

MORPHOLOGICAL, ANATOMICAL AND CYTOLOGICAL INVESTIGATIONS ON THREE TAXA OF *CENTAUREA* L. (ASTERACEAE) FROM TURKEY

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Abstract

Morphological, anatomical and cytological features of three Turkish taxa of *Centaurea* L., viz. *C. polypodiifolia* Boiss. var. *polypodiifolia*, *C. urvillei* DC. subsp. *urvillei* and *C. urvillei* subsp. *armata* Wagenitz were investigated. Stem anatomy revealed the presence of a thick cuticle layer outside the stem, and epidermis with dense hairs was observed in the lower part. Investigation on leaf anatomy showed that different types of hairs on the outside of the leaf were very intense. Palisade parenchyma was observed below the upper and lower epidermis. In *Centaurea polypodiifolia* var. *polypodiifolia*, chromosome number was found to be $2n=16$, while in *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata* $2n=20$. Total karyotype length of *C. polypodiifolia* var. *polypodiifolia*, *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata* was 22.9 μm , 37.84 μm and 40.01 μm , respectively. Among the investigated taxa the karyotype asymmetry index was found lowest in *C. urvillei* subsp. *armata*. Satellite was detected in *C. urvillei* subsp. *urvillei* and subsp. *armata*, while it was absent in *C. polypodiifolia* Boiss. var. *polypodiifolia*.

Introduction

Centaurea L. (Asteraceae) consists of about 700 species and distributed in the Mediterranean region and the Near East with a few species reaching northern Eurasia, north and east Africa, North America, and Australia (Bancheva *et al.*, 2014; Behçet *et al.*, 2017). Turkey is one of the main centers of this genus, particularly the Southwest and East of the country (Wagenitz, 1986), and regarded as the third largest genus in Turkey (Davis, 1975). In Turkey, *Centaurea* is represented by 194 species, of which 118 are endemic (Guner *et al.*, 2012). The systematics of the genus *Centaurea* is problematic, and the sectional classification of *Centaurea* relies heavily on the morphology of the appendage of phyllary and achenes (Garcia-Jacas *et al.*, 2001). The taxonomic complexity of *Centaurea*, especially in the Near East, has stirred much research (Duran and Duman, 2002; Türkoglu *et al.*, 2003; Yüzbaşıoğlu *et al.*, 2015). The status of species and infraspecific taxa included in *Centaurea* has been revised in several taxonomic treatments (Rahiminejad *et al.*, 2010; Ranjbar and Negaresh, 2013; Negaresh and Rahiminejad, 2014). In the recent past, several authors concentrated on morphological studies of *Centaurea* alongside describing new species from Turkey (Kose *et al.*, 2010; Hayta *et al.*, 2016; Behçet *et al.*, 2017).

Anatomical and cytological characters play pivotal role in plant systematics, and quite often offer tools for species delimitation. Stem and leaf anatomy provide many characters which have

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already been proven to be of importance in classification (Lu *et al.*, 2008), and have been widely used in taxonomic treatments and systematic studies (Noman *et al.*, 2014). Though some taxa of *Centaurea* were investigated based on anatomical (Ozcan, 2013; Uysal *et al.*, 2016) and cytological (Martin *et al.*, 2006; Uysal *et al.*, 2009) characters, many taxa of this genus occurring in Turkey remain unexplored using these important tools. Since *Centaurea* is taxonomically a complicated genus as it contains many taxa that show a great degree of morphological variations, anatomical and cytological data should be involved to resolve taxonomic limits. Therefore, the present study aimed at exploring the anatomical and cytological features of three *Centaurea* taxa from Turkey and to enrich the knowledge of these features for better understanding of systematics of *Centaurea*.

Materials and Methods

Plant materials

Plant materials were collected from natural habitats from 2011 to 2012. The voucher specimens have been deposited at the Firat University Herbarium (FUH). The taxonomic identifications of the taxa were confirmed following Davis (1975). The list of the taxa investigated in this study along with their localities and vouchers are provided in Table 1.

Table 1. List of the investigated taxa with their localities and voucher specimens.

Taxa	Locality	Vouchers
<i>Centaurea polypodiifolia</i> Boiss. var. <i>polypodiifolia</i>	B7/Elazığ; Çemişgezek, Danbüken, Avşan Köyü, 1090 m. 16.07.2012	Tasar, 1005
<i>C. urvillei</i> DC. subsp. <i>urvillei</i>	B7/Elazığ; Harput, Anguza Baba, Kayalık alan, 1400 m. 13.06.2011	Tasar, 1008
<i>C. urvillei</i> subsp. <i>armata</i> Wagenitz	B7/ Elazığ, Baskil, Yukarı Kuluşağı Köyü, Kayalık alan, 1400 m. 13.06.2011	Tasar, 1009

Anatomical investigation

Cross sections of stem, root, leaf and surface sections of leaf of three taxa of *Centaurea* were made after fixing in 70% ethanol (Yakar-Tan, 1982). The sections were observed under light microscope Olympus BX51 and photographed with Olympus Camedia C-4000 digital camera.

Cytological study

The cytological studies were conducted on root tips meristematic cells. The seeds were germinated on moist filter paper in Petri dishes at 25°C. The actively growing root tips were pretreated with aqueous colchicine (0.05%) for 3–3.5 h at room temperature. Afterwards, the root tips were fixed with Carnoy (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 15 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least 5 well-spread metaphase plates were taken using an Olympus BX51 microscope, and were recorded with an Olympus Camedia C-4000 digital camera. Diploid chromosome number (2n), ploidy level, karyotype formula, chromosome length range and total karyotype length (TKL) were determined. Chromosomes were classified according to nomenclature given by Levan *et al.* (1964). The intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2) followed Romero-Zarco (1986), while the karyotype symmetry nomenclature followed Stebbins (1971). For calculating coefficient of variation of chromosome length (CV_{CL}), coefficient of variation of the centromeric index (CV_{CI}) and asymmetry index (AI) Paszko (2006) was tailed.

Results and Discussion

Morphological characteristics

Centaurea polypodiifolia Boiss., Diagn. Pl. Orient. Ser. 1, 6: 126 (1846), var. ***polypodiifolia***. *C. euphratica* Boiss., Diagn. Pl. Orient. Ser. 1, 6: 125 (1845). *Microlophus polypodiifolius* (Boiss.) Agadshyanov, Fl. Azerb. 8: 443 (1961). (Fig. 1A-B).

Biennial or perennial, 33-44 cm long, with numerous branches and thickened taproot. Root 16-23 cm long. Stem, branches and lower leaves with crisp articulate hairs, other leaves almost glabrescent. Lower leaves 120-180×50-80 mm, median leaves 50-90×10-18 mm. Capitula corymbosely arranged, 2.5-3.5 cm long. Flowers yellow. Appendage very small (0.5-2.5 mm) and easily deciduous, a simple spinule or with 1-2 pairs of minute teeth. Involucre 16-20×10-13 mm, rarely broader, upper leaves narrowly lanceolate, not concealing involucre. Outer phyllaries 8-12 x 5-7 mm, median phyllaries 10-14×4-7 mm, inner phyllaries 15-18×2-4 mm. Achenes 4-5 mm, pappus 5-8 mm long.

Flowering: June to August.

Habitat: Rocky slopes, steppe, fallow fields, at 800-2500 m above the sea level.

Centaurea urvillei DC., Prodr. 6: 592 (1838), subsp. ***urvillei***. *Aegialophila longispina* Cand. in Bull. Soc. Bot. Fr. 44: 146 (1897). *C. urvillei* DC. var. *leptacantha* Bornm. in Beih. Bot. Centr. 38(2): 464 (1921). *C. chiosicola* Beauv. & Topali in Bull. Soc. Bot. Geneve Ser. 2, 26: 156 (1936). (Fig. 1C-D).

Short-lived perennial, 12-28 cm tall. Root 3-5 cm long. Stem simple or branched from near base, very short or elongate, mostly rather slender. Lower leaves 90-12 x 30-50 mm, median leaves 150-210 x 23-42 mm, slightly arachnoid to distinctly tomentose, lyrate with triangular, rhombic or oblong-ovate terminal segments, few to numerous lanceolate, lateral segments of leaves entire or dentate. Capitula 1-4, c. 5-7 cm long. Flowers rose-purple or whitish. Appendages (excluding cilia) 2-4(-5) mm broad at the base, rarely more than 20 mm long. Involucre 30-40×17-38 mm, ovoid to globose. Outer phyllaries 7-12×5-9 mm, median phyllaries 13-15×5-7 mm, inner phyllaries 15-22 × 5-7 mm. Achenes 4-6 mm long, pappus 7-12 mm long.

Flowering: June to July.

Habitat: Rocky slopes, macchie, open *Pinus* forests, at 2000 m above sea level.

Centaurea urvillei DC., Prodr. 6: 592 (1838), subsp. ***armata*** Wagenitz., Willdenowia 6(3): 491 (1972). *C. urvillei* DC. var. *platyacantha* Bornm. in Beih. Centr. 38(2): 464 (1921) (Fig. 1E-F).

Short-lived perennial or biennial, 11-20 cm long. Root 4-10 cm long. Stem simple or branched from near the base, short or elongated, stout. Lower leaves 90-120×25-30 mm, median leaves 110-160×30-50 mm, slightly arachnoid to distinctly tomentose, lyrate with triangular, rhombic or oblong-ovate terminal segments, few to numerous lanceolate, oblong or lyrate, interspersed with small lobes. Capitulum 5.0-5.5 cm long. Flowers rose-purple or whitish. Appendages 4-7 mm broad at the base, with 8-12 cilia on each side. Involucre 30-35×30-40 mm, ovoid to globose. Outer phyllaries 15-24×8-10 mm, median phyllaries 15-18×5-10 mm, inner phyllaries 20-25×6-10 mm. Achenes 4-6 mm long, pappus 6-13 mm long.

Flowering: June to August.

Habitat: Dry stony slopes, scree, rocks, at 50-2800 m above sea level.

Notes: The present morphological investigation deals with in-depth study including the several quantitative characters of three taxa of *Centaurea*, viz, size of root, lower and median leaves, outer, median and inner phyllaries and capitulum, where these characters are missing in the Flora of Turkey (Davis, 1975).

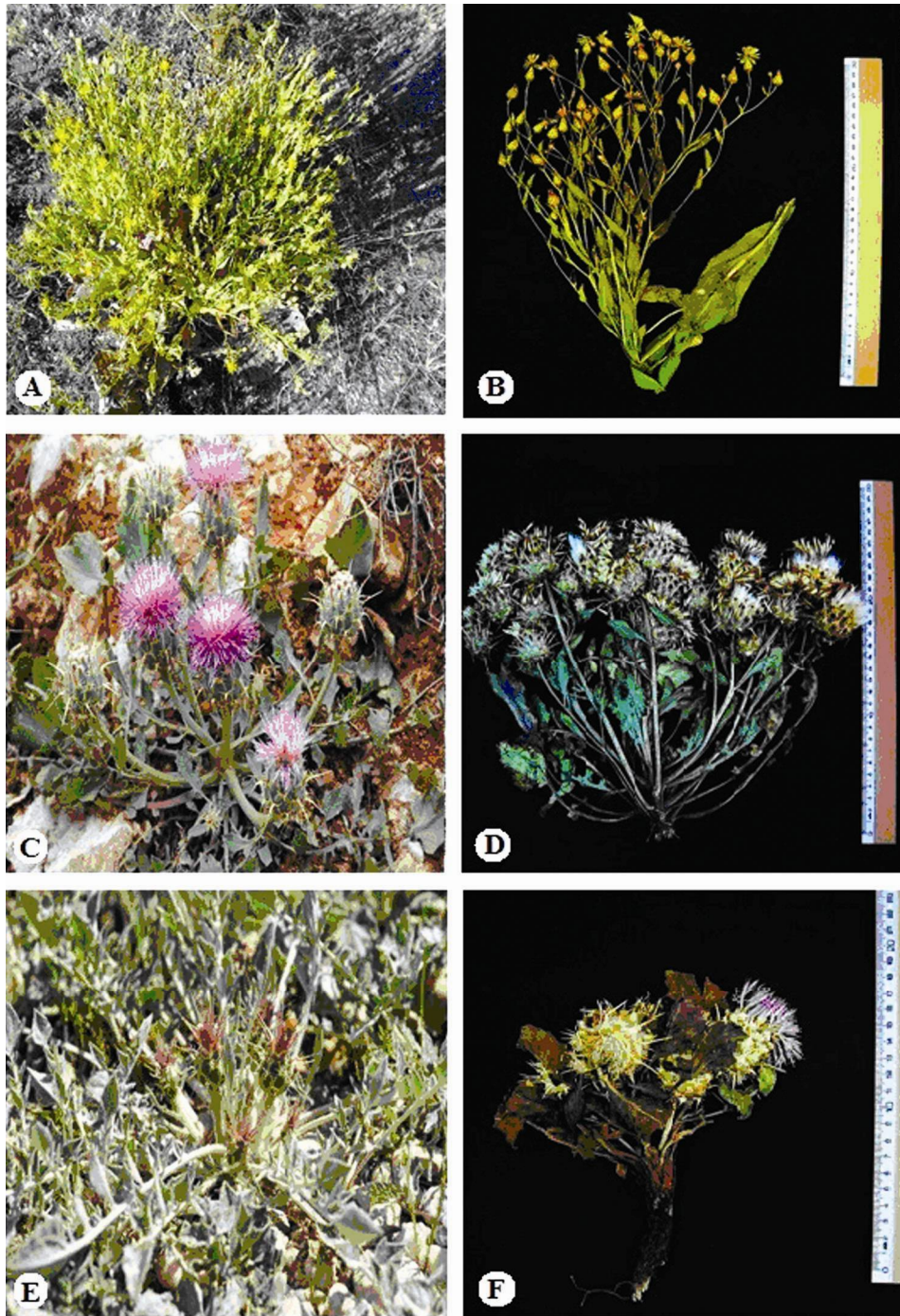


Fig. 1. A-B: *Centaurea polypodiifolia* var. *polypodiifolia*; C-D: *C. urvillei* subsp. *urvillei*, E-F: *C. urvillei* subsp. *armata*; A,C,E: Habitat, B,D,F: Herbarium specimens.

Anatomical characters

Root anatomy

The outer layer contains a periderm in the form of an epidermis (protective tissue). The crushed epidermis is found below the peridermis. Under the epidermis is a cortex layer consisting of small, ovoid, thick-walled parenchyma cells. Sclerenchymatous bundles are partially embedded in the cortex layer. Under the cortex, there is cambium trapped in a narrow area. Next to the cortex there are well developed vascular bundles. Although the number of phloem cells is less, xylem cells occupy a broader space. The xylem expanded to cover its own pith region (Fig. 2).

Stem anatomy

Stem anatomy revealed the presence of a thick cuticle layer outside the stem, and in the lower part of it, epidermis with dense hairs was observed. Collenchyma and chlorenchyma occurred in alternating segments below epidermis. There are sclerenchymatous bundles between chlorenchyma and collenchyma. Scleranchyma cells are in the order of 4-5, and there are intercellular spaces between these cells. Under this layer, phloem and xylem are present, respectively. It has been observed that the vascular bundles under the collenchymatic tissue are larger. The vascular bundles in the stem of studied taxa are arranged in two rings and the type of vascular bundles is bicollateral. The cambium is seen as crushed. The phloem in the inner side of xylem is less visible. The collenchyma tissue was generally located very close to the epidermis with 3-4 rows in *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata* and 4-6 rows in *C. polypodiifolia* var. *polypodiifolia*. In addition, a chlorenchymatous tissue below the epidermis was observed in the stem cortex with 3-4 rows in *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata* and 4-5 rows in *C. polypodiifolia* var. *polypodiifolia* (Fig. 2).

Leaf anatomy

Investigation on leaf anatomy showed different types of hairs on the outside of the leaf that were very intense. On the outer side, there is a cuticular layer framing the epidermis. Under the upper and lower epidermis, there is palisade parenchyma. Because the palisade parenchyma is present on both sides, it can be concluded that the leaf type is isolateral (equifacial). Between the two palisade parenchyma, there is an irregularly arranged spongy parenchyma which occupies less area. All three taxa have equifacial leaves. In *C. polypodiifolia* var. *polypodiifolia*, mesophyll tissue consists of 2-3 layers of palisade and 2-3 layers of spongy parenchymatous cells, whereas in *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata* it is composed of 3 layers of palisade parenchymatous cells and 2 layers of spongy parenchymatous cells (Fig. 3). In surface section, stoma cells were found as amaryllis type. Stomata are surrounded by usually 3, and rarely 4 neighboring cells. The type of stomata is anisocytic (Fig. 3).

Cytological characters

Chromosome numbers and detailed chromosome morphology of three *Centaurea* taxa are reported in this study. In *Centaurea polypodiifolia* Boiss. var. *polypodiifolia*, the chromosome number was found to be $2n=16$ and the basic chromosome number of $x=8$. The shortest chromosome length is $1.92\ \mu\text{m}$, while the longest is $4.66\ \mu\text{m}$, and haploid chromosome length is $22.9\ \mu\text{m}$. The karyotype formula of this taxon is $1M+5m+2sm$ (Table 2).

In *C. urvillei* subsp. *urvillei*, chromosome number $2n=20$ and the basic chromosome number of $x=10$ is reported. The shortest chromosome length is $2.80\ \mu\text{m}$, the longest is $5.28\ \mu\text{m}$ and haploid chromosome length is $37.84\ \mu\text{m}$. The karyotype formula of this taxon is $2M+5m+3sm$ (Table 2). Satellite was detected on the short arm of chromosome 5 in this taxon. The chromosome number of *C. urvillei* subsp. *armata* is $2n=20$, where the basic chromosome number of $x=10$. The

shortest chromosome length is 3.05 μm , the longest 4.74 μm and haploid chromosome length is 41.01 μm . The karyotype formula of this taxon is $2M+5m+3sm$ (Table 2). Satellite was detected on the short arm of chromosome 3. Other karyotype parameters and asymmetries are given in Table 3. Somatic metaphase chromosomes and idiograms of all three taxa of *Centaurea* are presented in Figures 4 and 5.

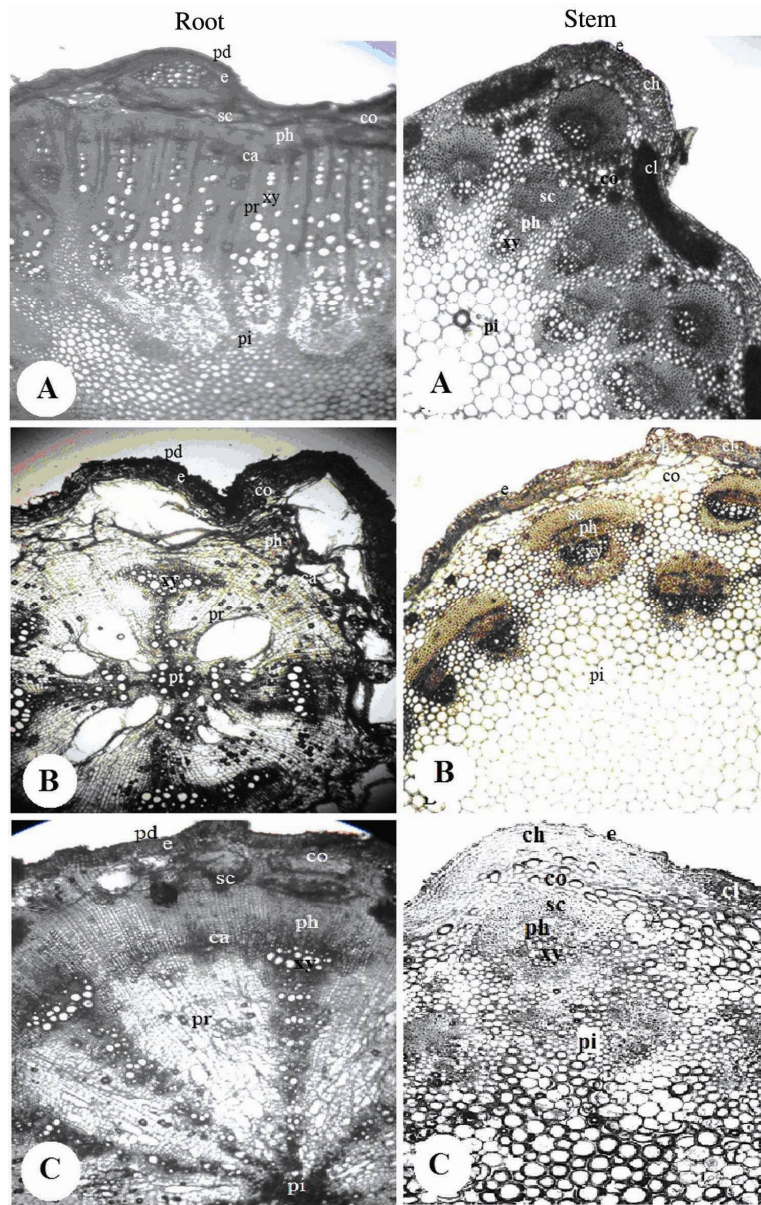


Fig. 2. Cross-sections of stems and roots. A. *Centaurea polypodiifolia* var. *polypodiifolia*; B. *C. urvillei* subsp. *urvillei*; C. *C. urvillei* subsp. *armata*. (pd=periderm, e=epidermis, co=cortex, sc=sclerenchyma, ph=phloem, ca=cambium, xy=xylem, pr=pith ray, pi=pith, ch=collenchyma, cl=chlorenchyma).

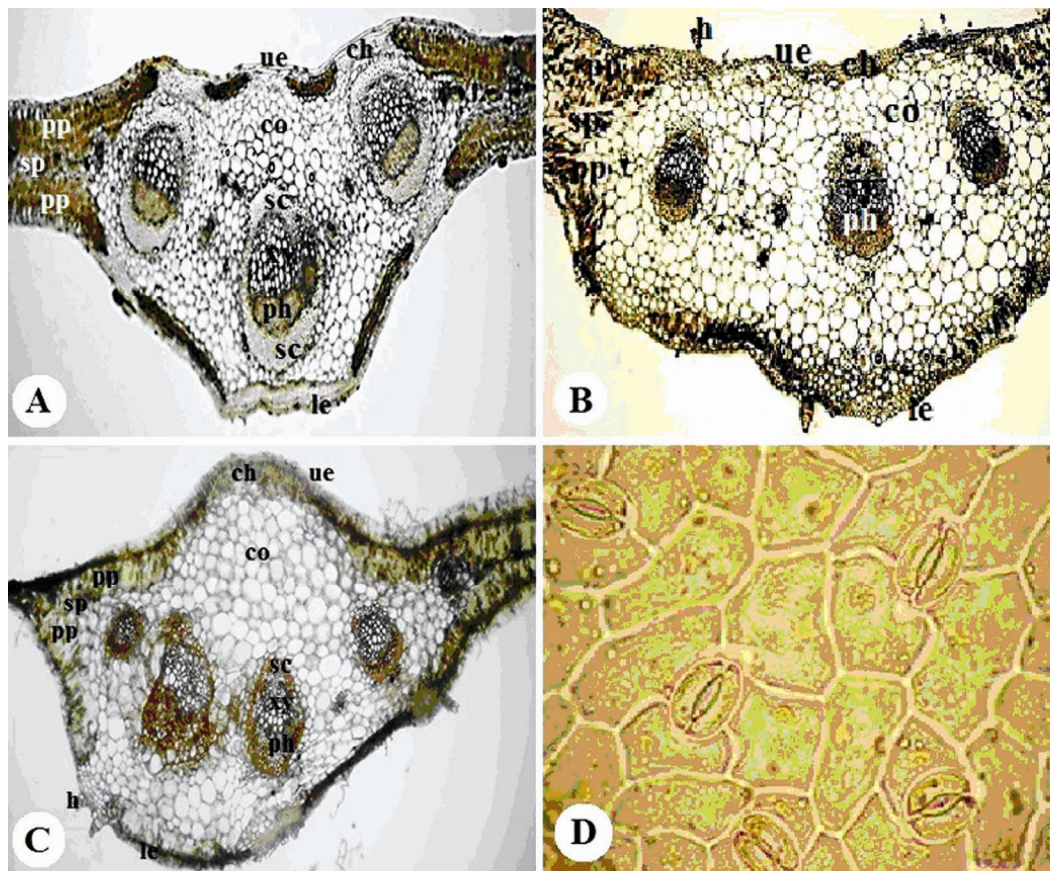


Fig. 3. Cross-sections of leaves of three *Centaurea* taxa. A. *C. polypodiifolia* var. *polypodiifolia*; B. *C. urvillei* subsp. *urvillei*; C. *C. urvillei* subsp. *armata*; D. Surface section of leaf of *C. polypodiifolia* var. *polypodiifolia* (other two taxa similar) (h=hair, ue=upper epidermis, ch=collenchyma, co=cortex, sc=sclerenchyma, ph=phloem, xy=xylem, pp=palisade parenchyma, sp=spongy parenchyma, le=lower epidermis).

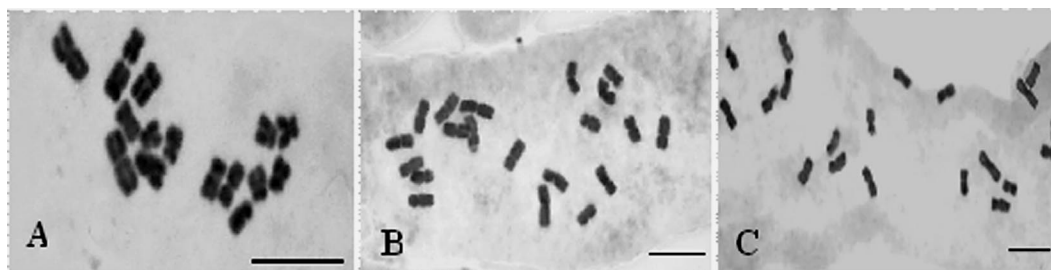


Fig. 4. Somatic metaphase chromosomes of three *Centaurea* taxa. A. *C. polypodiifolia* var. *polypodiifolia* ($2n=16$); B. *C. urvillei* subsp. *urvillei* ($2n=20$); C. *C. urvillei* subsp. *armata* ($2n=20$) (Scale bars: 10 μm).

Table 2. Karyological features of three taxa of *Centaurea* studied.

Cytological characters	<i>C. polypodiifolia</i> var. <i>polypodiifolia</i>	<i>C. urvillei</i> subsp. <i>urvillei</i>	<i>C. urvillei</i> subsp. <i>armata</i>
Chromosome number (2n)	16	20	20
Ploidy level	2x	2x	2x
Karyotype formule	1M+5m+2sm	2M+5m+3sm	2M+5m+3sm
Chromosome length (μm)	1.92-4.66	2.80-5.28	4.74-3.05
Total karyotype length (TKL) (μm)	22.9	37.84	41.01
Intrachromosomal asymmetry index (A_1)	0.29	0.31	0.27
Interchromosomal asymmetry index (A_2)	0.32	0.21	0.14
Karyotype symmetry nomenclature followed Stebbins (SC)	3B	3A	3A
Coefficient of variation of chromosome length (CV_{CL})	32.75	21.70	14.36
Coefficient of variation of centromeric index (CV_{CI})	15.02	22.57	19.80
Karyotype asymmetry index (AI)	4.92	4.89	2.84

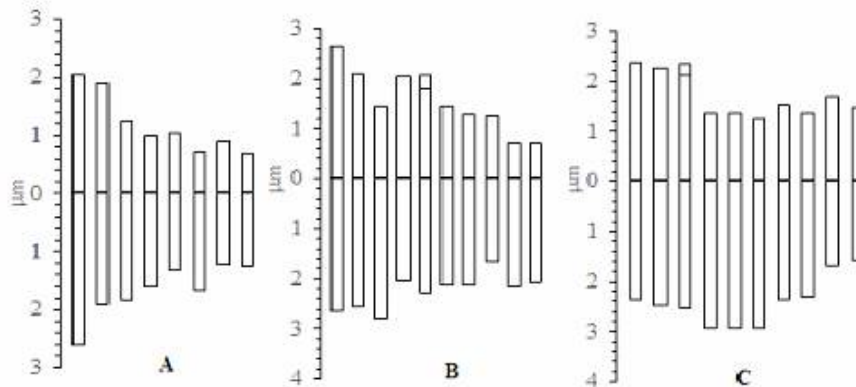


Fig. 5. Idiogram of three *Centaurea* taxa. A. *C. polypodiifolia* var. *polypodiifolia*; B. *C. urvillei* subsp. *urvillei*, C. *C. urvillei* subsp. *armata*.

The present study was carried out in order to provide useful and additional morphological, anatomical and cytological data for three *Centaurea* taxa of Turkey, namely *C. polypodiifolia* var. *polypodiifolia*, *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata*. Several attempts have been made to subdivide the genus *Centaurea* (*s.l.*) comprising about 300 problematic species (Garcia-Jacas *et al.*, 2006). Wagenitz (1975) placed these taxa in the two distinct sections of the genus *Centaurea*, viz. section *Acrocentron* (*C. urvillei* subsp. *urvillei*, *C. urvillei* subsp. *armata*) and section *Microlophus* (*C. polypodiifolia* var. *polypodiifolia*) in the Flora of Turkey. *Centaurea polypodiifolia* Boiss. is one of the six species included in the section *Microlophus* (Cass.) DC., and is divided into three varieties based on length of appendages and width of involucre. The appendages of *C. polypodiifolia* var. *polypodiifolia* are very small and easily deciduous. The type has narrow upper leaves but larger involucre than most of the other material of this variety and approaches var. *szovitsiana* in this respect. The appendages of *C. polypodiifolia* var. *pseudobehen*

are very variable and the upper leaves are narrowly lanceolate. The following gathering is almost intermediate between var. *polypodiifolia* and var. *pseudobehen* (Davis, 1975).

Centaurea urvillei DC. is one of the seventeen species included in the section *Acrocentron* (Cass.) DC. and is a very polymorphic species with five distinct subspecies, connected by transitional forms although the extremes (subsp. *urvillei* and subsp. *hayekiana*) look very different and could easily be placed at species rank. The geographical differentiation is only partial, especially where the areas of subsp. *urvillei* and subsp. *armata* overlap. *C. urvillei* is endemic to Turkey and closely related to *C. lydia* and *C. raphanina sensu lato* (Davis, 1975). *C. urvillei* subsp. *urvillei* shows considerable variation in the length of the appendages (spines) and appendages have been found shortest in some samples collected from Sout-western Anatolia of Turkey.

Wagenitz (1975) explained morphological characters in the genus *Centaurea*, and Metcalfe and Chalk (1979) provided information on anatomical characteristics of the family Asteraceae. In addition to some morphological and anatomical investigations, studies on palynology, phytogeographic distribution and ecology of some *Centaurea s.l.* species in Turkey were presented (Ozler *et al.*, 2009). However, no detailed morphological and anatomical studies of the investigated taxa are available so far.

In the present study we investigated root, stem and leaf anatomy of *C. polypodiifolia* var. *polypodiifolia*, *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata*. The general root, stem and leaf structure are almost similar in all examined taxa, but a few differences were determined as shown in Figures 2 and 3. The anatomical characters of all studied taxa have been presented here for the first time. In root of all examined taxa, the crushed epidermis is found below the peridermis. Although the number of phloem cells is less, xylem cells occupy a broader space. The xylem expanded to cover its own pith region.

The collenchyma tissue was generally located very close to the epidermis with 3-4 rows in *C. urvillei* subsp. *urvillei* and subsp. *armata* and 4-6 rows in *C. polypodiifolia* var. *polypodiifolia*. It is well established that the position and the average number of row in collenchyma tissue is important for comparative anatomical studies in plants (Lersten and Curtis, 1997; Makbul *et al.*, 2008). Additionally, a chlorenchymatous tissue below the epidermis was observed in the stem cortex with 3-4 rows in *C. urvillei* subsp. *urvillei* and subsp. *armata* and 4-5 rows in *C. polypodiifolia* var. *polypodiifolia*. This tissue was reported in the genus *Centaurea* in some earlier studies (Uysal *et al.*, 2005; Celik *et al.*, 2005, 2008; Kaya *et al.*, 2010). The vascular bundles in stem of *Centaurea* are generally arranged in 2 rings. Our investigated taxa revealed 2 rings, however, they are sometimes arranged in 1 ring in some taxa of *Centaurea*. Celik *et al.* (2005, 2008) and Kaya *et al.* (2010) reported that vascular bundles are scattered in a circular manner in a single ring in the stem of some *Centaurea* species.

Leaf anatomical properties are used as significant distinctive characters in plant taxonomy (Uysal *et al.*, 2005; Kaya *et al.*, 2010). In cross-sections of leaves we found that vascular bundle was capped by sclerenchymatic fibres at both sides in all the examined taxa (Fig. 3). However, the sclerenchymatic fibres were more prominent in *C. polypodiifolia* var. *polypodiifolia*. All studied taxa have equifacial leaves, with a mesophyll tissue that consists of 2-3 layers of palisade and 2-3 layers of spongy parenchymatic cells of *C. polypodiifolia* var. *polypodiifolia*, 3 layers of palisade parenchymatic cells and 2 layers of spongy parenchymatic cells in *C. urvillei* subsp. *urvillei* and *C. urvillei* subsp. *armata*, respectively.

The importance of karyomorphology in *Centaurea* has long been realized (Garcia-Jacas and Susanna, 1992). In the genus *Centaurea* in Turkey the somatic chromosome number varies from $2n=16$ to 66 (Wagenitz, 1975; Martin *et al.*, 2006; Inceer *et al.*, 2007). The basic chromosome

number of *Centaurea* species varies from $x=7$ to 16 and four ploidy levels ($2x$, $3x$, $4x$, $6x$) have been determined (Gomurgen *et al.*, 2010; Meric *et al.*, 2010; Kocyigit and Bona, 2013). The chromosome number of *C. polypodiifolia* var. *polypodiifolia* in the present study was found to be $2n=2x=16$, and this finding was found consistent with Ghaffari and Shahraki (2001). In *C. urvillei* subsp. *urvillei* and subsp. *armmata* $2n=2x=20$ were found and our results were supported by previous studies (Gardou and Tchehrehgosha, 1975; Garcia-Jacas *et al.*, 1997), although different chromosomal counts for *C. urvillei* subsp. *urvillei* ($2n=40$) have also been made (Martin *et al.*, 2009). In this study we found anatomical and cytological characters as useful in the taxonomy of three *Centaurea* taxa in Turkey. Investigation of anatomical features were first of its nature among the studied taxa. The findings of the present study would be useful for contributing to systematics of this genus *Centaurea* and to infer interspecific relationships based on anatomical and karyological characters.

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