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Anatomical, Palynological and Achene Micromorphological Characteristics of *Cousinia boissieri* Buhse (Sect. *Leiocaules*, Asteraceae) Growing in Turkey

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Abstract

Cousinia boissieri Buhse, belonging to section *Leiocaules* Bunge, is biennial or perennial herbaceous usually growing dry, stony slopes and steppe in the Iran, Turkey and Iraq. In present study, the micromorphological, anatomical and palynological features of this species have been examined. In addition, distribution map and coloured photographs of *Cousinia boissieri* are presented. Number of cortex cells, number of vascular bundles in midrib and midrib shape are found to be significant characters. Pollen grains are tricolporate aperture and prolate-spheroidal shape. Scanning Electron Microscopy investigations showed that exine sculpturing pattern of pollen grains is verrucate. Pollen features can be useful character for separating to species belonging to *Cousinia* genus. Morever in this study first time of a species belonging to *Cousinia* genus is examined achenes micromorphological and surface ornamentation of achene coat is found as reticulate. This micromorphologic features of achene can be used for taxonomic purposes.

Key words: Asteraceae, Anatomy, *Cousinia boissieri*, Micromorphology, Turkey

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Türkiye de Yetişen *Cousinia boissieri* türünün Anatomik, Palinolojik ve Mikromorfolojik Özellikleri

Özet

Seksiyon *Leiocaules* Bunge içinde yer alan *C. boissieri* Buhse türü iki yıllık veya çok yıllık otsudurlar, genellikle İran, Türkiye ve Irak'ın kuru, taşlık yamaçlar, ve bozkır alanlarında yetişir. Bu çalışmada, *C. boissieri* türünün mikromorfolojik, anatomik ve palinolojik özellikleri incelenmiştir. Ayrıca, renkli fotoğraf ve dağılış haritası sunulmuştur. Korteks hücrelerinin saysısı, orta damardaki iletim demetlerinin sayısı ve orta damar şeklinin önemli bir sistematik karakter olduğu bulunmuştur. Polen tanelerinin apertür tipi trikolporat ve polen şekli prolat-siferoidaldir. SEM mikroskop incelemeleri gösterdiki polen ekzin yüzey süsleri verrukattır. Ayrıca bu çalışmada ilk kez *Cousinia* cinsine ait olan bir türün akenleri mikromorfolojik yönden incelenmiş ve aken yüzey süsleri ağsı olarak tespit edilmiştir. Akenin bu mikromorfolojik özelliği taksonomik amaçlar için kullanılabilir.

Anahtar kelimeler: Asteraceae, Anatomi, *Cousinia boissieri*, Mikromorfoloji, Türkiye

1. Introduction

Cousinia Cass. (Asteraceae, Cardueae) is one of the largest genus in the Asteraceae and comprises approximately 700 species [1; 2; 3; 4].

Most of its species are centred in the mountainous region covering from Turkestan westwards across Afghanistan and Iran to Turkey [1; 5; 6; 7]. It is exceptional in containing so many species in a comparatively small area [8].

The genus *Cousinia* [9] has been investigated from different point such as taxonomical [2; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20], anatomical [21], palynological [12; 22; 23; 24; 25; 26], phylogenetical [6; 27; 28; 29] and cytogenetical [29; 30; 31; 32; 33; 34]

Some of *Cousinia* species are used as dropsy, hematuria, vomiting diuretic, antiseptic, chronic diarrhea and dysentery using both roots and Fresh juice. Morever, it is also used liver complaints and asthma [35].

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According to [36], *Cousinia* has six sections and 38 taxa in Turkey, of which 26 are endemic. *Cousinia* sect. *Leiocaules* is totally represented so far by 22 taxa in SSSR, Turkey and Iran [7]. Sect. *Leiocaules* is represented by only one species in Turkey (*C. boissieri* Buhse) [36].

Cousinia sect. *Leiocaules* Bunge is biennial and perennial herbs, growing mainly on dry stony slopes and steppe [36]. Morphologically, sect. *Leiocaules* is characterized by the presence of leaves leathery, stem leaves is not decurrent, capitula solitary, involucre ovoid-globose, phyllaries 25-110, acuminate from an enlarged adpressed base into a short or elongated erect or reflexed spine. Flowers yellow, pink or white, achenes obovate, ribbed lengthwise, denticulate at apex [36].

There is no knowledge about anatomy of *Cousinia. boissieri* Buhse. This study aims to present as a first detailed data of the anatomical, micromorphological and palynological characteristics of *C. boissieri* and to determine if these data will contribute to taxonomy of the genus.

2. Materials and methods

2.1 Plant Materials

Cousinia boissieri were gathered from Kurubaş pass in Van province, during the flower time. Collected specimens have been kept in Selcuk University Herbarium (KNYA). The herbarium samples were examined using Flora of Turkey under the stereo-binocular microscope.

2.2. Palynological and Micromorphological Methods

For palynological examination, pollen materials was taken from herbarium samples. Wodehouse's technique was used to prepare pollen slides. 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. The pollen micromorphology of *C. boissieri* was examined using scanning electron microscopy (SEM) techniques. SEM micrographs were used to determine exine sculpturing of the pollen. For pollen morphology, [37] terminology was followed.

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

2.3. Anatomical Methods

Concerning anatomical studies, collected samples in field was stored in 70 % ethanol. The paraffin method was used for transverse sections of examined samples. The paraffin wax method was worked for preparing cross-sections of middle parts of mature stems, leaf blades and midrib. The samples were sectioned between 5 and 12 µm thickness with a Leica RM2125RT rotary microtome. All sections were stained with safranin-fast green and then mounted with Entellan [38]. Measurements and photos were made using a Leica DM1000 binocular ligth microscope with a Leica DFC280 camera. Anatomical studies were carried out using specimens from Van, Kurubaş pass 1960 m, 24 June 2013, O.Tugay 8.439 (KNYA).

3. Results

Cousinia boissieri [39:42] (Figs. 1–7)
Type:—N.W. Iran in montosis apricis prope Tabris prov. Aderbidjan Persiae, 25 vii 1847, Buhse 646 (holo. G!).

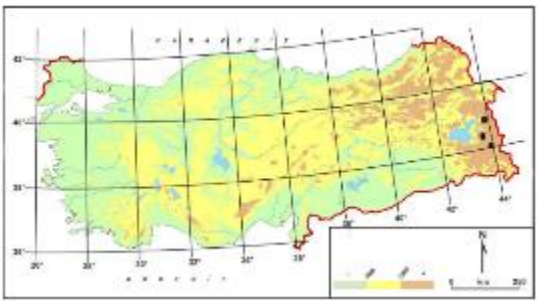

Figure 1. Distribution map of *C. boissieri* (■) distributed Turkey

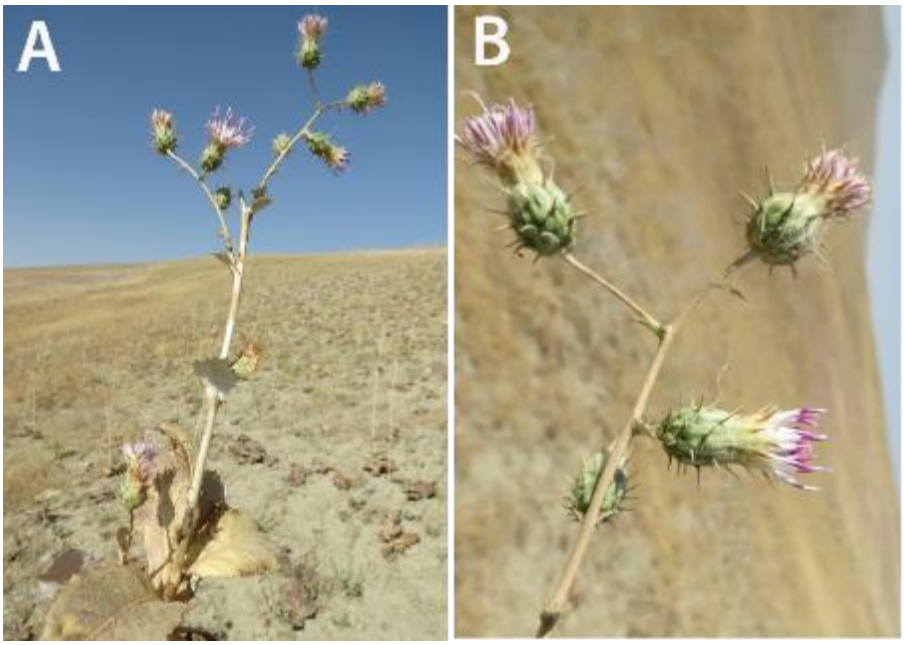

Figure 2. A−B. General view and flowers: *C. boissieri*

3.1. Pollen characteristics

Pollen grains of *C. boissieri* is tricolporate, radially symmetrical, isopolar and pollen shape is prolate-spheroidal. Polar axis (P) and equatorial axis (E) are 35.20 ± 1.25 (mean ± standard deviation) μm and 30.00 ± 0.82 μm, respectively. The ratio of P/E of pollen grains is 1.21. Colpus length is 31.22 ± 1.53 μm and colpus width is 3.52 ± 0.50 μm. The exine thickness is 1.77 ± 0.27 μm and the intine thickness is 0.61 ± 0.09 μm. Exine ornamentation is densely verrucose (Fig. 3A–B).

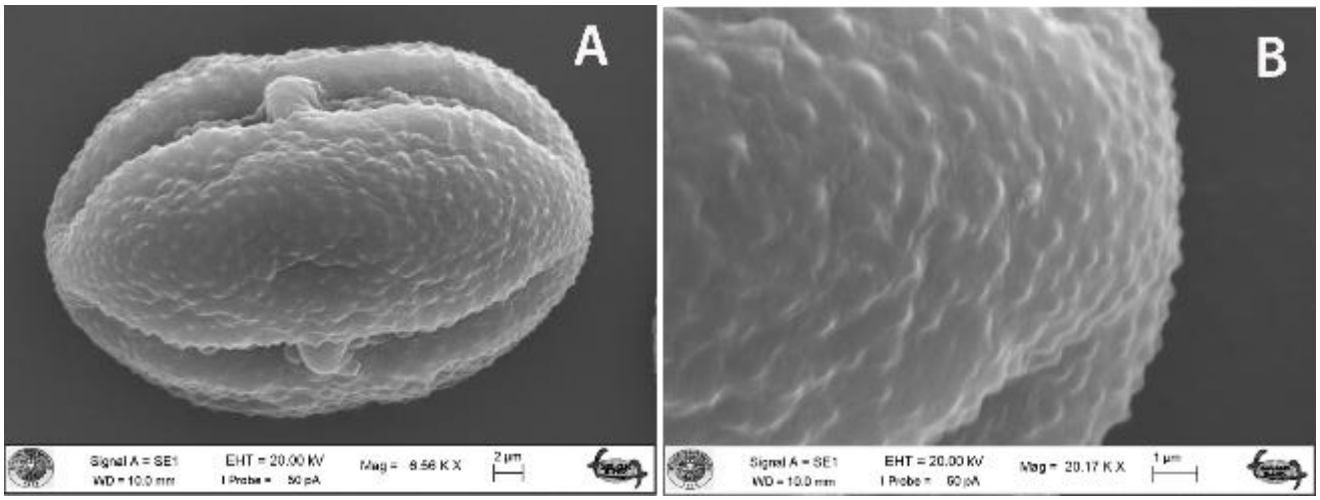

Figure 3. A−B. SEM micrographs of the pollen grains A, B equatorial view and exine sculpturing of *C. boissieri* (O.Tugay 8568 & D.Ulukuş)

3.2 Achene characteristics

In this study, achens features of *C. boissieri* was investigated for the first time. It was observed that *C. boissierii* has obovate achene shape with light brown colour. Achenes are 4.85–3.76 mm length × 2.22-1.37 mm width and the range of the L/W ratio 2.36 ± 0.42 mm. *Cousinia boissierii* has longitudinally clearly striate. Surface ornamentation of achene coat is reticulate (Fig. 4A–B).

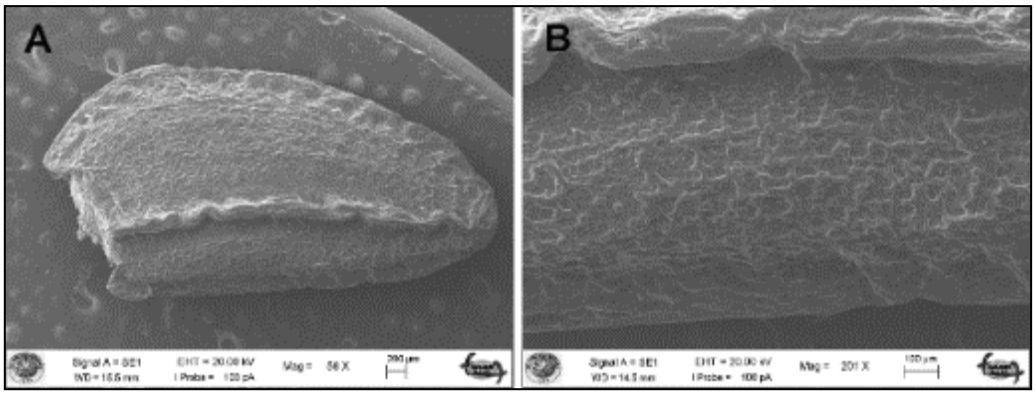

Figure 5. SEM photographs of achen *C. boissieri* A−B: general view and sculpturing

3.3. Anatomical characteristics

Stem

In transverse sections taken from the stem of C. *boissieri* shows that stem is nearly rounded, epidermis is 1 layered, contains oval or rectangular cells and covered by thick cuticle. The cortex is composed of 10–12 layers of oval, cylindrical, or rectangular parenchymatous cells. The phloem is encircled by sclerenchymatic cells. Sclrenchymatous fibres above outer and inner phloem 60–90 µm and 20–55 µm in thickness respectively. Vascular bundles is numerous and elliptic. The pith region comprises orbicular parenchymatous cells (Fig. 5A–B).

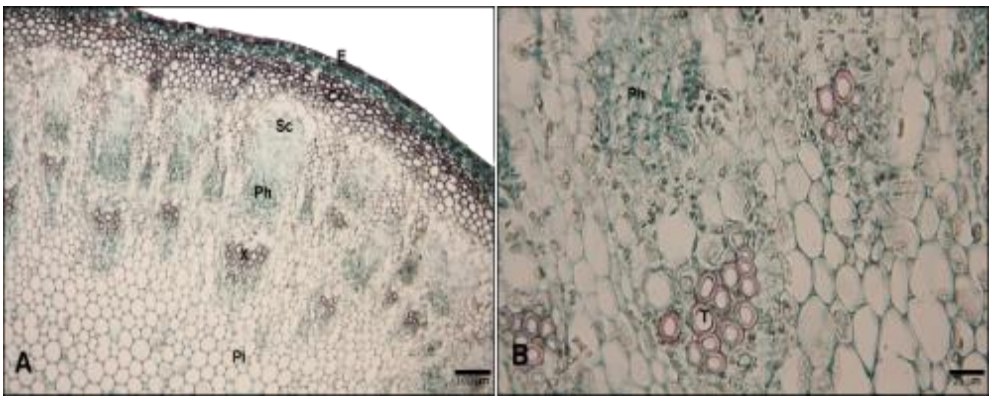

Figure 5. A−B. Transverse section of the stem: Co, cortex; E, epidermis; Pi, pith region; Ph, phloem; Sc, sclerenchyma; **T,** trachea**; X**, xylem;

Leaf

In transverse section the lamina shows the upper and the lower epidermis covered with a thin cuticle layer. Both epidermises contain uniseriate oval and rectangular cells. The mesophyll is consist of 3–4 layer of elongated palisade paranchyma cells. Spongy parenchyma cells are 1–2 layers lower side. Spongiose cells are irregular, compact, cubic or spherical (Fig. 6A–B).

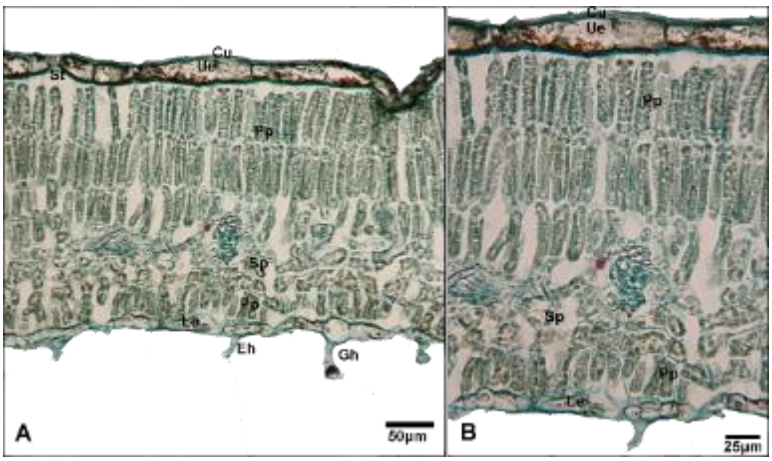

Figure 6. A−B. Transverse section of the lamina: Cu, cuticle; Eh, eglandular hair; Gh, glandular hair; Le, lower epidermis; **Pp**, palisade parenchyma; **Sp** Spongy parenchyma; **Ue**, upper epidermis

Midrib

The midrib shape is nearly rectangular. Totally, it has seven 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 300 µm and 120 µm respectively (Fig. 7A–B).

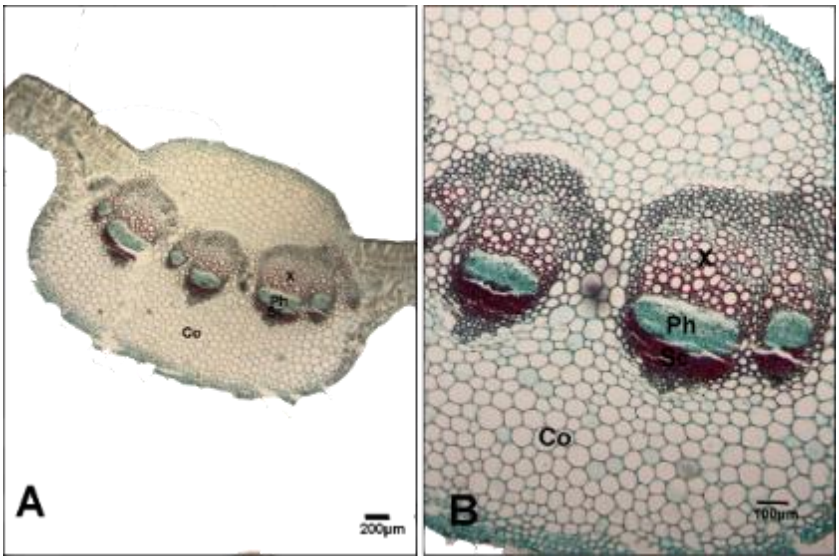

Figure 7. A−B. Transverse section of the midrib: Co, collenchyma; Sc, sclerenchyma; Ph, phloem; X, xylem

4. Conclusions and discussion

In this study, anatomical, micromorphological and palynological characteristics of *C. boissieri* belonging section *Leiocaules* distributing in Turkey are presented.

Morphological characteristics such as size of phyllaries, the number of phyllaries, bristles of receptacle and bristles of pappus have taxonomically significant to identify *C. boissieri.* The species morphologically differs from the other members of *Cousinia* with regard to its barbelluate bristles of receptacle and ivory white stems [36].

Up to now, there is one study related to anatomy of *Cousinia*. In this study, [21] made anatomical study relating to 14 species of *Cousinia* section *Serratuloideae* Bunge. 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 midrip shape. These plants have into two types; the leathery leaf type has one or two layers palisade parenchyma in lower surface of its blade and herbaceous leaf type has one palisade parenchyma on upper surface. In addition, midrip shape some of species are eliptic and nearly orbicular. However, our results showed that both upper and lower surface have palisade paranchyma layer. Besides, midrip shape is nearly triangular. Therefore, these characters used for leaves are important to separate studied species.

Concerning palynological studies, [12] defined two pollens type as *Arctium* and *Cousinia*. [25] investigated pollen morphological features 25 species belonging to sect. *Stenocephalae* from genus *Cousinia* and defined prolate, isopolar, tricolporate of pollen grains. Our finding showed that *C. boissieri* is prolate-spheroidal. [40] reported that pollen grains are verrucate pattern of exine. In present study is also found that pattern of exine is verrucose.

Recently, [26] have studied on pollens of *C. boissieri*. Our result do not mostly consistent with their finding. Because they reported that the of exine are 6.05 µm in examined fresh pollen and 6.11 µm respectively. And also, the intine thinckness is 1.24 µm. In our study, thickness of exine is 1.77 ± 0.27 µm and the intine is 0.61 ± 0.09 µm thick. On the other hand, they defined that ornamentation is reticulate. However, According to SEM microscopy, our finding demonstrated that exine sculpturing pattern is densely verrucate (Fig. 3A–B). In two studies, only pollen shape resembles.

In one study made about achene micromorhology of asteraceae, [41] recognised that achene coat ornamentation genus of *Cota* has reticulate-striate. In this work, achenes also was found reticulate-striate.

In achene morphology studies belonging to *Cousinia* genus. [42] reported that achenes are 5–6.5 mm long, winged-angulate, oblong-obpyramidal. [43] determined that achenes are ca. 5 mm long, ca. 2 mm wide, compressed, narrowly ovate, brown, irregularly darkly spotted, longitudinally obscurely striated. According to in this study, achenes are obovate, light brown colour ca. 4.85 mm long, 2.22 mm wide, longitudinally striated. Surface ornamentation of achene coat is reticulate.

Anatomical and achene micromorphological study on the *Cousinia boissieri* is given for the first time. So we argue for further anatomic and palynologic investigations of other *Cousinia* taxa, hoping that they could serve to their taxonomic status.

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