VI.1969, *Fitz & Spitz.*; [C3] Isparta: Davraz dağı, 1800 m, 30.V.1955, *A. 4301 & T. Baytop* (ISTE); Northeast of the Anamas, 1500 m, *Sorger 68-37-33*; [C4] Konya: Crossroad of Beyşehir-Başarakavak, woodland of the DSİ, stony slopes, 1330 m, 05.V.2005, *E. Yıldıztugay 743* (KNYA!).

C. reuteriana var. *phrygia*: [A4] Karabük: Keltepe, steppe, 1800 m, 09.VII.2015, *E. Şirin 582 & M. Yılmaz* (KNYA!); Keltepe, 2000 m, 12.VII.1984, *M. Demiros 1258* (ANK!); Keltepe from Karaağaç village, taşlı stony slopes, 1800-1900 m, 16.VII.2004, *A. Duran 6759 & E. Hamzaoğlu* (KNYA!); Zonguldak: Keltepe, 1950 m, *Davis 38916*; [A5] Amasya: Akdağ, 1900 m, 26.VI.1965, *C. Tobey 1208* (ISTO 4173!); Kastamonu: Devrekani, Yaralıgöz stream, subalpine, 2000 m, 09.VII.1991, *Yurdakulol 3532* (ANK!); Kastamonu: *Manissadjian 59* (ANK!); [A6] Amasya: Akdağ, 1600-1900 m; Sivas: Koyunhisar, *Tchihatcheff 817*; [B3] Afyon: Sultandağı, Büyükyayla, stony slopes, 1850 m, 21.V.2015, *E. Şirin 554 & M. Şirin* (KNYA!); Sultan Dağları, Kızıltepe, 2200 m, 26.VI.1985, *Y. Akman 13795* (ANK!); Akşehir, Sultandağı, 1890-1910 m, *Hub.-Mor. 8673*; Konya: Akşehir-Yalvaç, 1600 m, 03.VI.1974, *A. Baytop & Y. Doğantan* (ISTE 29296!); [C2] Denizli: Bozdağ North slopes, subalpine, 1700 m, 28.V.1995, *N. Özhatay et al. 95/142* (ISTE 70424!); Horoz Dağı, 1500 m, *Hub.-Mor. 5405*; [C3] Is-

parta: Davraz Dağı, stony slopes, 1800 m, 16.VI.2015, *E. Şirin 562 & M. Şirin* (KNYA!); Davraz Dağı, 1950 m, Mayıs 1845, *Heldreich*; Davraz, Davraz Dağı, 1700-1750 m, 05.VI.1981, *T. Ekim 5598 & B. Yıldız* (ANK!); Davraz Dağı, 1800 m, 30.V.1955, *A. Baytop & T. Baytop* (ISTE 4301!); Konya: Derebucak Çamlık, Kızıldağ, stony slopes, 1454 m, 22.V.2004, *H. Demirelma 2929* (KNYA!); [C4] Konya: Kızılören Dağı, South slopes, stony slopes, 1900 m, 25.V.1989, *A. Tatlı 8923 et al.* (KNYA!); [C5] Adana: East of Cilician Gates, 2000 m, *Sorger 62-71-31*.

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REZIME

Botanica SERBICA

Nova vrsta *Centaurea* L. subgen. *Cyanus* Mill. (Asteraceae) iz Turske

Emrah Şirin, Hasan Yildirim, Tuna Uysal i Kuddisi Ertuğrul

U radu je opisana *Centaurea hekimhanensis,* nova vrsta nađena u Hekimhanu (Turska, provincija Malatya). Ova *Centaurea* raste na kameno-šljunčanim padinama na planinskim stanama Yamadağa i morfološki je slična vrstama *C. reuteriana*, *C. bourgaei*, i *C. pichleri* u pogledu cvetnog stabla bočno u osnovi rozete, ali se razlikuje po oblicima listova stabljike i rozete, veličine listova rozete i srednjih dodataka; ornamenti ahena su nepravilno sulkatni. Broj hromozoma nove vrste je 2n = 20.

Ključne reči: ahene, Compositae, Centaureinae, *Cyanus*, kariologija