www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1257770
165/2 (2023) 140-151

Comparative anatomy and achene micromorphology assessment of two *Cousinia* Cass. (*Asteraceae*) species in view of taxonomy

Deniz ULUKUŞ [*1], Osman TUGAY [2]
ORCID: 0000-0002-9627-5492**;** 0000-0003-3980-7648

[†1] Department of Biotechnology, Faculty of Sciences, Selçuk University, Konya, Turkey
[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, Selçuk University, Konya, Turkey

Abstract

Cousinia is a member of the monophyletic tribe Cardueae, which is traditionally subdivided into four taxonomic groups; however, the rank and boundaries of these taxa are highly controversial. The purpose of this study was to contribute to the genus taxonomy by determining the comparative anatomical and achene micromorphological features of *C. aintabensis* Boiss. & Hausskn. and C. *birecikensis* Hub.-Mor. that are morphologically similar to each other. In anatomical studies, stem, leaf, and midrib features were determined. Paraffin embedding, microtome sectioning, and safranin-fast green staining were used for the samples. The obtained sections were photographed, and the leaf, stem, and midrib characters were measured. The importance of anatomical characters between species was determined by applying Independent sample T-test test to quantitative characters. In addition, box plot and heatmap analyses of the studied species were carried out. Our results showed that the stem epidermis, inner sclerenchyma and phloem layer; For leaves; lower and upper epidermis mesophyll thickness, palisade parenchyma; In midrib; number of vascular bundles, collenchyma, and phloem are important characters that can be used in the differentiation of species. SEM microscope was used for achene micromorphological examinations. *C. aintabensis* achene surface ornamentation is identified as striate-retipylate. It was observed that *C. birecikensis* had striate and irregular reticulate-faveolate surface ornamentation.

Keywords: *Asteraceae*, Anatomy, *Cousinia*, micromorphology, Türkiye

---------- * ----------

Taksonomik açıdan iki *Cousinia* türünün anatomik ve aken mikromorfolojik karşılaştırlması ve değerlendirilmesi

Özet

Cousinia, geleneksel olarak dört taksonomik gruba ayrılan monofiletik Cardueae tribusunun bir üyesidir; ancak bu taksonların sıralaması ve sınırları oldukça tartışmalıdır. Bu çalışma morfolojik olarak birbirine benzeyen *C. aintabensis* Boiss. & Hausskn. ve *C. birecikensis* Hub.-Mor. türlerinin karşılaştırmalı anatomik ve aken mikromorfolojik özellikleri belirlenerek cinsin taksonomisine katkı sağlamak amacıyla gerçekleştirilmiştir. Anatomik çalışmalarda gövde, yaprak ve orta damar özellikleri tespit edilmiştir. Örnekler için parafine gömme, mikrotomla kesit alma ve safranin-fast green boyama yöntemi uygulanmıştır. Elde edilen kesitler fotoğraflanıp yaprak, gövde ve orta damar karakterlerinin ölçümleri yapılmıştır. Nicel karakterlere bağımsız örneklem T-testi uygulanarak türler arasındaki anatomik karakterlerin önemi belirlenmiştir. Ayrıca çalışılan türler ile ilgili box plot ve heat map analizleri gerçekleştirilmiştir. Sonuçlarımız gövde epidermis, içteki sklerankima ve floem tabakası, yaprak için alt ve üst epidermis, mezofil kalınlığı, palizad parenkiması, orta damarda ise iletim demeti sayısı, kollenkima ve floem özellikleri bakımından türlerin ayrımında kullanılabilecek önemli karakterler olduğu belirlenmiştir. Aken mikromorfolojik incelemeler için SEM mikroskobu kullanılmış. *C. aintabensis* türü aken yüzeyi süsü striat-retipilat olarak belirlenmiştir. *C. birecikensis* türünde ise striat ve düzensiz retikulat-faveolat yüzey süsüne sahip olduğu görülmüştür.

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

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: + 90332)2410041; Fax.: +290332)2410041; E-mail: dulukus@selcuk.edu.tr
 Received: 20.03.2023; Published: 15.08.2023 BioDiCon. 1089-280323

1. Introduction

The genus *Cousinia* (*Asteraceae*, *Cardueae*) is the third largest genus of the *Asteraceae* family after *Senecio* and *Vernonia* and the largest genus of the Cardueae tribe [1; 2; 3; 4] and is the largest of the flowering plants. It is among the 50 genera (5). There are roughly 700 species in this genus, which is found throughout Central and Western Asia. *Cousinia* has a high percentage of endemic species and is a typical genus for the Irano-Turanian region [6].

The genus *Cousinia* is part of the monophyletic tribus *Cardueae*, which is usually divided into four taxonomic groups. However, the distinction within the tribus is quite problematic [7; 4].

The *Arctium-Cousinia* complex and the genus *Arctium* L. are both included in the non-monophyletic genus *Cousinia* [8; 9].

The genus *Cousinia* was described by Huber-Morath in the Flora of Turkey. In Turkey, this genus is represented by a total of 38 species in 6 sections, and 26 of them are endemic. According to the list of plants of Turkey, there are 39 species in our country [10]. With the newly described species (*Cousinia agridaghensis* Tugay, Ertuğrul & Ulukuş), the number of taxa in the genus *Cousinia* has reached 40 in Turkey [11].

Due to the wide morphological variability in the genus, the taxonomy of *Cousinia* is complex and controversial [12]. In their anatomical study of 14 species belonging to the *Cousinia* sect. *Serratuloideae*, [13] stated that these species can be divided taxonomically according to the midrib and leaf structure. Recent anatomical and achene micromorphological studies on the genus [14, 15; 16; 17] have emphasized the importance of midripetal anatomy for the genus *Cousinia*.

The genus *Cousinia* is divided into 70 sections in the world, the largest of which is the *Cynaroideae* Bunge section with 89 species [1]. There are a total of 8 species, 4 of which are endemic, in the *Cousinia* genus sect. *Cynaroideae* in Turkey [18].

In systematic study of the section *Cynaroideae*, It has also been done in molecular and palynological studies. [19] aimed to use pollen characteristics in determining and defining species boundaries. [20] investigated the molecular phylogenetic relationships of 50 *Cousinia* species belonging to the section *Cynaroideae* distributed in Iran. There is only one study of *Cynaroideae* anatomy, and [21] studied the leaf, stem, and root anatomy of *C. mobayenii*.

Even after molecular studies [8, 21, 9, 12], it is still not clear how to identify and define species, how to group them into sections, and how they are related to each other.

In this study, it was aimed to determine these characteristics of *C. aintabensis and C. birecikensis*, whose anatomical and achene micromorphological features have not been determined until now, and to contribute to the use of these characters in the taxonomy of the genus.

2. Materials and methods

The materials utilized in this investigation were photographed and gathered from their distribution locations. For anatomical studies, live tissue was stored in 70% ethanol. Using the paraffin technique, we slice cross-sections of the stems and leaves. Using a Leica RM2125RT rotary microtome, sections between 5 and 12 µm thick were cut from paraffin wax-embedded materials. The safranin-fast green stain was used on all parts before they were mounted in Entellan [22]. The measurements and images were captured using a Leica DM1000 binocular light microscope and a Leica DFC280 camera.

At least 30 cell measurements were recorded, and the minimum, mean, maximum, and standard deviation were determined, so that stem, leaf and midrib anatomy could be compared based on cell size (Table 1). R 4.1.2 software was utilized for all statistical tests [23]. The stem, leaf and midrib features of each species were measured quantitatively, and box plots were provided (Figure 7,8,9). The heat map was created by using the cluster method (R 4.1.2 with library pheatmap) of the anatomical features of the species (Figure a). Independent sample T-test were used to assess the statistical significance of quantitative stem, midrib and leaf features (R 4.1.2). P-values <0.05 were regarded statistically significant (Table 2).

The texture of the achene coat was analyzed using scanning electron micrographs. In order to explain the scanning electron microscopy (SEM) features of the achene coat, we used the terminology of [24].

3. Results

Along with the anatomical features of the species, micromorphological studies of the achenes and photos of the species features were also given (Fig. 1).

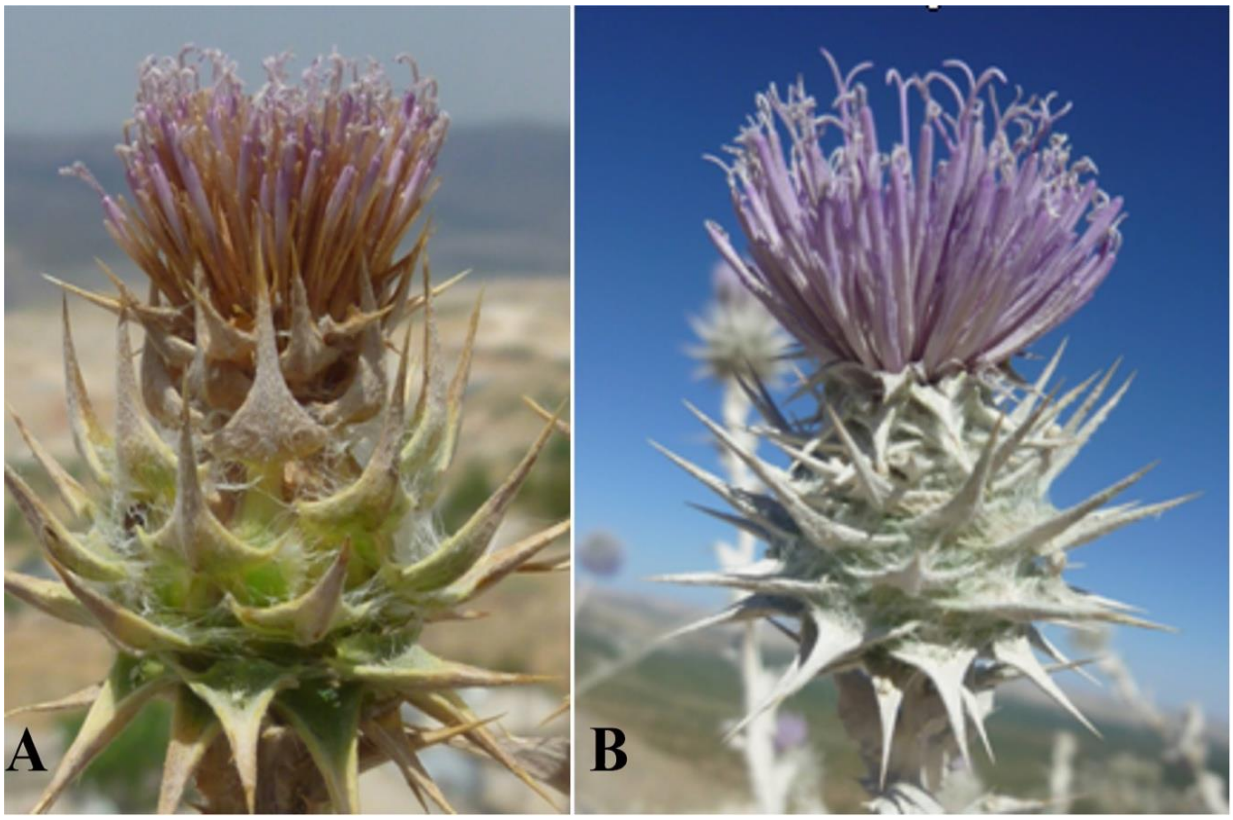

Figure 1. Photographs of studied *Cousinia aintabensis* (A) and *C. birecikensis* (B)

3.1. Anatomical properties

Stem
C. aintabensis

The *C. aintabensis* stem has a roughly round cross-section. The epidermis includes a single layer of cuticle-covered, oval or rectangular-shaped cells. Ovate, rectangular, and orbicular parenchymatous cells make up the cortex (9,88-74,07 µm), which has 8-11 layers. Sclerenchymatous cells surround the phloem. The thickness of sclerenchymatous fibers is between 26,08 and 156,50 above the external phloem and between 17,39 and 191,30 µm above the internal phloem. The cambium is not easily identifiable. Phloem dimensions are between 27,32 and 67,08 µm. It's estimated that the xylem ranges in size from 55,07 to 243,40 µm. There are many elliptical vascular bundles. The pith is made up of large parenchymatous cells that can be hexagonal, polygonal, or round (Table 1, Fig. 2A–B).

C. birecikensis

The *C. birecikensis* stem has a roughly round cross-section. The epidermis includes a single layer of cuticle-covered, oval or rectangular-shaped cells. Ovate, rectangular, and orbicular parenchymatous cells make up the cortex (14,46-35,74 µm), which has 6-9 layers. Sclerenchymatous cells surround the phloem. The thickness of sclerenchymatous fibers is between 65,18 and 133,30 µm above the external phloem and between 23,70 and 154 µm above the internal phloem. The cambium is not easily identifiable. Phloem dimensions are between 10,63 and 53,19 µm. It's estimated that the xylem ranges in size from 56,29 to 257,70 µm. There are many elliptical vascular bundles. The pith is made up of large parenchymatous cells that can be hexagonal, polygonal, or round (Table 1, Fig. 2C–D).

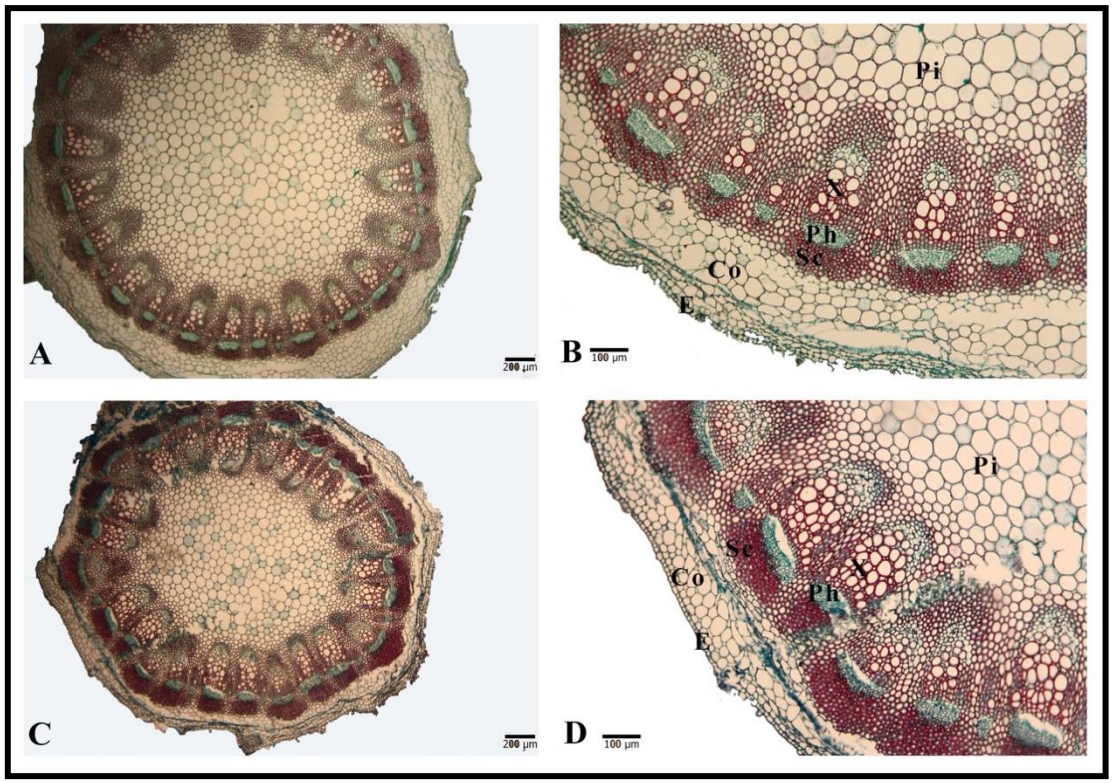

Figure 2. Transverse section of the stem; (A, B) *Cousinia aintabensis*, (C, D) *C. birecikensis*. (E epidermis, Co cortex, Sc sclerenchyma, Ph phloem, X xylem, Pi pith region)

Leaf

C. aintabensis

Lamina transverse sections of *C. aintabensis* reveal that the upper and lower epidermis are coated with a thin cuticle layer and eglandular hairs. Each epidermis is made up of a mix of uniseriate oval and rectangular cells. The mesophyll (178–205,60 µm) is composed of elongated palisade and spongy parenchyma cells. Palisade parenchyma is 1–2-rowed under the upper epidermis and 1-rowed under the lower epidermis. Spongiose cells can be round, irregular, compact, or cubic in shape (Table 1, Fig. 3A–B).

C. birecikensis

Transverse sections of the lamina of *C. birecikensis* show that the upper and lower epidermises have a thin layer of cuticle and eglandular hairs. Each epidermis is made up of a mix of uniseriate oval and rectangular cells. The mesophyll (227,50–312,80 µm) is composed of elongated palisade parenchyma cells. Palisade parenchyma is 1–2-rowed under the upper epidermis and 1-2-rowed under the lower epidermis. Spongiose cells can be round, irregular, compact, or cubic in shape (Table 1, Fig. 3C–D).

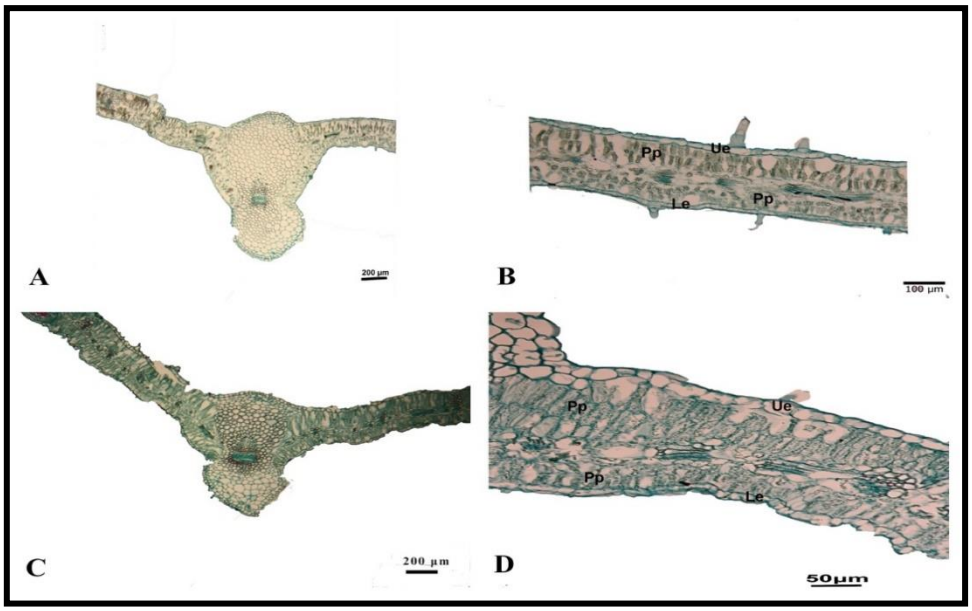

Figure 3. Transverse section of the lamina; (A, B) *Cousinia aintabensis*, (C, D) *C. birecikensis*. (Le lower epidermis, Pp palisade parenchyma, Ue upper epidermis).

Midrib

C. aintabensis

In the cross-sections of the leaf, there are six vascular bundles and a roughly semicircular midrib. One major vascular bundle is located in the middle, and it is encased by a parenchymatic bundle sheath on all sides. A tangential pattern is formed by the collenchyma beneath the lower epidermis. The thickness of the collenchyma beneath the lower and upper epidermis is 282,30-1011 µm and 382,30-900 µm, respectively (Table 1, Fig. 4A–B).

C. birecikensis

In the cross-sections of the leaf, There are nine vascular bundles and a roughly semicircular midrib. One major vascular bundle is located in the middle, and it is encased by a parenchymatic bundle sheath on all sides. A tangential pattern is formed by the collenchyma beneath the lower epidermis. The thickness of the collenchyma beneath the lower and upper epidermis is 38,29-868 µm and 76,59-578,70 µm, respectively (Table 1, Fig. 4C–D).

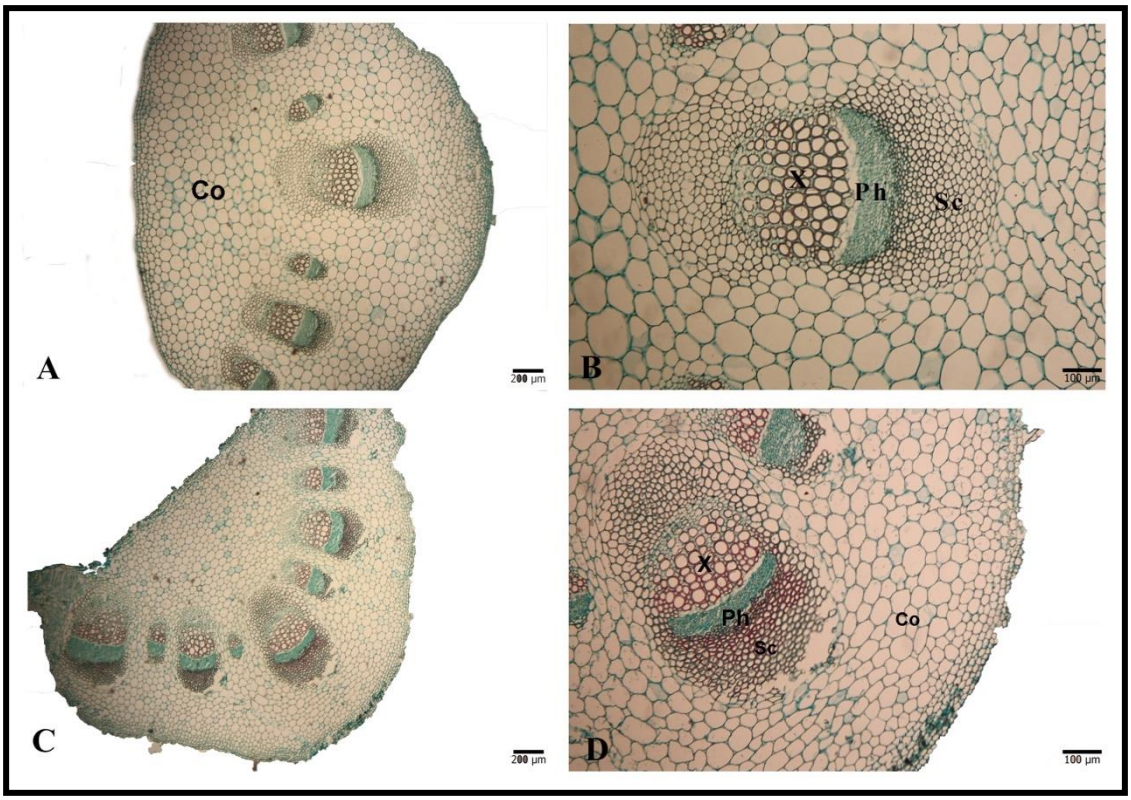

Figure 4. Transverse section of the midrib; (A, B) *Cousinia aintabensis*, (C, D) *C. birecikensis*. (Co collenchyma, Ph phloem, Sc sclerenchyma, X xylem).

TABLE 1. Comparative anatomy of the, stem, leaves and midrip *C. aintabensis* and *C. birecikensis.* Abbreviations: Mean: Average, SD: Standart deviation, Min: Minimum, Max: Maximum, µm: Micrometer

		C. aintabensis				*C. birecikensis*			
		Width (µm)		Length (µm)		Width (µm)		Length (µm)	
		min-max	mean± SD	min-max	mean± SD	min-max	mean ± SD	min-max	mean ± SD
Stem	Epidermis cell	7,41 - 28,39	15,76 ± 6,28	1,23 - 28,38	14,40± 6,61	4,79 - 23,93	9,48± 4,09	4,79 -12,23	7,96 ± 1,89
	Cortex cell	9,88 - 74,07	36,11 ± 18,10			14,46 - 35,74	24,68 ± 5,32		
	Outer sclerenchyma layer	26,08 - 156,50	86,92 ± 34,24			65,18 - 133,30	101,01 ± 19,75		
	Inner sclerenchyma layer	17,39 -191,30	96,93 ± 48,14			23,70 - 154,00	73,66 ± 31,95		
	Phloem layer	27,32 - 67,08	43,66 ± 9,49			10,63 - 53,19	30,99 ± 9,27		
	Xylem layer	55,07 - 243,40	150,41 ± 60,53			56,29 - 257,70	169,93 ± 53,89		
	Pith	9,37 - 111,80	54,32 ± 28,84			13,82 - 75,53	53,04± 13,81		
Leaf	Upper epidermis	15,85 – 89,28	41,07 ± 16,76	12,19 - 50,00	28,78 ± 9,61	11,55 - 41,77	21,71± 6,71	8,00 - 42,66	21,39 ± 9,23
	Lower epidermis	6,50 - 18,69	11,97 ± 3,76	7,32 - 16,26	10,46± 2,39	8,89- 44,44	21,77 ± 7,28	8,89 - 24,00	15,46 ± 3,57
	Mesophyll	178,00 - 205,60	192,50 ± 8,59			227,50 - 312,80	268,63± 21,87		
	Palisade parenchyma	8,87 - 17,07	12,17± 2,23	13,00 - 45,52	29,64 ± 8,42	8,00 - 23,11	16,14 ± 3,55	32,88 - 82,66	49,92 ± 9,60
Midrib	Upper collenchyma	282,30 - 1011,00	751,69 ± 214,71			38,29 - 868,00	454,28 ± 242,63		
	Lower collenchyma	382,30 - 900,00	569,56 ± 144,73			76,59 - 578,70	325,48± 129,51		
	Upper sclerenchyma	35,29 - 223,50	103,90 ± 65,40			25,53 - 161,70	97,70 ± 37,68		
	Lower sclerenchyma	41,17 - 258,80	97,05 ± 76,20			25,53 - 161,70	95,99 ± 38,16		
	Phloem layer	47,05 - 105,80	41,17 - 258,80			46,80 - 106,30	72,04 ± 17,61		
	Xylem layer	94,11 - 258,80	158,79 - 59,43			46,80 - 204,20	144,92 ± 49,65		

3.2. Achene micromorphology

C. aintabensis
Achenes are broadly obovate prominent margins at the wrinkled end and clearly toothed. Their achene surface pattern is striate and retipilate (Fig. 5A–B).

C. birecikensis
Achenes are oblong-obovate with prominent margins at the wrinkled end and are not clearly toothed. Their achene surface pattern is striate and irregularly reticulate-faveolate (Fig. 5C–D).

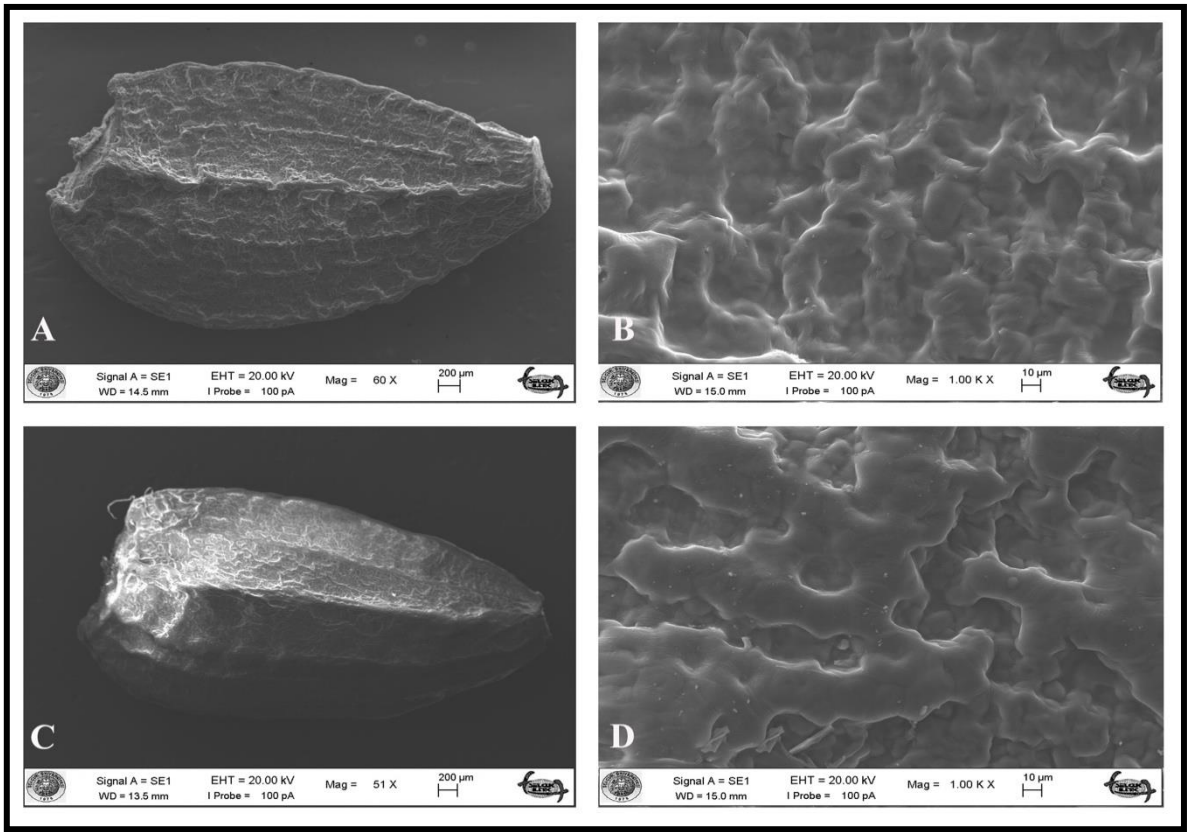

Figure 5. SEM micrographs of achenes of *Cousinia* species; (A, B), *Cousinia aintabensis*, (C, D) *C. birecikensis.*

3.3. Statistical analysis

According to the heatmap analyses made with the anatomical features of the stem, leaf, and midrib. *C. birecikensis* is clustered in terms of features such as leaf lower epirdemis, palisade parenchyma, and mesophyll thickness (Figure 6).

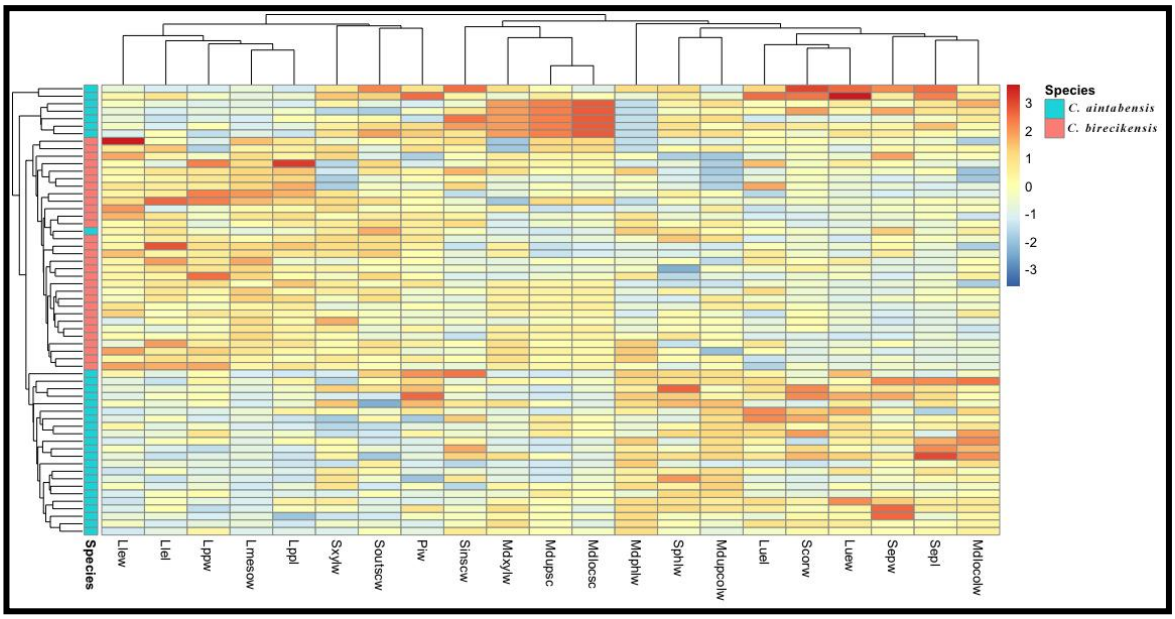

Figure 6. Heatmap for examined *Cousinia* species

Independent sample T-test show that stem epidermal cell length and width, cortex cell width, inner schylerenchyma width, and phloem layer width are all substantially different between *C. aintabensis* and *C. birecikensis* (Table 2, P<0.05). All of the leaf features used in this study were shown to be significant for the *C.aintabensis* and C. *birecikensis* (Table 2, P<0.05). *C. aintabensis* and *C. birecikensis* were significantly different from each other in terms of the midrib upper and lower collenchyma width, and phloem width (Table 2, P<0.05).

Table 2. Independent sample T-test based on the anatomical characters of the studied species

	Characteristics	*C. aintabensis-C. birecikensis*
Stem	Sepw	P< 0.05 *
	Sepl	P< 0.05 *
	Scorw	P< 0.05 *
	Soutscw	P>0.05 NS
	Sinscw	P< 0.05 *
	Sphlw	P< 0.05 *
	Sxylw	P>0.05 NS
	Piw	P>0.05 NS
Leaf	Luew	P< 0.05 *
	Luel	P< 0.05 *
	Llew	P< 0.05 *
	Llel	P< 0.05 *
	Lmesow	P< 0.05 *
	Lppw	P< 0.05 *
	Lppl	P< 0.05 *
Midrib	Mdupcolw	P< 0.05 *
	Mdlocolw	P< 0.05 *
	Mdupscw	P>0.05 NS
	Mdloscw	P>0.05 NS
	Mdphlw	P< 0.05 *
	Mdxylw	P>0.05 NS

NS = non-significant. * Significant at the level of 0.05.

Sepw: epidermis cell width of stem, Sepl: epidermis cell length of stem, Scorw: cortex cell width of stem, Soutscw: outer schylerenchyma width of stem, Sinscw: inner schylerenchyma width of stem, Sphlw: phloem width of stem, Sxylw: xylem width of stem, Piw: pith cell width of stem, Luew: upper epidermis width. Luew: upper epidermis width of leaf, Luel: upper epidermis length of leaf, Llew: lower epidermis width of leaf, Llel: lower epidermis length of leaf, Lmesow: mesophyll width, Lppw: palisade parenchyma cells width, Lppl: palisade parenchyma cells length. Mdupcolw: upper collenchyma width of midrib, Mdlocolw: lower collenchyma width of midrib, Mdupscw: upper schylerenchyma width of midrib, Mdloscw: lower schylerenchyma width of midrib, , Mphlw: phloem width of midrib, Mxylw: xylem width of midrib.

The results of the independent t-test were consistent with the results of the box plots. The mean trends of the stem (epidermis, schylerenchyma, and phloem layer), midrib (collenchyma, and phloem layer), and all leaf characters revealed significant differences between the taxonomic pairs (Table 2, Figs. 7-9).

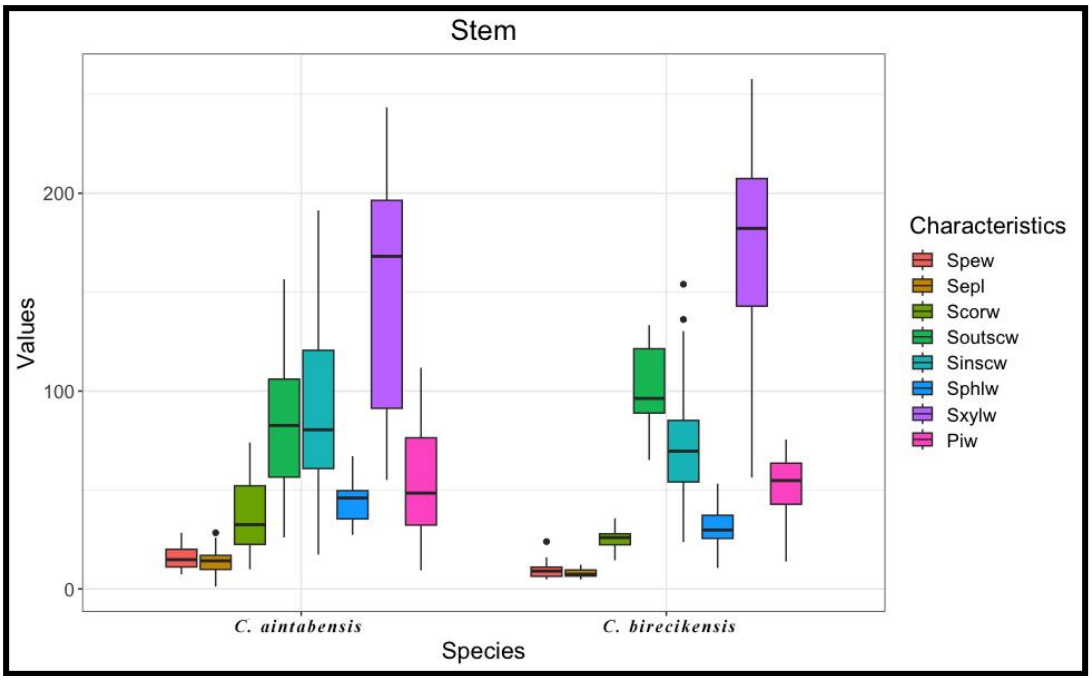

Figure 7. Box plots of examined stem characters

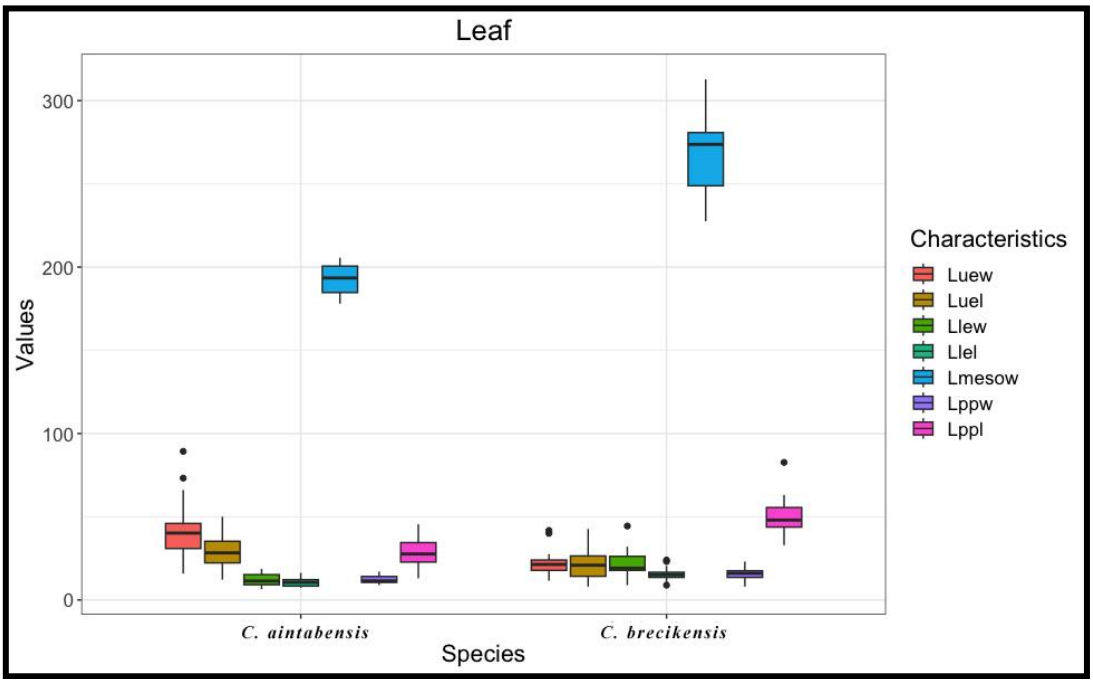

Figure 8. Box plots of examined leaf characters

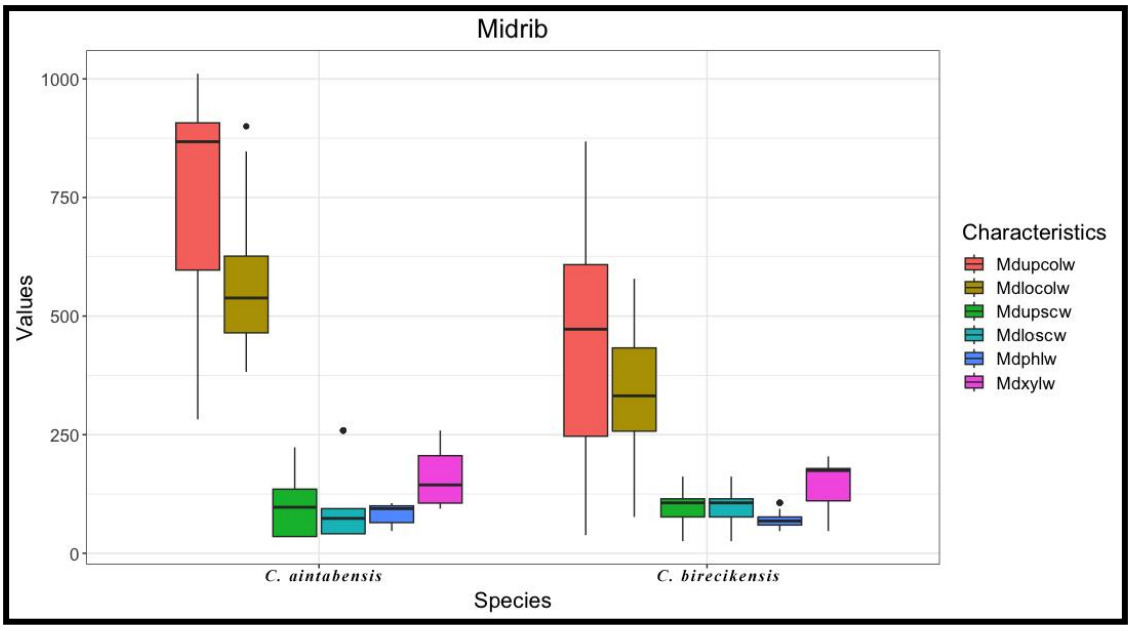

Figure 9. Box plots of examined midrib characters

4. Conclusions and Discussion

According to the stem anatomy results, the size of the epidermis cells, cortex layers, inner schylerenchyma, and phloem are taxonomically significant characters (Table 1). Moreover, it has been found that all anatomical characteristics of the leaves are significant taxonomic characters in distinguishing the investigated species (Table 1). According to midrib anatomy, the collenchyma and phloem characteristics of the examined species are important taxonomic characters (Table 1).

The findings of the study, which were obtained by anatomical, and achene micromorphological examinations, were analyzed and discussed with reference to the relevant literature. According to the literature, some anatomical studies have been done recently on the genus *Cousinia*. In these studies, [11] studied the stem and leaf midrib anatomy of *C. urumiensis* Bornm. and *C. agridaghensis*. In terms of the anatomy of the stem, leaf, and midrib, our findings partially concur with their conclusions. [14] reported that the midrib shape of *C. halysensis* Hub.-Mor. was semi-circular, and the number of vascular bundles was 10. [25] stated that *C. decolarans* Freyn & Sint. have 3 vascular bundles in the midrib. In our study, the midrib shape was also semicircular, but in the midrib, the number of vascular bundles was 6 in *C. aintabensis* and 9 in *C. birecikensis*, respectively. According to [15, 21], the mesophyll type is bifacial in leaf anatomy. In our study, we observed that all species examined have equifacial leaves. [11], [14], and [25] reported that midrib shape and the number of vascular bundles are important taxonomic characters. Our findings showed that they were consistent with theirs.

In the micromorphological examinations of achene belonging to the *Cousinia* genus, *C. iconica* Hub.-Mor. is reticulate-sitriate [15], *C. agridaghensis* and *C. urumiensis* are reticulate-faveolate [11], *C. boissieri* Buhse is reticulate [26], and *C. decolorans* is retipilate [25] (surface ornaments were encountered). According to our study, we observed that *C. aintabensis* seeds were retipilated, while *C. birecikensis* seeds were reticulate-faveolate. We predict that these variations in achene surface ornamentation will serve as a useful criterion for distinguishing species within the genus.

In this study, a total of eight stem, seven leaf, and six midrib anatomical characters were evaluated for their taxonomic significance in relation to *C. aintabensis* and *C. birecikensis*. The present study verified that comparative root and leaf anatomical traits can be used as an additional tool for correct species identification and to clarify the taxonomy of *C. aintabensis* and *C. birecikensis*. This study demonstrated that comparing stem, leaf, and midrib anatomical features can be utilized as an additional tool for accurate species identification and to clarify the taxonomy of *C. aintabensis* and *C. birecikensis*. In particular, the transverse section of the leaf displayed a remarkable amount of significant variation. Hence, the anatomical characteristics of the leaf have a greater potential for use in taxonomy than the stem or the midrib.

Acknowledgements

We would like to extend our gratitude for the financial support provided by the Scientific and Technological Research Council of Turkey (TÜBTAK-TBAG Project No. 111T364). We want to express our gratitude to the curators of the herbaria AEF, ANK, E, G, GAZI, HUB, ISTE, ISTF, K, and LE who gave us permission to examine the *Cousinia* specimens in their collections.

References

[1] Rechinger, K. H. (1986). *Cousinia*: morphology, taxonomy, distribution and phytogeographical ımplications. *Proceedings of the Royal Society of Edinburgh Section B-Biological Sciences,* 89, 45–58. doi: 10.1017/S0269727000008897

[2] Bremer, K. (1994). *Asteraceae, Cladistics and Classification*. Portland Oregan:Timber Press,

[3] Petit, D.P. (1997). Generic interrelationships of the Cardueae (Compositae): A cladistic analysis of morphological data. *Plantl Systematics and Evolution*, 207, 173-203.

[4] Susanna, A. & Garcia-Jacas, N. (2007). Tribe Cardueae. In Kubitzki, K. (Ed.), *The Families and Genera of Vascular Plants* (Vol. 8, pp. 123–146) Verlag Berlin Heidelberg: Springer.

[5] Frodin, D.G. (2004). History and concepts of big plant genera, *Taxon*, 53 (3), 753-766.

[6] Djamali, M., Baumel, A., Brewer, S., Jackson, S. T., Kadereit, J.W., Lopez-Vinyallonga, S., Mehregan, I., Shabanian, E. & Simakova, A. (2012). Ecological implications of *Cousinia* Cass. (Asteraceae) persistence through the last two glacial-interglacial cycles in the continental Middle East for the Irano-Turanian flora. *Review Palaeobotany and Palynology*, 172, 10-20.

[7] Garcia-Jacas, N., Garnatje, T., Susanna, A. & Vilatersana, R. (2002). Tribal and subtribal delimitation and phylogeny of the Cardueae (Asteraceae): A combined nuclear and chloroplast DNA analysis. *Molecular Phylogenetics and Evolution*, 22(1), 51-64.

[8] Susanna, A., Garcia-Jacas, N., Vilatersana, R., Garnatje, T., Valles, J., & Ghaffari, S. M. (2003). New chromosome counts in the genus *Cousinia* and the related genus *Schmalhausenia* (Asteraceae, Cardueae). *Botanical Journal of the Linnean Society*, 143(4), 411-418.
doi:10.1111/j.1095-8339.2003.00231.x

[9] Lopez-Vinyallonga, S., Mehregan, I., Garcia-Jacas, N., Tscherneva, O., Susanna, A. & Kadereit, J. W. (2009). Phylogeny and evolution of the *Arctium-Cousinia* complex (Compositae, Cardueae-Carduinae). *Taxon*, 58(1), 153-171.

[10] Tugay, O. (2012). *Cousinia*. In Güner, A, Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (Ed.), *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını.

[11] Tugay, O., Ulukuş, D., Ertuğrul, K., Uysal, T., Demirelma, H. & Dural, H. (2019). A new species of *Cousinia* (sect. *Cousinia*, Asteraceae) from the Ağrı Mountain (eastern Turkey): evidence from morphology, karyology and anatomy. *Phytotaxa,* 427(4), 259-269.

[12] Mehregan, I. & Assadi, M. (2016). A synopsis of *Cousinia* sect. *Pseudactinia* (Cardueae, Asteraceae) including a new species from NE Iran. *Phytotaxa,* 257(3), 271.

[13] Attar, F., Ghahreman, A., Mahdigholi, K., & Sheidai, M. (2004). Anatomy-Taxonomy studies of the species of section *Serratuloideae* (*Cousinia*, Compositae) in Iran. *Iranian Journal of Botany,* 10(2), 119-141.

[14] Ulukuş D, Tugay O (2019a). Endemik *Cousinia halysensis* Hub.-Mor. (Papatyagiller/Asteraceae) türünün anatomik, palinolojik ve mikromorfolojik yönden incelenmesi. *Bağbahçe Bilim Dergisi*, 6 (1), 59-65.

[15] Ulukuş D., Tugay O (2019b). Micromorphological, palynological and anatomical properties of endemic *Cousinia iconica* Hub.-Mor. (sect. Cousinia /Asteraceae). *Bağbahçe Bilim Dergisi*, 6(2), 58-63.

[16] Ulukuş D, Tugay O (2020). Morphology, anatomy and palynology of two endemic *Cousinia* Cass. species (Sect. *Cousinia*, Asteraceae) and their taxonomic implications. *Pakistan Journal of Botany*, 52(1): 297-304.

[17] Atasagun, B., Ulukuş, D., & Tugay, O. Endemik *Cousinia aucheri* DC. (İnce Kızan) Üzerine Anatomik, Palinolojik ve Mikromorfolojik Araştırmalar. *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi*, *47*(2), 181-191.

[18] Huber-Morath, A. (1975). *Cousinia*. In P. H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands* (Vol. 5, pp. 329–353). Edinburg: Edinburgh University Press.

[19] Atazadeh, N., Sheidai, M., Attar, F., Ghahremaninejad, F. & Koohdar, F. (2020). A palynological study of genus *Cousinia* Cass. (Family Asteraceae), sections *Cynaroideae* Bunge and Platyacanthae Rech. f. *Grana*, 59, 428-443.

[20] Atazadeh, N., Sheidai, M., Attar, F. & Koohdar, F. (2022). Molecular phylogeny and morphometric analyses in the genus *Cousinia* Cass.(Family Asteraceae), sections *Cynaroideae* Bunge and Platyacanthae Rech. f. *Caryologia*, 74(4): 39-50.

[21] Attar, F. & Ghahreman, A. (2000). Two new species and a new record of the genus *Cousinia* Casso Sect. *Cynaroideae* (Asteraceae) from West of Iran. *Iranian Journal of Botany*, 8 (2), 259-269.

[22] Johansen, D. A. (1940). *Plant microtechnique*. New York: McGraw-Hill.

[23] R Core Team. (2021). R 4.1.2. Vienna, Austria: R Foundation for Statistical Computing.
[24] Stearn, W. T (1983). Botanical latin. David & Charles, London, 566 pp.
[25] Ulukuş, D., Atasagun, B., & Tugay, O. (2021). Endemik *Cousinia decolorans* Freyn & Sint.(Asteraceae) Türünün Anatomik, Palinolojik ve Mikromorfolojik Özellikleri. *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi*, *47*(2), 192-202.
[26] Ulukuş, D. (2019). Anatomical, palynological and achene micromorphological characteristics of *Cousinia boissieri* Buhse (Sect. Leiocaules, Asteraceae) growing in Turkey. *Biodicon*, 12 (2), 119-125.