

MORPHOLOGY, ANATOMY AND PALYNOLOGY OF TWO ENDEMIC *COUSINIA* CASS. SPECIES (SECT. *COUSINIA*, ASTERACEAE) AND THEIR TAXONOMIC IMPLICATIONS

DENİZ ULUKUŞ^{1*} AND OSMAN TUGAY²

¹Department of Biotechnology, Faculty of Sciences, Selçuk University, Konya, Turkey.

²Department of Pharmaceutical Botany, Faculty of Pharmacy, Selçuk University, Konya, Turkey.

*Corresponding author's email: dulukus@selcuk.edu.tr

Abstract

The morphological, anatomical and palynological features of *Cousinia eleonorae* Hub.-Mor. and *Cousinia humilis* Boiss. are provided along with their taxonomic significance. Their expanded description, distribution map and coloured photographs are also given. The morphological characteristics of leaves, phyllaries are taxonomically significant for distinguishing *Cousinia* species. In addition, the micromorphological characteristics of pollen grains and achenes are provided with the use of scanning electron microscopy. Anatomical characters such as number of cortex cells, number of vascular bundles and midrib shape are found to be significant characters. Moreover, exine sculpturing pattern and shape of pollen grains can be used for taxonomic purposes.

Key words: Achene, Anatomy, *Cousinia*, Palynology, Taxonomy, Turkey.

Introduction

The genus *Cousinia* (Asteraceae, Cardueae) in terms of number of species is one of the richest genera in the Asteraceae family with about 630 species (Susanna & Garcia-Jacas, 2007; Mehregan & Kadereit, 2009). According to Frodin (2004), *Cousinia* locates among the first largest 50 genera in angiosperm plants. The genus is mainly distributed in Southwest and Central Asia (Susanna & Garcia-Jacas, 2007). *Cousinia* (Asteraceae) has more than 400 species in SW Asia (Rechinger, 1986).

Cousinia is not monophyletic within *Arctium-Cousinia* complex (Lopez-Vinyallonga *et al.*, 2009), owing to poor phylogenetic resolution in the genus, probably lack of characters and the high number of taxa in this species-rich and comparatively young (ca. 8.7 mya) lineage (Lopez-Vinyallonga *et al.*, 2009; Minaeifar *et al.*, 2016).

The genus *Cousinia* was first described by Cassini (1827) based on *Carduus orientalis* Adams. Since then, many authors have dealt in genus from different point of view. Such as taxonomical, (Boissier, 1875; Tscherneva & Vvedensky, 1962; Rechinger, 1964; Schepa, 1966; 1976; Tutin *et al.*, 1976; Feinbrun-Dothan, 1977; Rechinger, 1979; Kuprianova & Tscherneva, 1982; Rechinger, 1986; Tscherneva, 1988; Tamanian, 1999; Djavadi & Attar, 2005; Sennikov, 2010; 2011). Palynological (Kuprianova & Tscherneva, 1982; Djavadi *et al.*, 2007; Assadi, 2009; Saber *et al.*, 2009; Ahmad *et al.*, 2011; Ulukuş & Tugay, 2019; Ulukuş, 2019), point of view evolutionary and phylogenetic (Susanna *et al.*, 2003; Mehregan & Kadereit, 2008; Lopez-Vinyallonga *et al.*, 2009; Lopez-Vinyallonga *et al.*, 2011), cytogenetical (Djavadi & Ghaffari, 1999; Susanna *et al.*, 2003; Ghaffari *et al.*, 2006; Sheidai *et al.*, 2006; Lopez-Vinyallonga *et al.*, 2010; Sheidai *et al.*, 2012), anatomical (Attar *et al.*, 2004; Ulukuş & Tugay, 2019; Ulukuş, 2019).

Cousinia is represented in Turkey by 38 species distinguishing 6 section. Sect. *Cousinia* consists of 22 species, the representative section in Turkey, even more than Flora Iranica (ca. 10 species, (Rechinger, 1979). Hence, Turkey may be considered as main centers sect. *Cousinia* biodiversity. According to Turkey's Plants List, *Cousinia* genus is represented by 39 species in Turkey and the Turkish name of the genus is "Kızandikenî" (Tugay, 2012).

Cousinia sect. *Cousinia* species are perennial herbs, growing mainly on dry, limestone, volcanic and rocky slopes, salt marsh, fallow fields, serpentine hills, or steppe (Huber-Morath, 1975). Morphologically, sect. *Cousinia* is characterized by the presence of leaves leathery, stem leaves normally distinctly decurrent, involucre ovoid-oblong to broadly ovoid, phyllaries 45–175, with small, erect-spreading reflexed terminal sipe, flowers yellow or pink to purple, achenes striped lengthwise or angular, the apex truncate and finely denticulate (Huber-Morath, 1975). Palynologically, pollen grains of sect. *Cousinia* species are spherical to cylindrical, tricolpate, exine surface ornamentation is verrucated perforated (Ahmad *et al.*, 2011). Caryologically, some species of sect. *Cousinia* has $x=12$ (Susanna *et al.*, 2003; Ghaffari *et al.*, 2006). One study has anatomic related to *Cousinia* (Attar *et al.*, 2004). However, to date, anatomical study has not carried out about sect. *Cousinia*.

Some of *Cousinia* species are used as dropsy, hematuria, vomiting diuretic, antiseptic, chronic diarrhea and dysentery using both roots and Fresh juice. Moreover, it is also used liver complaints and asthma (Tareen *et al.*, 2010). Since 2011, we have studied on systematic revision of the *Cousinia* in Turkey based on extensive field and herbarium studies, and literature surveys on the species. In this study, first time we aim to give a detailed account of the morphological, anatomical and palynological characteristics of morphologically very similar to determine whether these data can be of value in the taxonomy of the species.

Material and Methods

Between 2011 and 2013, while we were performing taxonomic revision of the genus *Cousinia* in Turkey, the plant specimens were collected from different localities in Turkey. The specimens were kept in Selcuk University KONYA Herbarium (KNYA). The herbarium samples were examined using Flora of Turkey under the stereobinocular microscope.

In pollen investigations, pollen material were obtained from herbarium specimens, the pollen slides were prepared according to Wodehouse (1935) technique. The polar length (P), the equatorial length (E), the colpus length (CLG), colpus width (CLT), the exine and the intine thickness for 30 pollen grains were measured under the light microscope (1000x) and P/E ratios were calculated. To determine exine sculpturing of the pollen was used SEM microscope. Punt *et al.*, (2007) was followed for pollen terminology.

Morphometric measurements of achenes were made under a stereomicroscope (Leica S8AP0) coupled with Leica DFC 295 digital camera. The achene length and width (10–)30–35 achenes per specimen of each taxon were measured. Measurements were made using Image Tool software. Minimum-maximum ranges, mean, standard deviations of achene length and width as well as length/width ratio were calculated. SEM micrographs were used to determine achene coat sculpturing of the achenes. The terminology of Stearn (1983) was adopted to describe the SEM aspects of the achene coat.

For anatomical studies, living material was kept in 70% ethanol. The paraffin method was used for cross sections of stems and leaves. The specimens were embedded in paraffin wax and then sectioned between 5 and 10 µm thickness with a Leica RM2125RT rotary microtome. All sections were stained with safranin-fast green and then mounted with Entellan (Johansen, 1940). Measurements and photos were taken using a Leica DM1000 binocular light microscope with a Leica DFC280 camera.

Results

The morphological descriptions of the species were made according to Huber-Morath (1975). However, our field and herbarium studies displayed that some morphological characters should be amplified or expanded. The morphological differences from the literatures were given in (Table 1).

Distribution and Habitat

Cousinia eleonora Hub.-Mor.

Type: Turkey B6 Sivas: d. Kangal, Tecer to Gurun, limestone slopes nr Samakurd, 36 km S. of Tecer, 1520-1550 m, 21 vii 1958, *A.Huber-Morath* 15932 (holo. Hb. Hub.-Mor. G!).

B6 Sivas; Kangal, steppe, 1600 m, 28.07.2012, *O.Tugay* 7.855, *D.Ulukuş* & *M.A.Canbulat* (KNYA).

It is only known from a few locations in central Anatolia (Sivas) and it is a Irano-Turanian element (Fig. 1). It grows at

altitude of between 1450 and 1550 m in limestone steppe area with species such as: *Papaver argemone* L., *Helianthemum canum* (L.) Baumg., *Astragalus saganlugensis* Trautv., *Centaurea triumfettii* All., *Convolvulus calvertii* Boiss., *Gundelia tournefortii* L., *Helianthemum canum* (L.) Baumg., *Leontodon crispus* Vill. subsp. *asper* (Waldst. & Kit.) Rohl. var. *asper*, *Onobrychis armena* Boiss. & Huet, *Onosma aucheranum* DC., *Papaver argemone* L., *Senecio viscosus* L., *Scorzonera semicana* DC., *Scariola orientalis* (Boiss.) Soják, *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz., *Thymus cappadocicus* Boiss.

Cousinia humilis Boiss.

Type: [Turkey] in Cappadocia orientali, 1853, *Tchihatcheff* (holo. G!).

B6 Kayseri; Pınarbaşı, Ziyaret Pass, steppe, 1900 m, 25.08.2013, *O. Tugay* 8.615 & *D. Ulukuş* (KNYA).

It is only known from a few locations in central Anatolia (Kayseri) and it is a Irano-Turanian element (Fig. 1). It grows at altitude of between 1800 and 1900 m in steppe and erosion area with species such as: *Astragalus spruneri* Boiss., *Centaurea solstitialis* L., *Medicago papillosa* Boiss., *Potentilla argaea* Boiss. & Bal., *Saxifraga kotschyi* Boiss., *Scandix pecten-veneris* L., *Tetragonolobus maritimus* (L.) Roth., *Tragopogon latifolius* Boiss., *Tripleurospermum callosum* (Boiss. & Heldr.) E.Hossain, *Valeriana leucophaea* DC.

Morphologic properties

Cousinia eleonora Hub.-Mor. (Fig. 2a)

Perennial herbs, 18–40 cm, branched from base. Stem white tomentose hairy. Leaves narrowly lanceolate, woolly-arachnoid above, densely tomentose hairy; basal leaves oblong 6–15 × 2–4 cm, pinnatipartite, with 4–7 pairs of linear segments, segments 10–27 × 3 mm; stem leaves decurrent wings 14–26 mm, upper stem leaves lanceolate-linear, 24–40 × 18 mm, pinnatipartite, 2–4 pairs of prickly teeth. Capitula up to 20, 12–22 flowered. Involucre ovoid, 13–22 mm broad inclusive spines; Phyllaries 35–43, arachnoid-woolly at base, lower phyllaries reflexed, acuminate from 2 mm adpressed base into a 5–11 mm spreading part; Outer phyllaries 6–8 × 0.5–1 mm, middle phyllaries 11–12 × 1–1.5 mm, inner phyllaries 13–14 × 0.5–1 mm, bristles of receptacle smooth; 12–14 mm. Flowers yellow, 14–16 mm. Mature achenes dark brown, obovate, 8.06–4.99 × 3.38–1.81 mm, distinct longitudinally striped, indistinctly marginate and denticulate at apex. Bristles of pappus barbellate; 2–3 mm.

Table 1. Morphological differences between *C. eleonora* and *C. humilis*.

Diagnostic morphological characters	<i>C. eleonora</i>	<i>C. humilis</i>
Leaves indumentum	densely tomentose	densely white pannose
Basal leaves	pinnatipartite	dentate to pinnately lobed
Capitula number	up to 20	up to 16
Flowers number in each capitula	12–22	6–16
Involucre (mm)	13–22 with spines	13–18 with spines
Lower phyllaries	reflexed	spreading horizontally
Inner phyllaries (mm)	13–14 × 0.5–1	16–17 × 1–1.5
Bristles of receptacle (mm)	12–14	9–11
Bristles of pappus (mm)	2–3	2–2.5

Cousinia humilis Boiss. (Fig. 2b)

Perennial herbs, 5–22 cm, branched from base. Stem white woolly-tomentose hairy. Leaves narrowly lanceolate, loosely arachnoid above, densely white-pannose hairy; basal leaves up to 90 × 12 mm, dentate to pinnately lobed, with lanceolate to triangular segments; stem leaves gradually smaller, mostly dentate, narrowly and shortly decurrent. Capitula up to 16, 6–16 flowered. Involucre ovoid, 13–18 mm broad inclusive spines; Phyllaries 32–62, arachnoid-woolly at base, lanceolate-subulate, only lower ones spreading horizontally or somewhat decumbent, others erect-spreading, with lanceolate adpressed base 2–3 mm and spreading upper part 4–5 mm. Outer phyllaries 5–6 × 1 mm, middle phyllaries 10–13 × 1–1.5 mm, inner phyllaries 16–17 × 1–1.5 mm, bristles of receptacle smooth; 9–11 mm. Flowers yellow, 14–16 mm. Mature achenes dark brown, obovate, 7.71–6.04 × 3.66–2.73 mm, distinct longitudinally striped, indistinctly marginate and denticulate at apex. Bristles of pappus barbellate; 2–2.5 mm.

Anatomical properties

Significant properties were observed from transverse sections of the stem, leaf and midrib of the species. Anatomical measurement of various tissues of the studied species are given.

Stem anatomy

***Cousinia eleonora*:** Transverse sections taken from the stem of *C. eleonora* displays that stem is nearly rounded, epidermis is 1 layered, consists of oval or rectangular cells and covered by thick cuticle. The cortex (42–91 µm) is composed of 3–5 layers of oval, rectangular or orbicular parenchymatous cells. The phloem is encircled by sclerenchymatic cells. Sclerenchymatous fibres above the external and internal phloem 89–159 µm and 66–102 µm in thickness respectively. Cambium is not clearly distinguishable. Phloem size ranges from 57 to 88 µm. Size of the xylem is 171–264 µm. Vascular bundles are numerous and elliptic. The pith region comprises large hexagonal, polygonal or circular parenchymatous cells (Fig. 3a).

***Cousinia humilis*:** Transverse sections taken from the stem of *C. humilis* displays that stem is nearly rounded, epidermis is 1 layered, consists of oval or rectangular cells and covered by thick cuticle. The cortex (169–70 µm) is composed of 8–11 layers of oval, rectangular or orbicular parenchymatous cells. The phloem is encircled by sclerenchymatic cells. Sclerenchymatous fibres above the external and internal phloem are 30–56 µm and 20–32 µm in thickness respectively. Cambium is not clearly distinguishable. Phloem size ranges from 27 to 42 µm. Size of the xylem is 83–143 µm. Vascular bundles are numerous and elliptic. The pith region comprises large hexagonal, polygonal or circular parenchymatous cells (Fig. 4a).

Leaf anatomy

***Cousinia eleonora*:** Transverse sections of the lamina showed that the upper and lower epidermises are covered with a thin cuticle layer and eglandular hairs. Both epidermises consist of uniseriate oval and rectangular cells. The mesophyll (135–220 µm) consists of elongated palisade and spongy paranchyma cells. The Leaf is equifacial. Palisade parenchyma is 1–2 rowed under the upper epidermis and 1 rowed under the lower epidermis. Spongy parenchyma cells are 1 layer. Spongiouse cells are irregular, compact, cubic or spherical (Fig. 3b).

***Cousinia humilis*:** Transverse sections of the lamina showed that the upper and lower epidermises are covered with a thin cuticle layer and eglandular hairs. Both epidermises consist of uniseriate oval and rectangular cells. The mesophyll (190–281 µm) consists of elongated palisade and spongy paranchyma cells. The Leaf is equifacial. Palisade parenchyma is 1–3 rowed under the upper epidermis and 1–2 rowed under the lower epidermis. Spongy parenchyma cells are 1 layer. Spongiouse cells are irregular, compact, cubic or spherical (Fig. 4b).

Midrib anatomy

***Cousinia eleonora*:** The midrib shape is nearly circular. Belonging collenchyma of lower epidermis is tangential. Totally, it has 10 vascular bundle. There is 3 large vascular bundle in the center and is surrounded by a parenchymatic bundle sheat. Collenchymas of lower and upper epidermis are 515 µm and 395 µm respectively (Fig. 3c).

***Cousinia humilis*:** The midrib shape is almost triangular. Belonging collenchyma of lower epidermis is tangential. Totally, it has 4 vascular bundle. There is 1 large vascular bundle in the center and is surrounded by a parenchymatic bundle sheat. Collenchyma of lower and upper epidermis are 183 µm and 85 µm respectively (Fig. 4c).

Pollen properties

***Cousinia eleonora*:** Shape of pollen grains of *C. eleonora* is subprolate. Polar axis is 33.49 ± 3.09 µm and its equatorial axis is 25.78 ± 2.23 µm. The ratio of P/E is 1.30. Colpus length is 29.26 ± 3.96 µm and colpus width is 2.91 ± 0.66 µm. The exine thickness is 1.55 ± 0.29 µm and the intine thickness is 0.63 ± 0.15 µm. The exine sculpturing pattern is verrucose-perforate (Fig. 5a–b).

***Cousinia humilis*:** Shape of pollen grains of *C. humilis* is prolate-spheroidal. Polar axis is 36.22 ± 1.25 µm and equatorial axis is 30.02 ± 0.84 µm. The ratio of P/E is 1.20. Colpus length is 31.22 ± 1.53 µm and colpus width is 3.53 ± 0.52 µm. The exine thickness is 1.77 ± 0.27 µm and the intine thickness is 0.61 ± 0.09 µm. The exine sculpturing pattern is verrucose-perforate (Fig. 6a–b).

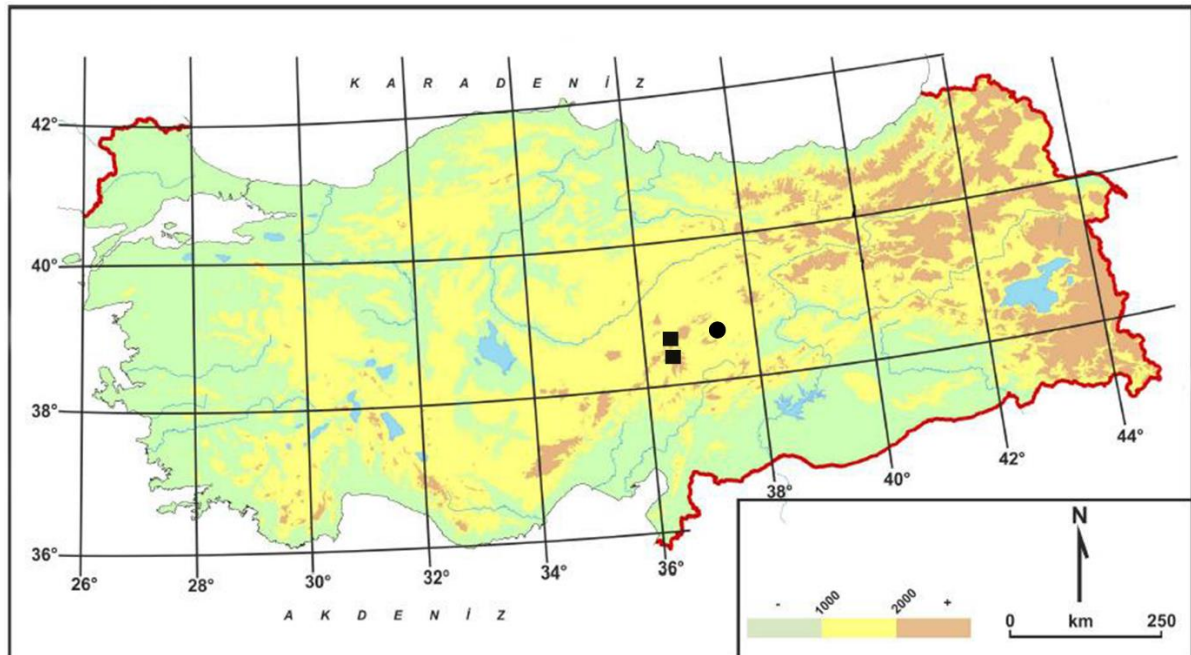


Fig. 1. Distribution map of *C. eleonoraae* (■) and *C. humilis* (●) in Turkey.

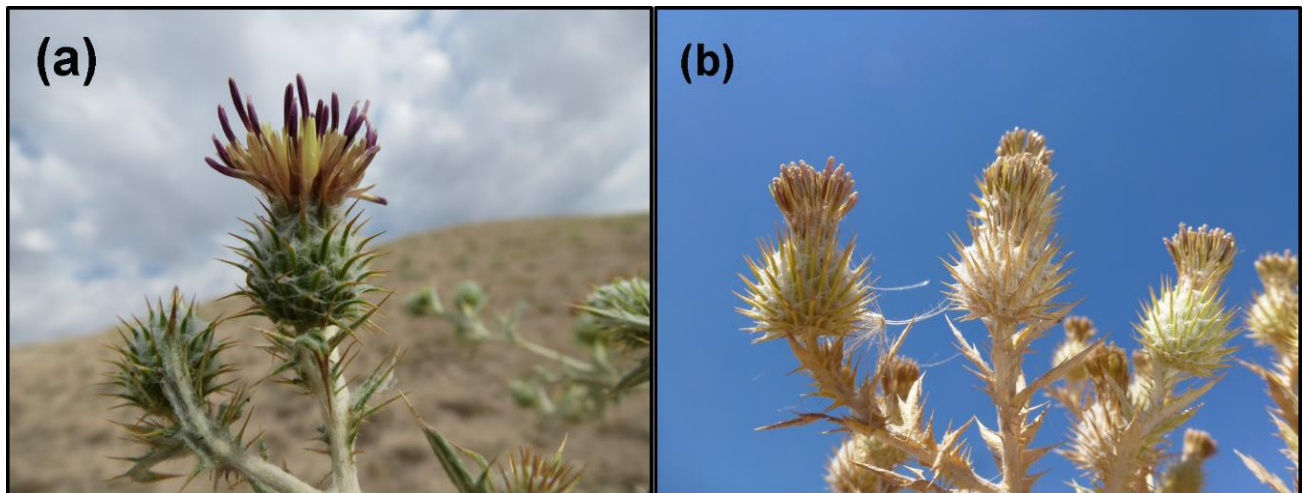


Fig. 2. General view of flowers: *C. eleonoraae* (a) and *C. humilis* (b)

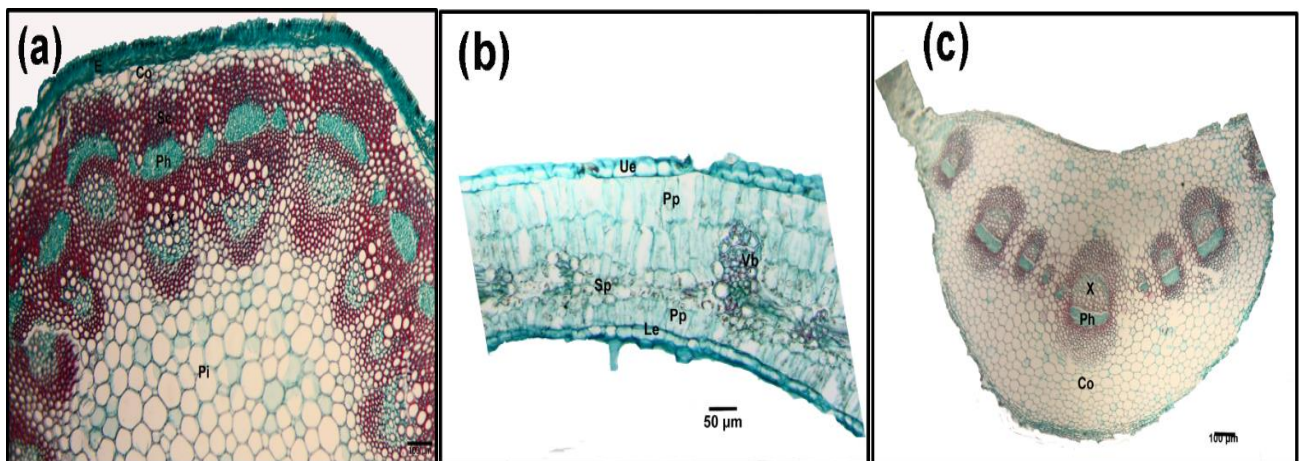


Fig. 3. Anatomical characters of *C. eleonoraae*: co: cortex, e: epidermis, le: lower epidermis, ph: phloem, pi: pith region, pp: palisade parenchyma, sc: sclerenchyma, sp: spongy parenchyma, x: xylem, ue: upper epidermis, vb: vascular bundle.

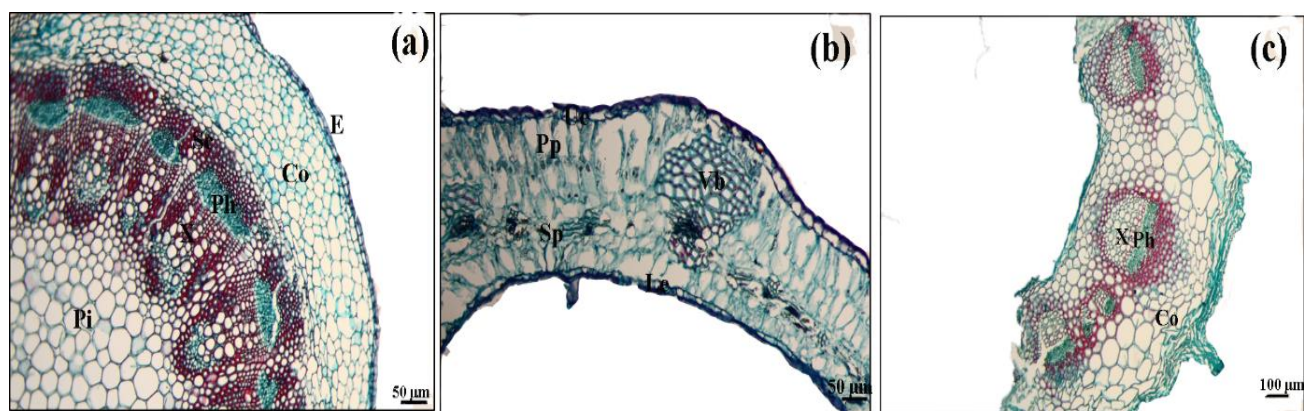


Fig. 4. Anatomical characters of *C. humilis*: co: cortex, e: epidermis, le: lower epidermis, ph: phloem, pi: pith region, pp: palisade parenchyma, sp: spongy parenchyma, x: xylem. ue: upper epidermis, vb: vascular bundle.

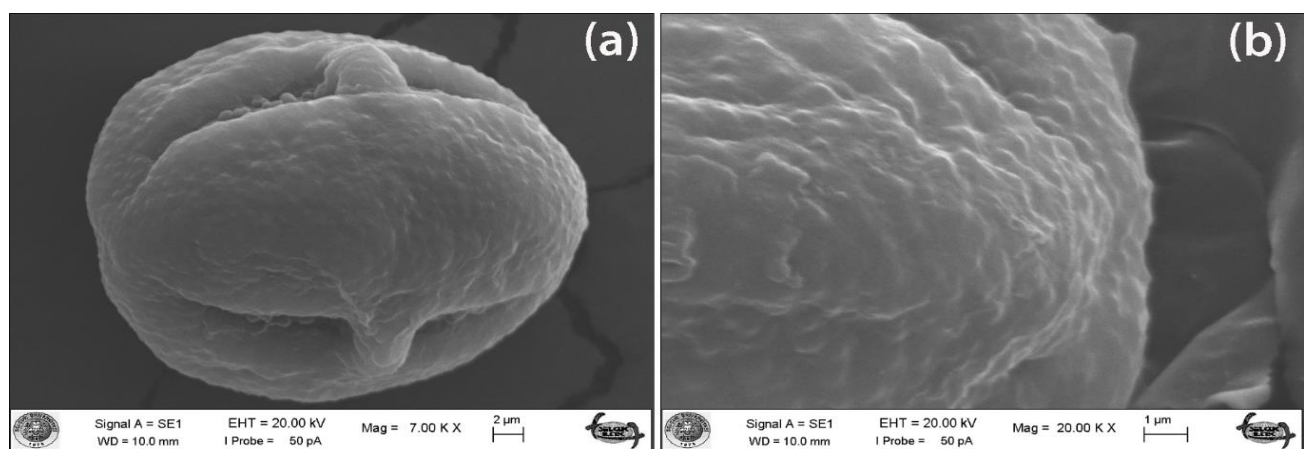


Fig. 5. SEM micrographs of the pollen grains; (a & b) equatorial view and exine sculpturing of *C. eleonora*.

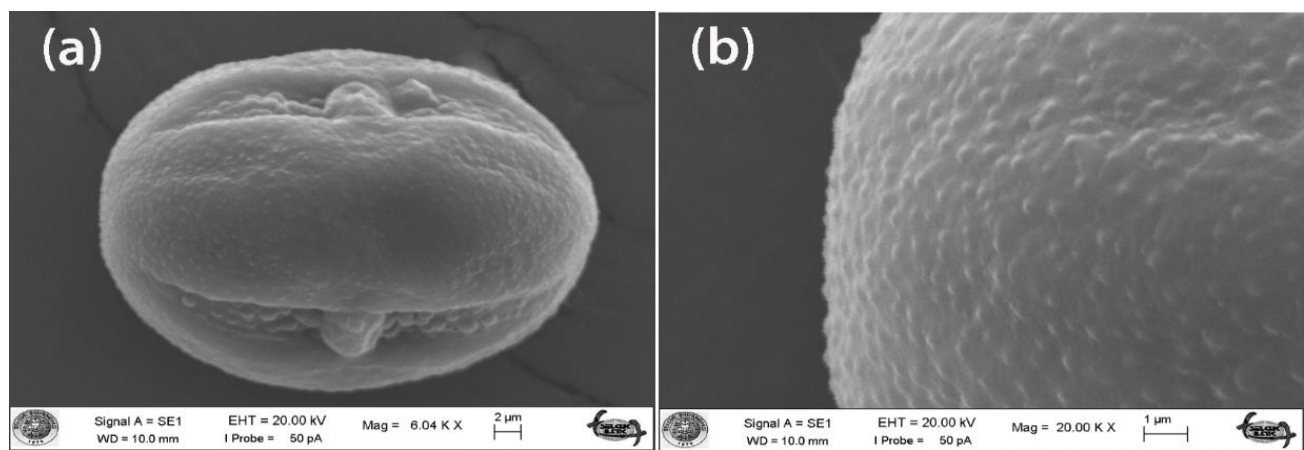


Fig. 6. SEM micrographs of the pollen grains; (a & b) equatorial view and exine sculpturing of *C. humilis*.

Achene morphology and micromorphology: The achenes shape of *C. eleonora* are obovate in their outline. Mature achenes of *C. eleonora* are 8.06–4.99 mm long and 3.38–1.81 mm wide and the range of the L/W ratio is 2.32 ± 0.32 . The achene surface pattern is striate and has irregular reticulate (Fig. 7a–b). The mature achenes are dark brown.

The achenes shape of *C. humilis* are obovate in their outline. Mature achenes of *C. humilis* are 7.71–6.04 mm long and 3.66–2.73 mm wide and the range of the L/W ratio

is 2.82 ± 1.86 . The achene surface pattern is striate and has irregular reticulate (Fig. 7c–d). The mature achene are light brown.

Discussion and Conclusions

The present study provides useful morphological, anatomical and palynological information of the examined *Cousinia* taxa. According to Huber-Morath (1975), *C. humilis* and *C. eleonora* are locating sect. *Cousinia* and differing from corolla colour with each other.

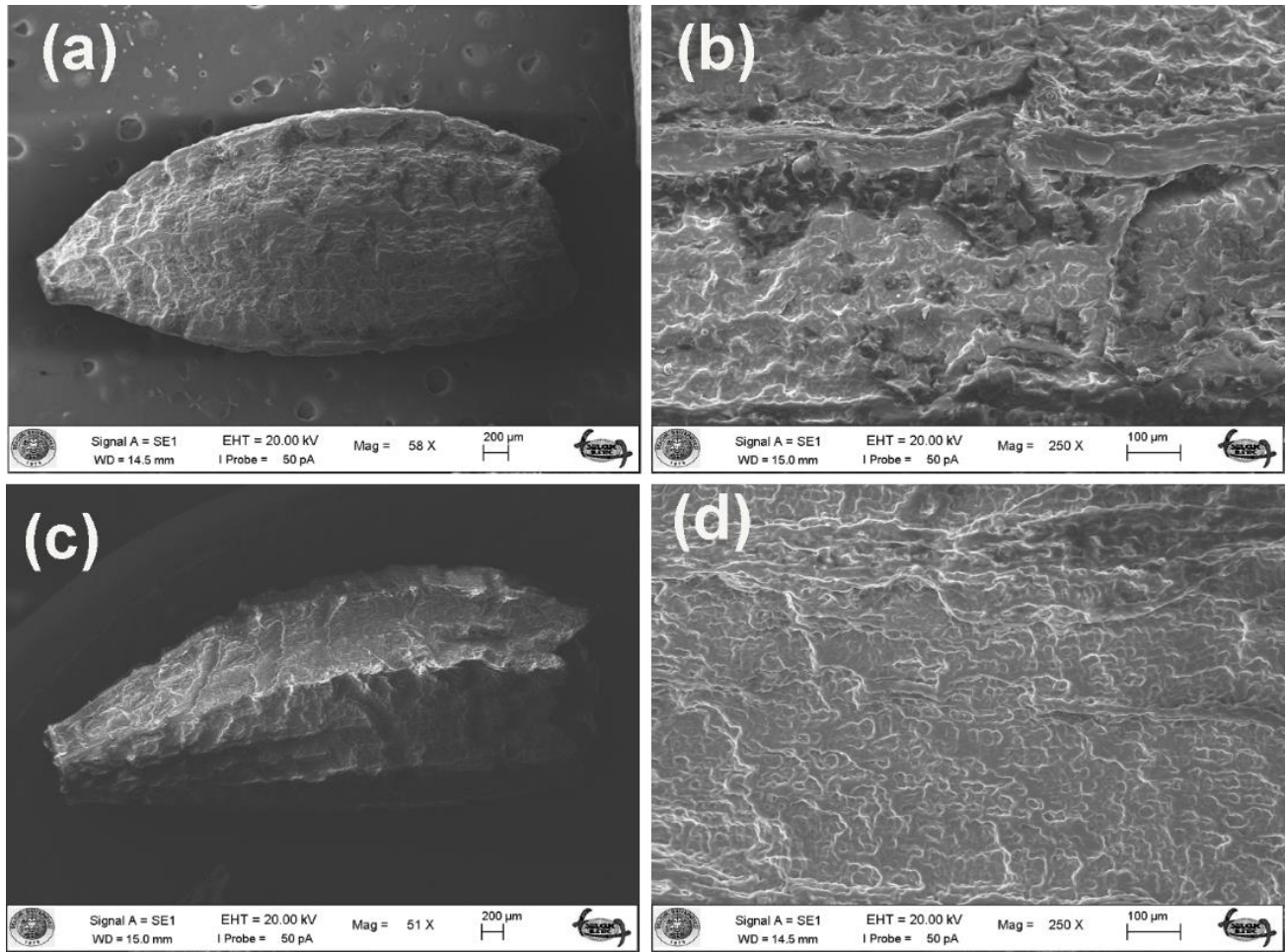


Fig. 7. Scanning electron micrographs of achenes of *Cousinia* species; (a&b) *C. eleonora*, (c&d) *C. humilis*.

Our finding indicate that among the morphological traits leaves and phyllaries characters are significant for distinguishing of taxa. With present study, we added new diagnostic morphological characters (Table 1). For instance *C. eleonora* differs from *C. humilis* by its densely tomentose hairs (not densely pannose), pinnatipartite on basal leaves (not dentate to pinnately lobed), number of capitula up to 20 (not up to 16), and lower phyllaries reflexed (not spreading horizontally), inner phyllaries (in *C. eleonora* 13–14 × 0.5–1 mm, in *C. humilis* 16–17 × 1–1.5 mm), bristles of receptacle (in *C. eleonora* 12–14 mm, in *C. humilis* 9–11 mm), achene size (in *C. eleonora* 8.06–4.99 × 3.38–1.81 mm, in *C. humilis* 7.71–6.04 × 3.66–2.73 mm). Our results are consistent with flora of Turkey.

It is considered that the palynological characters are significant in the taxonomy of the Asteraceae family (Wortley *et al.*, 2007). On the other hand, (Schtepa, (1966) and Kuprianova & Tscherneva, (1982) defined two pollens type as *Arctium* and *Cousinia*. Pollen grains some of the genera of Asteraceae are being tri-zono-colporate with a columellate exine ultrastructure (Wortley *et al.*, 2012). Saber *et al.*, (2009) investigated pollen morphological features 25 species belonging to sect. *Stenocephalae* from genus *Cousinia* and defined prolate, isopolar, tricolporate of pollen grains. Furthermore, they showed that exine ornamentation is densely or loosely verrucate. Meo & Khan (2003) reported that pollen grain shape of *Cousinia*

minuta is prolate to spheroidal. Pollen shape of this species is similar with *C. humilis* including in our study. Jafari & Ghanbarian (2007) studied pollen grains properties belonging 30 species of 24 genera in Asteraceae family containing *C. eriobasis* Bunge only species within *Cousinia* genus, with aid light microscopy, and reported that pollen grains shapes are subprolate and verrucate pattern of exine. According to Ahmad *et al.*, (2011), pollen grains of species belonging to sect. *Cousinia* of *Cousinia* genus were spherical to cylindrical shape. As for our study, while *C. humilis* was prolate-spheroidal, *C. eleonora* was subprolate. Our study showed that the pollen grains of studied species belong to sect. *Cousinia* have *Cousinia* pollen type and exine pattern of pollen is verrucose-perforate as previous study.

Anatomical studies showed that number of cortex layers and size of sclerenchymatous fibres both external and internal phloem respectively in stem are found to be important species characters. Hence, these characters could be used to distinguish the studied species. *C. eleonora* has 3–5 layered cortex tissue, while *C. humilis* has 8–11 layered cortex tissue. Moreover, sclerenchymatous fibres above the external and internal phloem of *C. eleonora* are 89–159 µm and 66–102 µm respectively. As for *C. humilis*, It has is 50–80 µm and 25–50 µm on phloem. On the other hand we observed that vascular bundle number and midrip shape in leaves are to be significant character for distinguishing

studied species. The midrib shape of *C. eleonora* is nearly circular and with 10 vascular bundle. However, midrib shape of *C. humilis* is triangular and with 4 vascular bundle (Fig. 3c, Fig. 4c).

To date, there is only one study about anatomy of *Cousinia* genus. According to this study, Attar *et al.*, (2004) made anatomical study relating to 14 species of *Cousinia* section *Serratuloideae*. They reported with the exception of some minor differences in stem, was the same for all of the species. Most variation among species was shown in structure belong to leaves and midrib shape. Therefore, these characters used for leaves are important to separate studied species. According to our findings, midrib shape is also found to be important leaves character for species of *Cousinia*. Besides, cortex layers number is observed to be significant character in stem.

While there are some phylogenetic, biogeographic and caryologic studies on the genus *Cousinia* (Ghaffari *et al.*, 2006; Lopez-Vinyallonga *et al.*, 2009; Lopez-Vinyallonga *et al.*, 2010; Sheidai *et al.*, 2012). In *Arctium-Cousinia* complex (Lopez-Vinyallonga *et al.*, 2009). Only *C. intertexta* located on them. Thus, we cannot deeply discuss the reliability of anatomical and palynological characters in terms of phylogeny. More detailed molecular phylogenetic study with a broader taxon sampling is required, particularly from Turkey, to find correlations between anatomical and palynological characters and classification of the genus.

Acknowledgements

We are grateful to the curators of herbaria AEF, ANK, E, G, GAZI, HUB, ISTE, ISTF, K and LE for permitting the examination of *Cousinia* specimens. We would like to thank TÜBİTAK (Project no: 111T364) and Selçuk University, BAP (Project no: 17401081) for financial support.

References

- Ahmad, K., M. Sheidai and F. Attar. 2011. Morphometry and palynological study of the genus *Cousinia* sect. *Cousinia* (Asteraceae) in Iran. *Iran. J. Bot.*, 17: 158-166.
- Assadi, M. 2009. Four new species of the genus *Cousinia* Cass. (Asteraceae) from Iran. *Iran. J. Bot.*, 15: 36-44.
- Attar, F., A. Ghahreman, K. Mahdigholi and M. Sheidai. 2004. Anatomy-Taxonomy studies of the species of section *Serratuloideae* (*Cousinia*, Compositae) in Iran. *Iran. J. Bot.*, 10: 119-141.
- Boissier, E. 1875. *Centaurea*. Flora orientalis 3. H. Georg, Geneva & Basilea (Basel), pp. 668-674.
- Cassini, A. 1827. Carlinées-Prototypes. Dictionnaire des sciences naturelles 47. Le mormant Paris, pp. 498-505.
- Djavadi, S. and S. Ghaffari. 1999. Distribution and chromosome studies of *Cousinia* section *Sphaerocephalae* (Asteraceae). *Iran. J. Bot.*, 8: 49-54.
- Djavadi, S. and F. Attar. 2005. Sect. *Lasiandra* from genus *Cousinia* Cass. (Compositae) with emphasis to a new species from east of Iran. *Feddes Repert.*, 116: 285-289.
- Djavadi, S., Z. Najafi and F. Atar. 2007. A new species of *Cousinia* sect. *Cousinia* from NW Iran. *Iran. J. Bot.*, 13: 43-46.
- Feinbrun-Dothan, N. 1977. Ericaceae to Compositae. The Israel Academy of sciences and humanities.
- Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon.*, 53: 753-776.
- Ghaffari, S.M., N. Garcia-Jacas and A. Susanna. 2006. New chromosome counts in the genus *Cousinia* (Asteraceae) from Iran. *Bot. J. Linn. Soc.*, 151: 411-419.
- Huber-Morath, A. 1975. *Cousinia*. Flora of Turkey and the East Aegean Islands 5. pp. 329-353.
- Jafari, E. and G. Ghanbarian. 2007. Pollen morphological studies on selected taxa of Asteraceae. *J. Plant Sci.*, 2: 195-201.
- Johansen, D.A. 1940. Plant microtechnique McGraw-Hill, New York.
- Kuprianova, L. and O. Tscherneva. 1982. Morfologija pyl'tzy I ultraskulptura palinodermi vidov roda *Cousinia* (Asteraceae) v svjazi s sistematikoi roda [Pollen morphology and ultrastructure of palynoderma in the species of the genus *Cousinia* (Asteraceae) in relation to the systematics of the genus]. *Bot. Zhurn.*, 67: 581-589.
- Lopez-Vinyallonga, S., I. Mehregan, N. Garcia-Jacas, O. Tscherneva, A. Susanna and J.W. Kadereit. 2009. Phylogeny and evolution of the *Arctium-Cousinia* complex (Compositae, Cardueae-Carduinae). *Taxon.*, 58: 153-171.
- Lopez-Vinyallonga, S., A. Susanna and N. Garcia-Jacas. 2010. Chromosome Numbers in the Genera *Cousinia*, *Olgaea* and *Syreitschikovia* (Compositae). *Folia Geobot.*, 45: 201-214.
- Lopez-Vinyallonga, S., K. Romaschenko, A. Susanna and N. Garcia-Jacas. 2011. Systematics of the Arctioid group: Disentangling *Arctium* and *Cousinia* (Cardueae, Carduinae). *Taxon.*, 60: 539-554.
- Mehregan, I. and J.W. Kadereit. 2008. taxonomic revision of *Cousinia* sect. *Cynaroideae* (Asteraceae, Cardueae). *Willdenowia.*, 38: 293-362.
- Mehregan, I. and J.W. Kadereit. 2009. The role of hybridization in the evolution of *Cousinia* s. str. (Asteraceae, Cardueae). *Willdenowia.* 39: 35-47.
- Meo, A.A. and M.A. Khan. 2003. Pollen morphology of *Achillea* (Compositae-Anthemoideae). *Pak. J. Weed Sci. Res.*, 9: 253-258.
- Minaeifar, A.A., M. Sheidai, F. Attar, Z. Noormohammadi and S. Ghasemzadeh-Baraki. 2016. Biosystematic study in the genus *Cousinia* Cass. (Asteraceae), section *Cousinia*. *Biochem. Syst. Ecol.*, 69: 252-260.
- Punt, W., P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas. 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.*, 143: 1-81.
- Rechinger, K.H. 1964. Flora of lowland Iraq.
- Rechinger, K. 1979. *Cousinia* In: Rechinger, KH (Eds.) *Flora Iranica*. Vol. 139.
- Rechinger, K.H. 1986. *Cousinia* - Morphology, Taxonomy, Distribution and Phytogeographical Implications. *Proceedings of the Royal Society of Edinburgh Section B-Biological Sciences.*, 89: 45-58.
- Saber, A., F. Attar and S. Djavadi. 2009. Studies of Pollen Grains in the Sect. *Stenocephalae* (*Cousinia* Cass.-Asteraceae) in Iran. *Iran. J. Bot.*, 15: 114-128.
- Schtepa, I. 1966. On the problem of the affinity between the genera *Arctium* L. & *Cousinia* Cass. of the family Compositae. The importance of palynological analysis for the stratigraphy and nuclear and chloroplast DNA analysis. *Collectanea Botanica.*, 26: 101-118.
- Schtepa, I. 1976. Palynological investigation of *Cousinia* sect. *Eriocousinia* (Compositae). In 'Proceedings of the 4th International Palynological Conference', pp. 31-33.
- Sennikov, A.N. 2010. A revision of *Cousinia* sections Alpinae (syn. Carduncellus), Subappendiculatae and Tianschanicae (Asteraceae) in the Kirghizian Tian-Shan and the neighbouring territories. *Phytotaxa.*, 5: 1-30.
- Sennikov, A.N. 2011. *Cousinia hystri-cocephala* (section Tianschanicae, Asteraceae), a new species from Central Asia. *Phytotaxa.*, 25: 23-30.

- Sheidai, M., K. Mehdigholi, A. Ghahreman and F. Attar. 2006. Cytogenetic study of the genus *Cousinia* (Asteraceae, section Serratuloideae) in Iran. *Genet. Mol. Biol.*, 29: 117-121.
- Sheidai, M., Z. Ahmad-Khanbeygi and F. Attar. 2012. New chromosome number reports in *Cousinia* species (Compositae). *Cytologia.*, 77: 11-16.
- Stearn, W.T. 1983. Botanical Latin. David & Charles, London, pp. 566
- Susanna, A., N. Garcia-Jacas. R. Vilatersana, T. Garnatje, J. Valles and S.M. Ghaffari. 2003. New chromosome counts in the genus *Cousinia* and the related genus *Schmalhausenia* (Asteraceae, Cardueae). *Bot. J. Linn. Soc.*, 143: 411-418.
- Susanna, A. and N. Garcia-Jacas. 2007. Tribe Cardueae. *The families and genera of vascular plants*. 8: 123-147.
- Tamanian, K. 1999. Synopsis of the Caucasian representatives of genus *Cousinia* (Asteraceae, Cardueae). *Feddes Repert.*, 110: 73-79.
- Tareen, R.B., T. Bibi, M.A. Khan, M. Ahmad, M. Zafar and S. Hina. 2010. Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan, Pakistan. *Pak. J. Bot.*, 42: 1465-1485.
- Tscherneva, O. and A. Vvedensky. 1962. *Cousinia* Cass. *Flora of the USSR*. 27: 108-357.
- Tscherneva, O. 1988. Konspekt sistemy roda *Cousinia* (Asteraceae) flory SSSR. *A synopsis of the Cousinia (Asteraceae) genus system in the flora of the USSR.* *Bot. Zhurn.*, 73: 870-876.
- Tugay, O. 2012. *Cousinia*. In: (Eds.): Güner, A., S. Aslan, T. Ekim, M. Vural & M.T. Babaç. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul.
- Tutin, T.G., V.H. Heywood, N.A. Burges and D. Valentine. 1976. *Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae)*. 4. Cambridge University Press.
- Ulukoş, D. and O. Tugay. 2019. Micromorphological, Palynological and Anatomical Properties of Endemic *Cousinia iconica* Hub.-Mor. (sect. *Cousinia* /Asteraceae). *Bağbahçe Bilim Dergisi.*, 6(2): 58.
- Ulukoş, D. 2019. Anatomical, Palynological and Achene Micromorphological Characteristics of *Cousinia boissieri* Buhse (Sect. *Leiocaules*, Asteraceae) Growing in Turkey. *Biodicon.*, 12 (2): 119-125
- Wodehouse, R.R. 1935. Pollen grains. McGraw-Hill, New York, pp. 574 pp.
- Wortley, A.H., V.A. Funk, H. Robinson, J.J. Skvarla and S. Blackmore. 2007. A search for pollen morphological synapomorphies to classify rogue genera in Compositae (Asteraceae). *Rev. Palaeobot. Palynol.*, 146: 169-181.
- Wortley, A.H., S. Blackmore, W.F. Chissoe and J.J. Skvarla. 2012. Recent advances in Compositae (Asteraceae) palynology, with emphasis on previously unstudied and unplaced taxa. *Grana.*, 51: 158-179.

(Received for publication 15 August 2018)