



## MORPHOLOGICAL, CYTOLOGICAL AND PALYNOLOGICAL FEATURES OF THREE CLOSELY RELATED *CENTAUREA* SPECIES (ASTERACEAE) FROM TURKEY

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**Abstract.** This study was carried out to determine the main distinctive morphological, cytological and palynological features of three closely related *Centaurea* L. taxa (Asteraceae) growing in Turkey: *C. aytugiana* Bancheva, Kaya et Binzet, *C. stenolepis* Kerner and *C. salicifolia* M. Bieb. ex Willd. The first species, recently described from Karabük province, Turkey (BANCHEVA *et al.* 2014), is an endemic of Karabük province, whereas the remaining taxa have larger distribution ranges. The results show that the morphological (including pollen-morphological) traits and chromosome numbers proved to be of high diagnostic value and are very useful for determination.

**Key words:** Asteraceae, *Centaurea*, endemic species, chromosome number, pollen morphology, SEM

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### Introduction

*Centaurea* L. s.l. is the fourth largest genus of the family Asteraceae comprising between 400 and 700 species with predominantly Old World distribution ranges (DITTRICH 1977; BREMER 1994; WAGENITZ & HELLOWIG 1996). Taxonomic complexity arises from the extensive variation in morphology, karyology and pollen diversity due to the rather complex evolution of the group including polyploidization, hybridization and introgression (BREMER 1994; GABRIELIAN 1995; SUSANNA *et al.* 1995; WAGENITZ & HELLOWIG 1996). In the Turkish flora the genus *Centaurea* s.str. consists of 156 species of which 103 (or 66%) are endemics (WAGENITZ 1975; DAVIS *et al.* 1988; GÜNER 2000; DURAN & DUMAN 2002; UZUNHISARCIKLI *et al.* 2005, 2007; VURAL *et al.* 2006; WAGENITZ *et al.* 2006; KAYA & VURAL 2007; UYSAL *et al.* 2007, 2008, 2009; AKSOY *et al.* 2008; UYSAL 2008; ARMAĞAN & ÜNAL 2009; HAMZAOĞLU & BUDAK 2009; UYSAL & KÖSE 2009; DOĞAN & DURAN 2009; DAŞKIN & YILMAZ 2009; KÜLTÜR 2010; ASLAN *et al.* 2011; DİNÇ & DOĞU 2012; YILDIRIMLI 2012; KÖSE & ALAN 2013; BANCHEVA *et al.* 2014).

The study deals with the investigation of the morphological, pollen morphological and karyological features of the recently described species *C. aytugiana* Bancheva, Kaya et Binzet from Karabük Province, Turkey (BANCHEVA *et al.* 2014) and its morphological relatives from sect. *Lepteranthus* (DC.) Dumort., *C. stenolepis* Kerner and *C. salicifolia* M. Bieb. ex Willd, in order to reveal the taxonomic position of *C. aytugiana* (Fig. 1).

### Material and methods

*C. aytugiana* was collected in 2007 and 2008 in the Karabük province, West Black Sea Region, Turkey. Morphological characters of this species were studied from the type collection and the personal collections of the authors, whereas morphological characters of the related species, *C. stenolepis* and *C. salicifolia* were observed in the following Herbaria: ISTE, MA, P, SOM, W and WU. The karyotype of *C. aytugiana* was studied from mitotic metaphase plates obtained from root tips of three plants collected at the type locality. Root tips were pretreated with 8-hydroxyquinoline for 30 min, then fixed in acetic alcohol (1:3) for 24 h at 4°C, hydrolysed

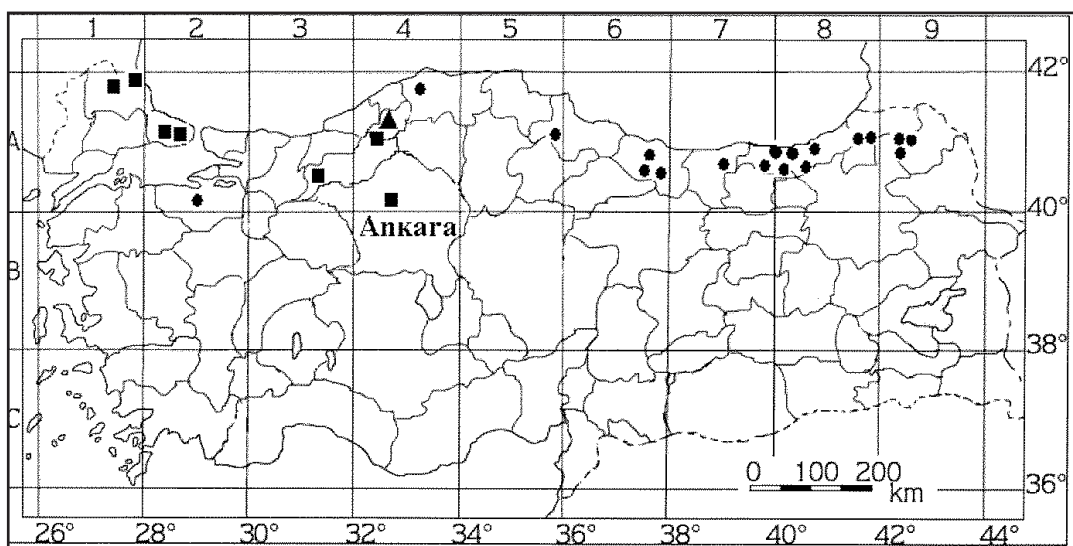


Fig. 1. Distribution map of *Centaurea aytugiana* (triangles), *C. stenolepis* (squares) and *C. salicifolia* (dots) in Turkey.

in 1 M HCl for 15 min at 60°C, stained with haematoxylin after Gomori (MELANDER & WINGSTRAND 1953) for 30 min at 60°C and then squashed in 45% acetic acid. The karyotype was determined according to LEVAN *et al.* (1964) on the basis of eight metaphase plates. The pollen morphology was studied by light microscope (LM) and scanning electronic microscope (SEM) according to ERDTMAN'S (1960) and HUTTUNEN & LAINE'S (1983) protocols, respectively. The pollen terminology follows PUNT *et al.* (2007).

## Results and discussion

### *Centaurea aytugiana* Bancheva, Kaya et Binzet.

**Morphology.** Perennial with woody rootstock. Stems 22-70 cm, erect, single to numerous, much-branched, puberulent to arachnoid-tomentose. Leaves pinnatisect, puberulent on both surfaces, with (3)4-7 pairs of lanceolate segments, acute on the top; upper leaves entire. Capitula numerous, 10-20 mm. Involucre narrowly ovoid-cylindric, 13-14(-16)×5-6 mm. appendages partly covering the basal part of the phyllaries, 1 mm broad at base, gradually narrowed into threadlike recurved tip, whole appendage 6-7 mm, straw-yellowish to pale brown, with (7)8-11 pairs, 2-3 mm long cilia

of the both sides. Florets white or very rare pale pink. Achenes 3×1.3-1.5 mm, brown and smooth. Pappus absent. *Fl. VIII.*

**Distribution and ecology.** Turkey, Karabük province, on 641 m a.s.l., at the edge of shady meadows in xerophilous forests dominated mainly by *Carpinus betulus* L. *C. orientalis* Miller, *Pinus nigra* J.F. Arnold and *P. sylvestris* L.

**Karyology.** Chromosome number: 2n=30+1B. The karyotype consists of small chromosomes, about 1.5 mμ in size. The submetacentric type prevails. Two pairs of chromosomes with satellites were also evident (Fig. 3 A).

**Pollen.** The pollen grains are of WAGENITZ'S *Jacea*-type (1955), subprolate, with correlation polar axis/equatorial diameter 1.18. The exine sculpture is microechinatae, with density of the sculpture elements 3-4 to 25 μm<sup>2</sup> and high of the microspines c. 1.5 μm. Equatorial diameter 22.5±1.5 μm; polar axis 26.3±3 μm; length of the colpus 20.1±1.4 μm; breadth of the colpus 3.3±0.6 μm (Fig. 3 B-D).

### *Centaurea stenolepis* Kerner.

**Morphology.** Perennial, 25-70(-100) cm, stem erect, branched in upper part. Leaves scabrous above, slightly arachnoid-tomentose below, entire (minutely denticulate), lower broadly lanceolate (2-6 cm broad) and petiolate,



**Fig. 2.** Outer, middle, and inner appendages (from left to right) in *Centaurea aytugiana* (A), *C. stenolepis* (B) and *C. salicifolia* (C), and capitulum of *C. aytugiana* (D).

median and upper lanceolate to oblong, sessile. Involucre 16-20×10-15 mm, oblong. Appendages small, only partly covering basal part of phyllaries, only 0.5-0.8(1) mm broad at base, gradually narrowed into threadlike recurved tip, whole appendage 6-12 mm, blackish-brown in lower part, tip light brown, with numerous distant 2.5-4 mm cilia on both sides. Flowers rose-purple, marginal radiant. Achenes 3.2-3.8 mm; pappus 0.5-1.5 mm. *Fl.* VII-VIII.

*Distribution and ecology.* Central Europe (S.E. part), Balkans, S. Russia, on 900-1350 m. a.s.l., on meadows and scrubs.

*Karyology.*  $2n=2x=2m+20sm=22$ . A pair of chromosomes with satellites was also evident (BANCHEVA 1998).

*Pollen.* The pollen grains are of WAGENITZ's *Jacea*-type (1955), subprolate, with correlation polar axis/equatorial diameter 1.1. The exine sculpture is microechinatae, with density of the sculpture elements 4-6 to 25  $\mu\text{m}^2$  and high of the microspines c. 2  $\mu\text{m}$ . Equatorial diameter 25.5±1.2  $\mu\text{m}$ ; polar axis 28.3±3  $\mu\text{m}$ ; length of the colpus 22.1±1  $\mu\text{m}$ ; breadth of the colpus 3.9±0.5  $\mu\text{m}$  (Fig. 4).

### ***C. salicifolia* M. Bieb. ex Willd.**

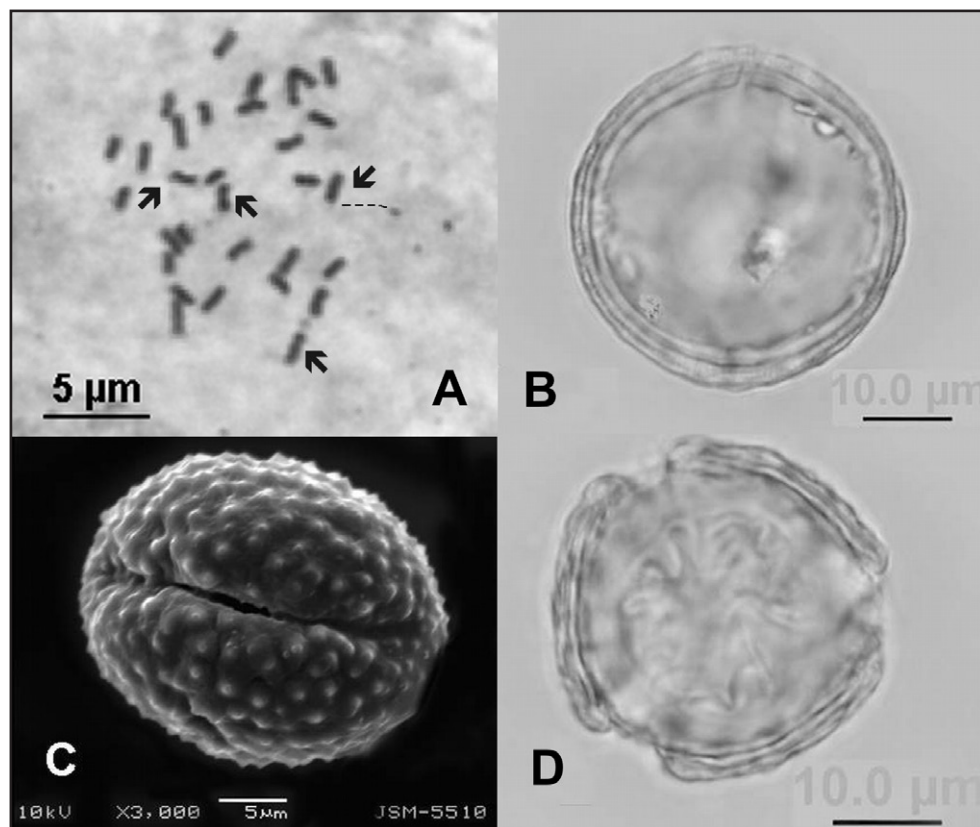
*Morphology.* Perennial. Stems erect, 25-75 cm, ± corymbosely branched in upper part. Leaves scabrous, sometimes slightly

arachnoid especially below, entire or denticulate, rarely dentate, lanceolate; basal and lower petiolate, median and upper sessile, sometimes slightly auriculate and half-clasping. Involucre 14-17×8-15 mm, nearly cylindrical to cup-shaped. Appendages brown or blackish brown, 3-5 mm, lanceolate to narrowly triangular, gradually tapering into an erect or slightly curved tip, cilia 10-15 on each side, ± crowded towards base, 1-3 mm. Flowers rose-purple, marginal distinctly radiant. Achenes 3-4 mm; pappus 0.3-1 mm. *Fl.* VII-VIII.

*Distribution and ecology.* Caucasia and Turkey, on meadows.

*Karyology.* Only one chromosome number,  $2n=22$ , is reported (TONIAN 1980).

Morphologically *C. aytugiana* resembles *C. stenolepis* and *C. salicifolia* by the similar shape of the appendage, which is gradually narrowed into a threadlike recurved tip and usually provided with more or less cilia on both sides (Fig. 2). Cilia are more numerous in *C. stenolepis*, with 12 to 18 sparse pairs, and in *C. salicifolia*, with 10 to 15 pairs crowded toward the base, but fewer in *C. aytugiana*, with eight to 11 sparse pairs. Appendages are darker in similar species: brown to light brown apically in *C. stenolepis* and brown to blackish brown in *C. salicifolia* versus a lighter stramineous color in the new species. Appendages are the longest



**Fig. 3.** Mitotic metaphase root tip cell and pollen grains of *Centaurea aytugiana*: **A** – mitotic metaphase root tip cell showing  $2n=30+1B$ ; **B** – LM photo, equatorial view of pollen grain; **C** – SEM photo of pollen grain shape and ornamentation; **D** – LM photo, polar view of pollen grain.

**Table 1.** Diagnostic characters of *Centaurea aytugiana* and its morphologically closest relatives *C. stenolepis* and *C. salicifolia*.

Character	<i>C. aytugiana</i>	<i>C. stenolepis</i>	<i>C. salicifolia</i>
Stem height (cm)	22-70	(5)25-70(-100)	25-75
Leaves	lyrate to pinnatisect	entire (minutely denticulate), lanceolate to lanceolate	entire or denticulate, rarely dentate, lanceolate
Involucrum size (mm)	13-14(16)×5-6	16-20×10-15	14-17×8-10(15)
Appendage length (mm)	6-7	6-12	3-5
Appendage colour	Straw-yellowish to pale brown at the base	Brown to light brown at the tip	Brown to blackish brown
Number of cilia	(7)8-11	12-18	10-15
Achene size (mm)	3×1.3-1.5	3.2-3.8×3	3-4×3
Pappus length (mm)	Absent	0.5-1.5	0.3-1
Colour of the florets	White (rare pale pink)	Rose-purple	Rose-purple
Chromosome number	$2n=30+1B$	$2n=22, 44$	$2n=22$
Habitat	at the edge of shady meadows in xerophilous forests	meadows	meadows

in *C. stenolepis*, 6-12 mm; of medium length, 6-7 mm, in the new species; and the shortest in *C. salicifolia*, 3-5 mm. The involucre is narrower in *C. aytugiana*, 5-6 mm, versus 10-15 mm in *C. stenolepis* and 8-10(12) mm in *C. salicifolia*. Leaves are lyrate to pinnatisect in the new species, but are entire (minutely denticulate) and broadly lanceolate to lanceolate in *C. stenolepis* and entire or denticulate (rarely dentate) and lanceolate in *C. salicifolia*. Florets are usually white in *C. aytugiana*, but a darker rose-purple in both *C. stenolepis* and *C. salicifolia* (Tab. 1).

The chromosome number of  $2n=30+1B$  of *C. aytugiana* does not coincide with the chromosome numbers of *Centaurea* sect. *Lepteranthus* and *Centaurea* sect. *Jacea* (L.) DC ( $x=11$ ), where *C. stenolepis* and *C. salicifolia* are assigned to (WAGENITZ 1975; DOSTÁL 1976). Two chromosome reports ( $2n=22$  and  $2n=44$ ) are known for *C. stenolepis* (KUZMANOV & GEORGIEVA 1977; LOVRIC 1982; SHARKOVA 1996; BANCHEVA 1998), and only one report for *C. salicifolia* ( $2n = 22$ ) (TONIAN 1980). The base chromosome number of all representatives of sect. *Lepteranthus* is  $x=11$ , whereas in *C. aytugiana* it is  $x=15$ .

Pollen morphology together with base chromosome number and DNA sequences are the most important data used in a taxonomy of *Centaurea* s.l. and the subtribe *Centaureinae* Dumort. (WAGENITZ & HELLWIG 1996; HELLWIG 2004). WAGENITZ (1955) delimited eight *Centaurea* pollen types corresponding very well to the main taxonomic clusters of the group. The pollen grains of *C. aytugiana* belong to Wagenitz's *Jacea*-type, typical for sections *Jacea*, *Lepteranthus*, and *Acrolophus* (Cass.) DC., and characterized by a reduction of the spines and inner layer of columellae, thus apparently standing at the end of an evolutionary line (WAGENITZ 1955). *C. stenolepis* has the same pollen type (BANCHEVA 1999), whereas *C. salicifolia* has not been investigated so far.

### Conclusions

The results show that the morphological (including pollen-morphological) traits and chromosome numbers proved to be of

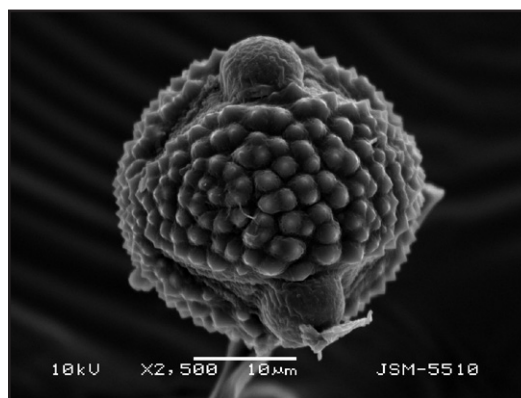


Fig. 4. Pollen grain of *Centaurea aytugiana*.

high diagnostic value and are very useful in determination of the target *Centaurea* species. In the same time the results do not confirm our first hypothesis, based only on morphological similarity of the studied taxa, for the position of *C. aytugiana* into sect. *Lepteranthus*. Further molecular investigations for detection the section affiliation of the taxon are necessary.

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