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Original Article

Taxonomic significance of anatomy and achene micromorphology of selected *Cousinia* Cass. species (Asteraceae)

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ABSTRACT

Background and Aims: The genus *Cousinia* has about 700 taxa all over the world. It is a hard and controversial group to classify in terms of taxonomy. This study aims to determine the achene micromorphological and anatomical characteristics of two selected *Cousinia* species, as well as their taxonomic significance.

Methods: In anatomical studies, the sections were set in paraffin, cut with a microtome, and stained with safranin-fast green. For both *C. eriocephala* Boiss. & Hausskn. and *C. calocephala* Jaub. & Spach species selected, an independent sample T-test analysis was performed using quantitative data to determine the importance of anatomical characters. In addition, PCA analysis and heatmap analyses were performed. SEM images were taken to determine the micromorphological features of the achenes.

Results: In the transver section of stems in *C. eriocephala*, from the epidermis to the center, there are 9–12 rows of cortexs layers composed of parenchymatic cells. In the transver section of stem in *C. calocephala* from the epidermis to the center, there are 5–8 rows of cortical layers composed of parenchymatic cells. In the cross-sections of the leaf in *C. eriocephala*, it was determined that the midrib shape was semi-orbicular, and a total of 9 vascular bundles, 3 large and 6 small, were counted. In the cross-sections of the leaf in *C. calocephala*, it was determined that the midrib shape was semi-orbicular, and a total of 6 vascular bundles, 3 large and 3 small, were counted. The achene surface ornamentation of *C. eriocephala* is striate-scrobiculate, while *C. calocephala* is striate and scrobiculate-faveolate.

Conclusion: According to the findings, it was determined that anatomical characters are important in the differentiation of species, as supported by both PCA and heatmap analysis.

Keywords: Asteraceae, *Cousinia eriocephala, C. calocephala*, Plant anatomy, Principal component analysis, Turkey

INTRODUCTION

In the Asteraceae family, the genus *Cousinia* (Asteraceae, Cardueae) is comprised of approximately 700 taxa, which are distributed in Turkey, Iran, Afghanistan, and Central Asia. The genus *Cousinia* has high species diversity and endemism and is characterized by the Iranian Turan phytogeographic region (Djamali et al., 2012).

The first detailed studies of the genus *Cousinia* were made by Bunge (1865) based on morphological data. Bunge (1865) found 126 species of the *Cousinia* genus in 23 sections, Boissier (1875) found 141 species in 14 sections, based on Bunge's studies, Tscherneva (1962) evaluated 260 species in 50 sections. The genus *Cousinia* has been evaluated with more than 350 species in 58 sections of the Iranian flora, including the Pakistan mountains, Iranian plateaus, Turkmenistan and Afghanistan (Rechinger, 1972). According to Rechinger (1986), *Cousinia* probably has a high proportion of species in a limited range with a unique degree of differentiation.

The *Arctium-Cousinia* complex and the genus *Arctium L.* are both included in the non-monophyletic genus *Cousinia* (Susanna et al., 2003; Lopez-Vinyallonga et al., 2009).

The genus *Cousinia* was first described by Cassini in 1827 as *Carduus orientalis* Adams. It is defined based on its type. The genus *Cousinia* is represented by a total of 38 species, 26 of which are endemic, within 6 sections in the Flora of Turkey (Huber-Morath, 1975). According to the list of plants in Turkey, there are 39 species (Tugay, 2012). With the recently published *Cousinia agridaghensis* Tugay, Ertuğrul & Ulukuş, the total number of species of *Cousinia* in Turkey has reached 40 (Tugay et al., 2019).

Cousinia sect. *Cynaroideae* Bunge contains 89 species from

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all over the world (Rechinger, 1986). There are a total of 8 species, 4 of which are endemic, in the *Cousinia* genus, sect. *Cynaroideae*, in Turkey (Huber-Morath, 1975).

To date, a great deal of taxonomical studies have been conducted on the genus *Cousinia* sect. *Cynaroideae* (Tscherneva, 1962; Huber-Morath, 1975; Rechinger 1972, 1979; Winkler 1892, 1897; Mehregan & Kadereit 2008; Attar & Ghahreman 2000, 2006; Attar & Djavidi, 2010, Attar & Rad, 2019). Recently, palynological and molecular studies have been carried out on sect. *Cynaroideae* (Atazadeh, Sheidai, Attar, Ghahremaninejad & Koohdar, 2020; Atazadeh, Sheidai, Attar & Koohdar, 2021).

There has been only one study of *Cynaroideae* anatomy. In this study, Attar & Ghahreman (2000) studied the leaf, stem, and root anatomy of *C. mobayenii* Ghahr. & Attar. The aim of this research is to reveal the taxonomic importance of the stem, leaf anatomy and achene micromorphology of *C. eriocephala* and *C. calocephala* distributed in Turkey and to contribute to future taxonomic research on the genus *Cousinia*.

MATERIALS AND METHODS

Plant Material

Between the years of 2011 and 2013, while taxonomic revision of the genus *Cousinia* was being carried out in Turkey, plant samples were collected from various places around the country (O.Tugay-8461 & O.Tugay-8471). The KNYA Herbarium at Selcuk University was in charge of storing the specimens. The herbarium specimens were analyzed using the Flora of Turkey and East Aegean Islands with a stereobinocular microscope.

Anatomy

Living material was preserved in a 70% ethanol solution for the purpose of anatomical research. When cutting cross sections of the stems and leaves, the paraffin process was utilized. Following the embedding of the specimens in paraffin wax, a Leica RM2125RTS rotary microtome was used to cut sections with a thickness ranging from 5 to 10 micrometers. After staining with safranin-fast green, each section was mounted with Entellan (Johansen, 1940). The Leica DM1000 binocular light microscope with the Leica DFC280 camera was used to take the measurements as well as the photographs.

Achene Micromorphology

Seed surface ornamentation was identified using scanning electron microscopy images. The surface ornamentation of seeds was evaluated using the terminology proposed by Stearn (1983).

Statistical Analysis

In order to examine the anatomy of the stem, leaf, and midrib based on cell size, at least thirty cell measurements were taken and the minimum, mean, maximum, and standard deviation were calculated (Table 1). For all statistical tests, R 4.1.2 software was utilized. (R core Team, 2021). PCA analysis was conducted using the quantitative characters of anatomical stem, leaf, and midrib characteristics in the species studied. The heat map was created by using the cluster method (R 4.1.2 with library heatmap) of the anatomical features of the species (Figure 7). Independent sample T-tests were used to assess the statistical significance of quantitative stem, midrib, and leaf features (R 4.1.2). P-values <0.05 were regarded as statistically significant (Table 2).

RESULTS

In addition to showing the anatomical and micromorphological features of the species studied, also photographs of the flowers of the species was featured (Figure 1).

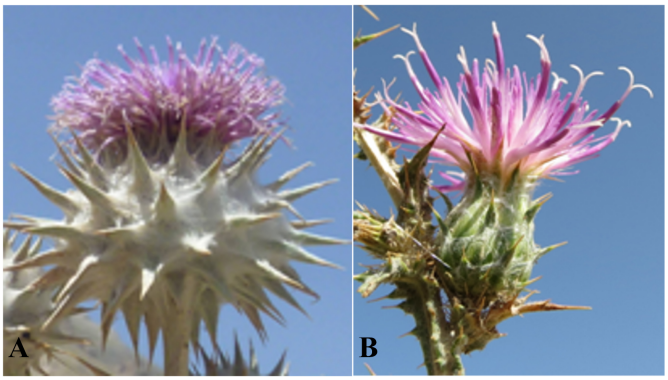

Figure 1. Photo: Prof. Dr. Osman TUGAY
Photographs of studied *Cousinia eriocephala* (A) *and C. calocephala* (B)

Stem Anatomy

Cousinia ericocephala

In the cross-sections of the stem, there is a single layer of protective epidermis tissue on the outermost. Epidermis cells consist of oval and rectangular cells with cell dimensions of 7.11-33.77 × 4.44-20.44 µm. On the epidermis, there is a thin layer of cuticle. From the epidermis to the center, there are 9–12 rows of cortexs layers composed of parenchymatic cells of 17.77-65.77 µm in rectangular, pentagonal, and oval shapes. The thickness of sclerenchymatous fibers is between 67.82 and 135.60 above the external phloem and between 34.78 and 113.00 µm above the internal phloem. The vascular bundles are arranged parallel to the stem axis and are well developed. The phloem layer is composed of dense small cells and its dimensions are be-

tween 32.22 and 91.11 µm. The cambium layer is not clearly visible. Xylem ranges in size from 56.52 to 146.3 µm. There are many elliptical vascular bundles. In the center, there is the pith region, which is usually composed of pentagonal-shaped parenchymatic cells (Table 1. Figure 2A-B).

Cousinia calocephala

In the cross-sections of the stem, there is a single layer of protective epidermis tissue on the outside. Epidermis cells consist of oval and rectangular cells with cell dimensions of 4.89-16.3 × 5.43-14.67 µm. On the epidermis, there is a thick layer of cuticle. From the epidermis to the center, there are 5-8 rows of cortical layers composed of parenchymatic cells of 13.91-145.2 µm in rectangular, pentagonal, and oval shapes. The thickness of sclerenchymatous fibers is between 76.52 and 168.6 µm above the external phloem and between 29.56 and 120.00 µm above the internal phloem The vascular bundles are arranged parallel to the stem axis and are well developed. The phloem layer is composed of dense small cells, and its dimensions are between 49.27 and 107.20 µm. The cambium layer is not clearly visible. Xylem ranges in size from 21.73 to 105.70 µm. There are many elliptical vascular bundles. In the center, there is the pith region, which is usually occupied by pentagonal-shaped parenchymatic cells (Table 1. Figure. 2C-D).

Leaf Anatomy

Cousinia ericocephala

In the cross section of the leaf, there are the upper and lower epidermis layers arranged in a single row. The upper epidermis layer is mostly rectangular, and its dimensions are between 10.37-28.14 × 10.37-24.44 µm. The cells of the lower epidermis are slightly smaller than the upper ones. Lower epidermis cell sizes range from 8.00-32.00 × 4.44-21.33 µm. The mesophyll layer (275.50-400.00 µm) between the lower and upper epidermis is parenchymatic palisade, sponge, and palisade. 2-3 rows of palisade parenchyma cells contain abundant chloroplasts, and their dimensions are between 11.11-47.77 × 26.66-112.20 µm. The leaves are equifacial and there is a large collateral vascular bundle consisting of phloem and xylem in the midrib (Table 1, Figure 3A-B).

Cousinia calocephala

In the cross section of the leaf, there are the upper and lower epidermis layers arranged in a single row. The upper epidermis layer is mostly rectangular, and its dimensions are between 15.51-57.75 × 6.03-25.00 µm. The cells of the lower epidermis are slightly smaller than the upper ones. Lower epidermis cell sizes range from 10.75-32.75 × 6.03-17.24 µm. The mesophyll layer (208.60–296.50 µm) between the lower and upper epidermis is parenchymatic palisade, sponge, and palisade. 2-3 rows of palisade parenchyma cells contain abundant chloroplasts, and their dimensions are between 5.17-14.65 × 43.96-66.37 µm. The leaves are equifacial, and there is a large collateral vascular bundle consisting of phloem and xylem in the midrib (Table 1, Figure 3C-D).

Midrib

Cousinia ericocephala

In the cross-sections of the leaf, it was determined that the midrib shape was semi-orbicular, and a total of 9 vascular bundles, 3 large and 6 small, were counted. Phloem and xylem tissues are surrounded by dense sclerenchyma cells. There are collenchyma and parenchymatic cells up to the epidermis in both the upper and lower parts of the conducting bundles. Parenchymatic cells are pentagonal and hexagonal in shape. The phloem layer is composed of very small cells, the size of the layer is between 69.56-108.6 µm. The xylem tissue is well developed, and sizes range from 134.7-260.8 µm (Table 1, Figure 4A-B).

Cousinia calocephala

In the cross-sections of the leaf, it was determined that the midrib shape was semi-orbicular, and a total of 6 vascular bundles, 3 large and 3 small, were counted. Phloem and xylem tissues are surrounded by dense sclerenchyma cells. There are collenchyma and parenchymatic cells up to the epidermis in both the upper and lower parts of the conducting bundles. Parenchymatic cells are pentagonal and hexagonal in shape. The phloem layer is composed of very small cells, the size of the layer is between 65.21-102.1 µm. The xylem tissue is well developed, and sizes range from 63.04-193.4 µm (Table 1, Figure 4C-D).

Achene micromorphology

Cousinia eriocephala

Achenes are broadly obovate prominent margins at the wrinkled end and are not clearly toothed. Their achene surface pattern is striate and scrobiculate. The surface of cells are hollow and anticlinal walls are flat. Periclinal walls are concave or flat (Figure 5A-B).

Cousinia calocephala

Achenes are oblong-obovate with prominent margins at the wrinkled end and are clearly toothed. Their achene surface pattern is striate and scrobiculate-faveolate. The surface of cells are hollow and anticlinal walls are flat. Periclinal walls are concave or flat (Figure 5C-D).

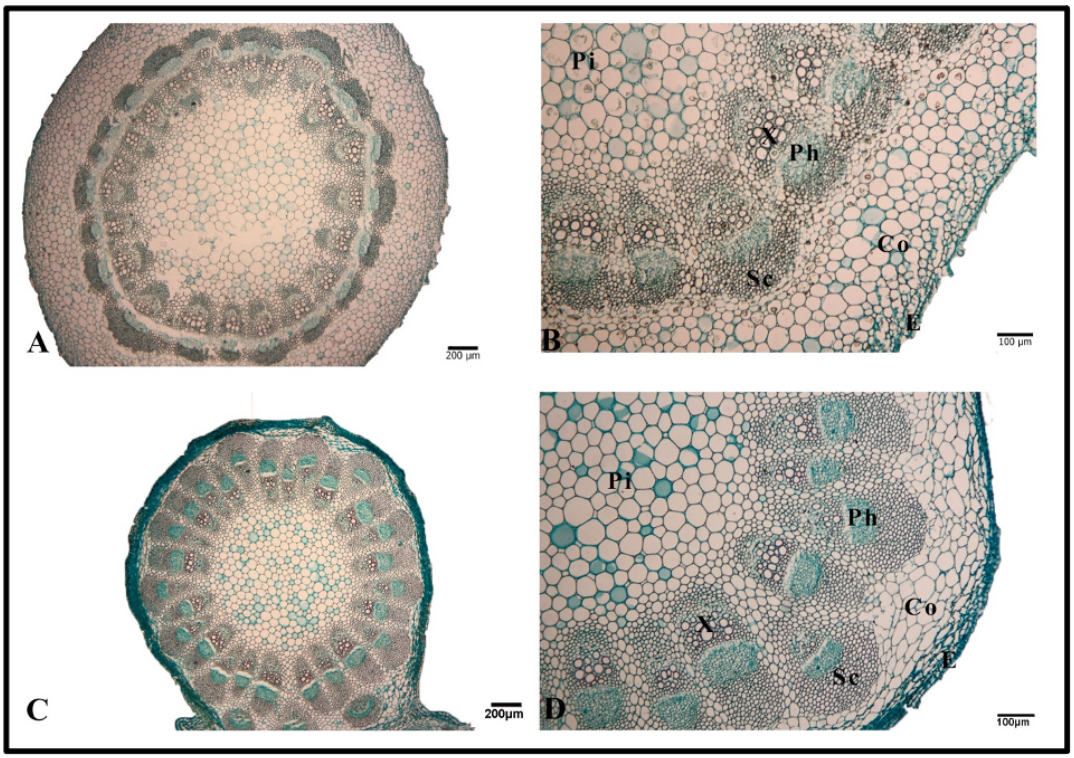

Figure 2. Transverse section of the stem; (A, B) *Cousinia eriocephala*, (C, D) C. *calocephala*. (E: epidermis, Co: cortex, Sc: sclerenchyma, Ph: phloem, X: xylem, Pi: pith region).

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

		C. eriocephala				*C. calocephala*			
		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	7.11 – 33.77	16.05 ± 6.48	4.44 – 20.44	11.13 ± 3.78	4.89 – 16.3	9.14 ± 2.32	5.43 – 14.67	9.58 ± 2.32
	Cortex cell	17.77 – 65.77	39.52 ± 12.95			13.91 – 145.2	29.67 ± 23.54		
	Outer sclerenchyma layer	67.82 – 135.60	83.40 ± 17.23			76.52 – 168.60	122.22 ± 19.87		
	Inner sclerenchyma layer	34.78 – 113.00	71.73 ± 21.79			29.56 – 120.00	61.38 ± 23.30		
	Phloem layer	32.22 – 91.11	55.17 ± 14.88			49.27 – 107.20	79.99 ± 13.05		
	Xylem layer	56.52 – 146.30	105.99 ± 21.26			21.73 – 105.70	55.88 ± 18.49		
	Pith	23.33 – 93.33	63.40 ± 17.23			19.13 – 69.56	45.32 ± 12.24		
Leaf	Upper epidermis	10.37 – 28.14	19.50 ± 4.08	10.37 – 24.44	17.22 ± 2.65	15.51 – 57.75	26.00 ± 10.64	6.03 – 25.00	15.62 ± 4.84
	Lower epidermis	8.00 – 32.00	14.12 ± 4.85	4.44 – 21.33	10.63 ± 3.33	10,75 - 32,75	16.17 ± 5.45	6.03 – 17.24	12.15 ± 2.82
	Mesophyll	275.50 – 400.00	329.87 ± 32.24			208.60 – 296.50	249.83 ± 27.98		
	Palisade parenchyma	11.11 – 47.77	21.47 ± 8.74	26.66 – 112.20	68.73 ± 20.00	5.17 – 14.65	11.05 ± 2.55	43.96 – 66.37	54.33 ± 6.29
Midrib	Lower collenchyma	343.4 – 943.4	662.27 ± 235.84			143.40 – 760.80	445.02 ± 196.43		
	Upper sclerenchyma	101.80 – 308.60	162.83 ± 89.74			132.60 – 132.60	132.60 ± 5.78		
	Lower sclerenchyma	113.00 – 423,10	272.10 ± 111.75			45.65 – 93.47	72.46 ± 20.28		
	Phloem layer	69.56 – 108.60	89.39 ± 16.06			65.21 – 102.10	78.67 ± 13.77		
	Xylem layer	134.7 – 260.8	209.20 ± 47.93			63.04 – 193.40	124.74 ± 49.34		

Statistical analysis

According to PCA analyses based on stem, leaf, and midrib characters of *C. calocephala* and *C. eriocephala*, the two species were distinguished from each other (Figure 6).

The independent sample T-test show that stem epidermal cell width, cortex cell width, outer schylerenchyma width, xylem layer width, pith width, and phloem layer width are all substantially different between *C. calocephala* and *C. eriocephala* (Table 2, P<0.05). Leaf mesophyll, palisade length and width characteristics seem to be important in the differentiation of *C. calocephala* and *C. eriocephala* (Table 2, P<0.05). Except for the upper schylerenchyma width, the remaining midrib fea-

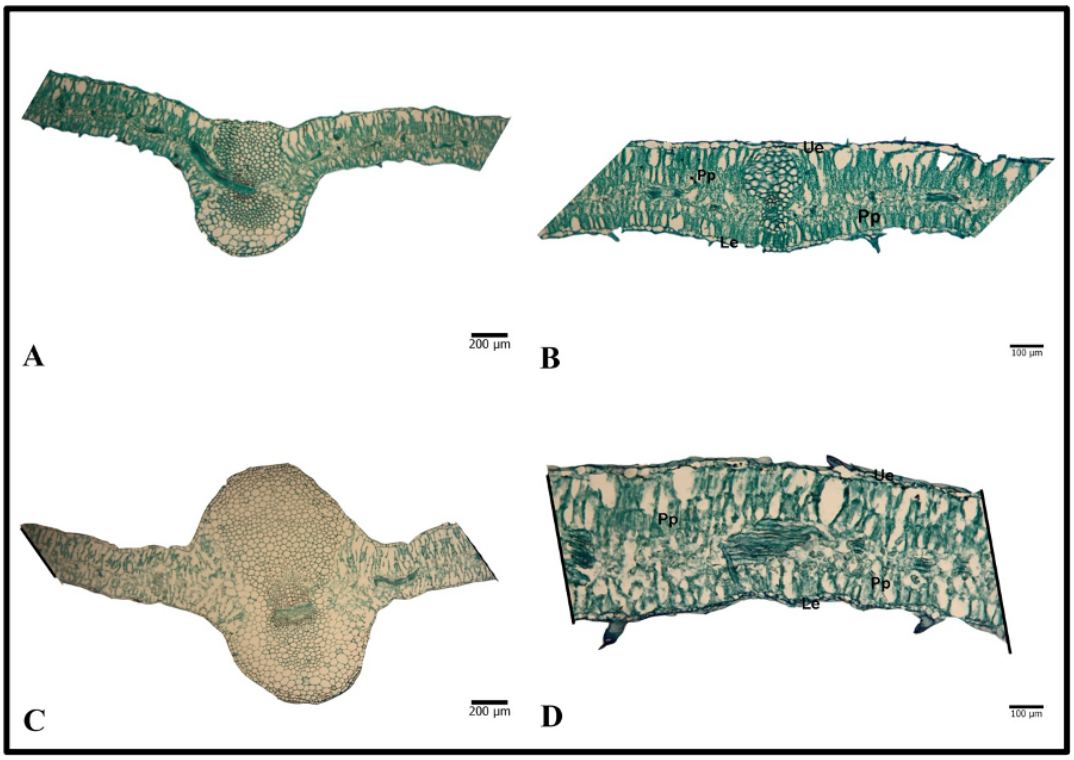

Figure 3. Transverse section of the lamina; (A, B) *Cousinia eriocephala*, (C, D) C. *calocephala*. (Le: lower epidermis, Pp: palisade parenchyma, Ue: upper epidermis).

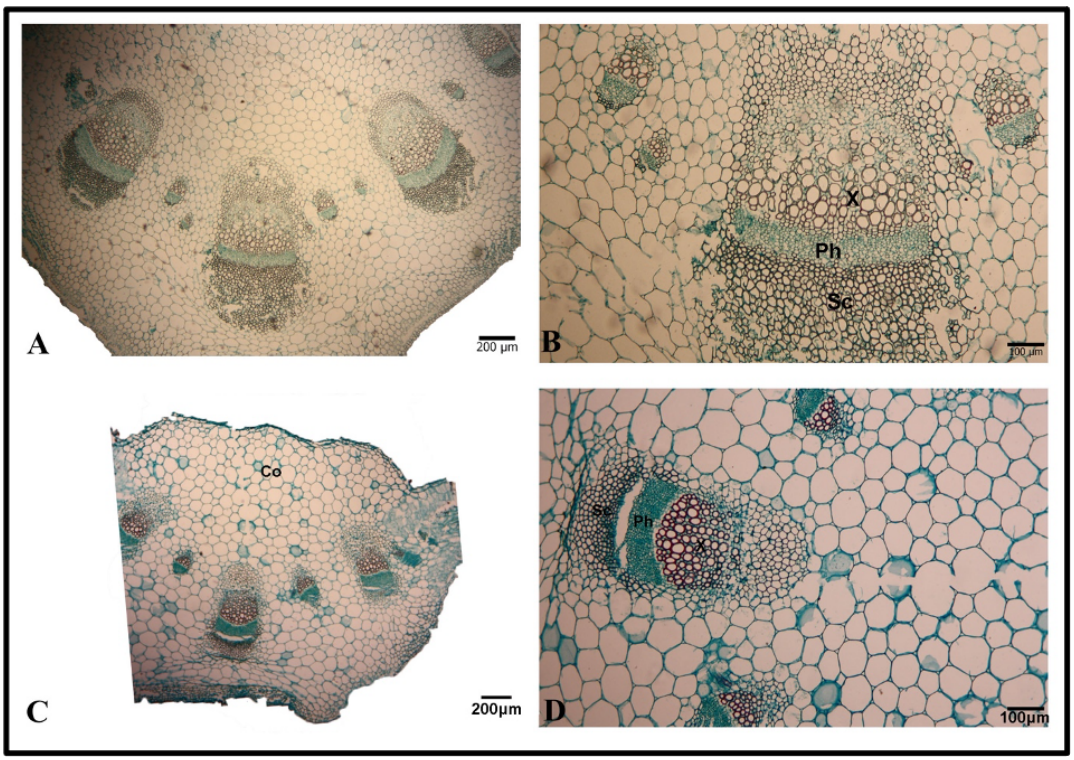

Figure 4. Transverse section of the midrib; (A, B) *Cousinia eriocephala*, (C, D) C. *calocephala*. (Co: collenchyma, Ph: phloem, Sc: sclerenchyma, X: xylem).

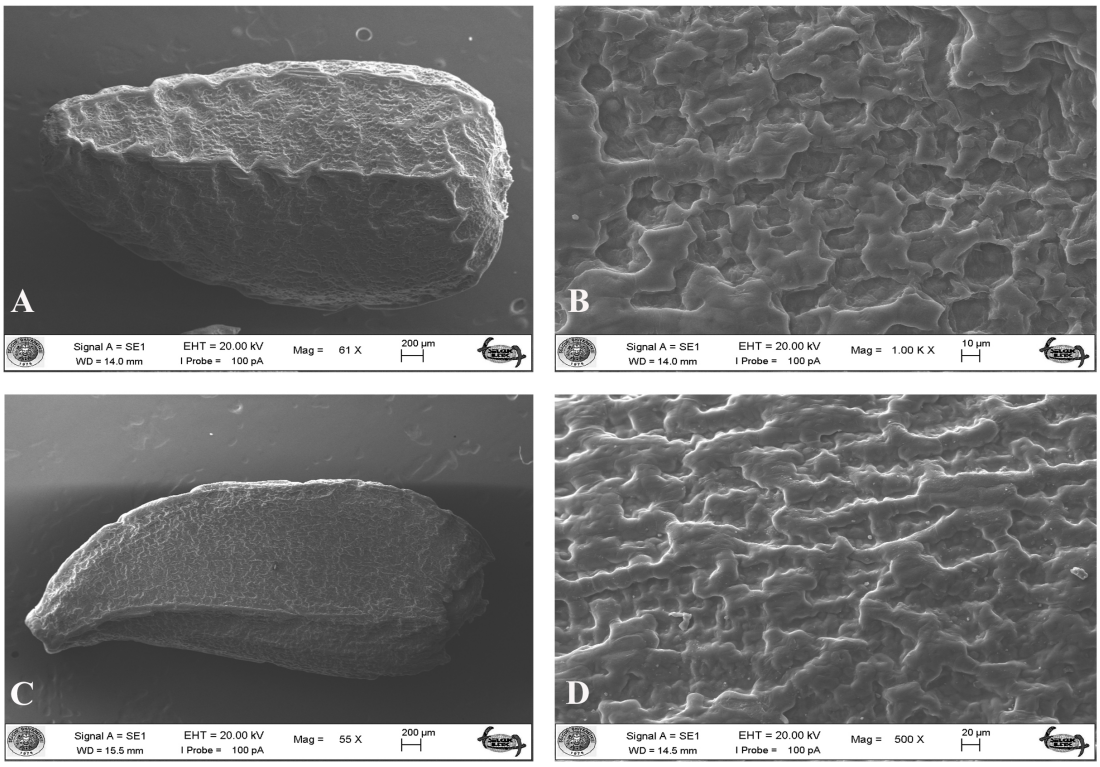

Figure 5. SEM micrographs of achenes of *Cousinia eriocephala* (A, B) and C. *calocephala* (C, D).

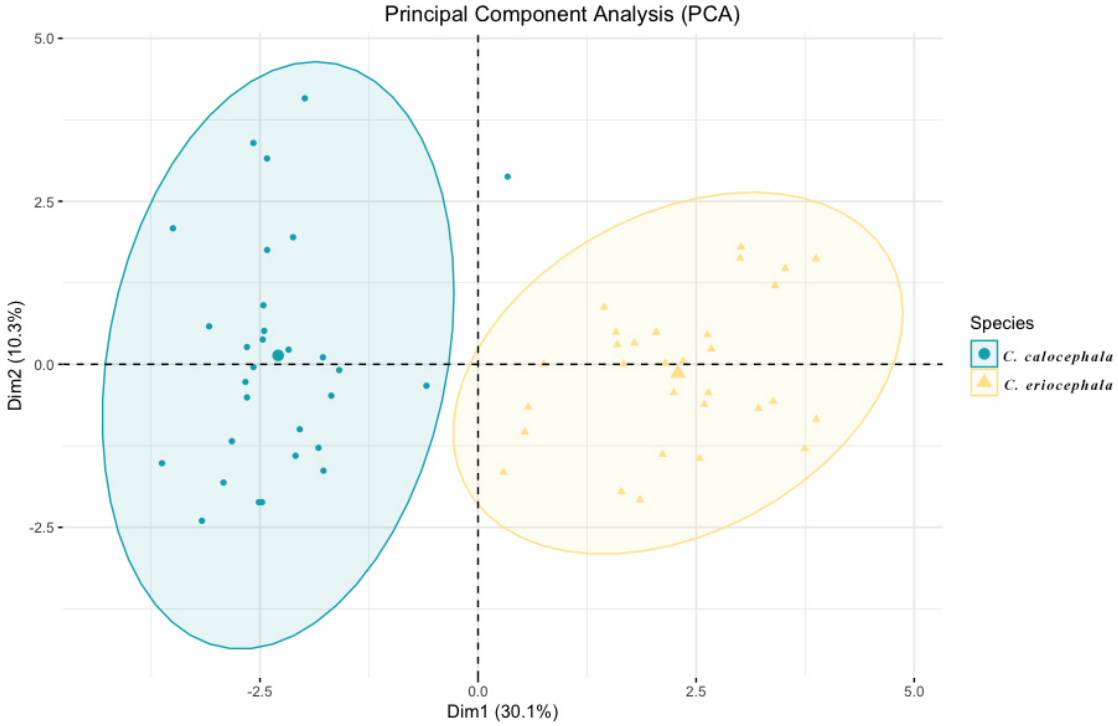

Figure 6. PCA for examined *Cousinia* species

tures were found to be important in the differentiation of *C. calocephala* and *C. eriocephala* (Table 2, P<0.05).

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

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

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, Sphlw: phloem width of stem, Sxylw: xylem width of stem, Piw: pith cell width of stem, 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, Mdlocolw: lower collenchyma width of midrib, Mdupscw: upper schylerenchyma width of midrib, Mdloscw: lower schylerenchyma width of midrib, Mdphlw: phloem width of midrib, Mdxylw: xylem width of midrib.

The results of the heat map analysis, which was based on anatomical characteristics, demonstrated that the two species that were analyzed could be distinguished from one another (Figure 7).

DISCUSSION

The data provided from stem, leaf, and midrib anatomical findings in this research indicated significant results that will contribute to the identification of the studied species within the *Cousinia* sect. *Cynaroideae* (Table 2). According to the stem anatomy findings, the size of the epidermis cells, cortex layers, outer schylerenchyma, phloem, xylem, and pith cells have taxonomically significant characters (Table 2). These stem anatomical characteristics can be integrated with morphological characteristics to identify species. Depending on the studied stem anatomical features, *C. eriocephala* differs from *C. calocephala* by its 9–12 layered parenchyma cells in cortex (Figure 2). According to the leaf anatomy results, the size of the epidermis cells, the size of the mesophyll layer width, and the size of the palisade parenchyma cells are taxonomically significant characters (Table 2, Figure 3). According to midrib anatomical characters, except for the upper schylerenchyma, other midrib characters are taxonomically important (Table 2). Recently, some anatomical studies have been carried out related to *Cousinia*. In these studies, Ulukuş & Tugay (2019b) investigated the stem, leaf, and midrib anatomy of *C. iconica* Hub.-Mor. Our anatomy findings partially concur with their findings. Ulukuş & Tugay (2019b) stated that mesophyll type is bifacial; in our study, we observed that the species examined are equifacial. Ulukuş & Tugay (2019b) reported that the number of vascular bundles of *C. iconica* in the midrib is 10. According to our study, while there are nine vascular bundles in *C. eriocephala*, respectively, there are six vascular bundles in *C. calocephala* (Figure 4). Ulukuş & Tugay (2019a) studied the anatomy of *C. halysensis*. Our findings partially accord with theirs concerning anatomy. Ulukuş (2019) stated that number of midrib vascular bundles have 10. However, we observed that the number of vascular bundles has nine and six studied species, respectively (Figure 4A-D). According to Atasagun, Ulukuş & Tugay (2021), *C. aucheri* DC. has 7-8 layered parenchyma cells in the cortex, but in our study, we found that *C. eriocephala* has 9–12 layered parenchyma cells in the cortex (Figure 2A–B). Ulukuş, Atasagun & Tugay (2021) stated that *C. decolarans* have 3 vascular bundles, However, in this study, we observed that the number of vascular bundles was nine and six in *C. eriocephala* and *C. calocephala*, respectively (Figure 4A–D).

In achene micromorphology studies related to *Cousinia*, Ulukuş & Tugay (2019) found that theachene structure pattern is reticulate in *C. iconica*, According to our study, we concluded that the achene structure patterns of *C. eriocephala* and *C. calocephala* are scrobiculate and scrobiculate-faveolate, respectively (Figures 5B–D). Ulukuş & Tugay (2019) stated that *C. halysensis* has a retipilate achene structure pattern. Both Atasagun et al. (2021) and Ulukuş et al. (2021) reported that studied species have a retipilate of achene surface ornamentation. However, we found that achene surface ornamentation is scrobiculate and scrobiculate-faveolate in the studied species (Figure 5B-D).

CONCLUSION

In this study, it was seen in both heatmap and PCA analyzes that the anatomical features used could be an important taxonomic character in the differentiation of species with the support of the statistical analysis results.

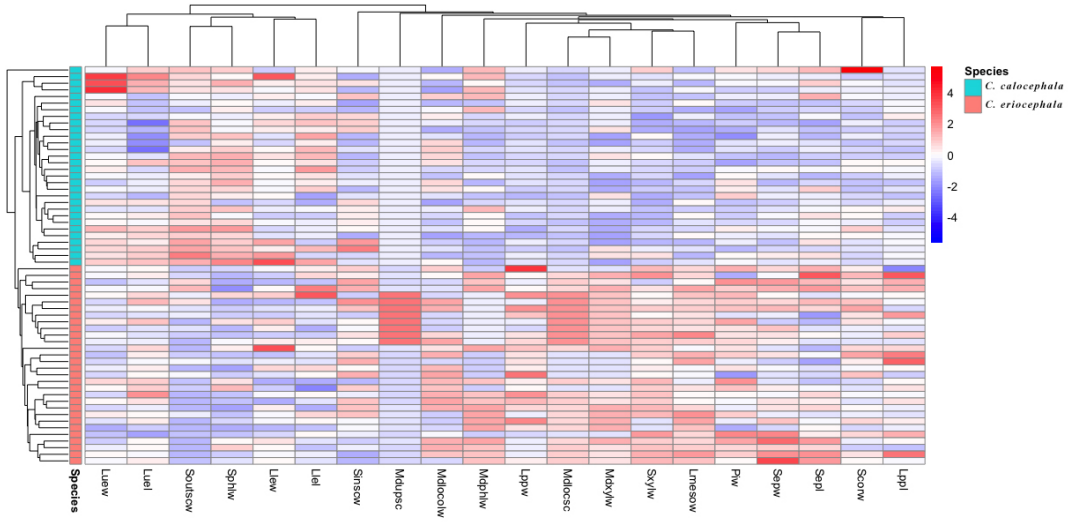

Figure 7. Heatmap for *C. calocephala* and *C. eriocephala* examined

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Author Contributions: Conception/Design of Study- D.U. & O.T.; Data Acquisition- D.U.; Data Analysis/Interpretation- D.U.; Drafting Manuscript- D.U.; Critical Revision of Manuscript- D.U.; Final Approval and Accountability- D.U., O.T.

Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

Atasagun, B., Ulukuş, D., & Tugay, O. (2021). Endemik *Cousinia aucheri* DC. (İnce Kızan) Üzerine Anatomik, Palinolojik ve Mikromorfolojik Araştırmalar. *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi, 47*(2), 181-191.

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

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*, 119-141.

Attar, F., & Ghahreman, A. (2006). A synopsis of sect. *Cynaroides* (Cousinia, Compositae), distribution patterns and diversity centers. -*Rostaniha, 7 (Supplement 2)*, 315-342.

Attar, F., & Djavadi, S.B. (2010). A taxonomic revision of *Cousinia*, sect. *Cynaroides* (Asteraceae, Cardueae) in the flora of Iran. *Iranian Journal of Botany, 16* (1), 130-184.

Attar, F., & Rad, M. A. (2019). *Cousinia sharifii*, a new species of *Cousinia* sect. *Cynaroideae* (Asteraceae) from NW Iran. *Phytotaxa*, 395(4), 287-295.

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.

Boissier, E. (1875). *Centaurea; Flora orientalis* 3. H. Georg, Geneva & Basilea (Basel), 668-674.

Bunge, A. (1865). *Übersichtliche Zusammenstellung der Arten der Gattung Cousinia Cass. Mémoires de l'Académie Impériale des Sciences de.* Saint-Pétersbourg Ser. 7, 9(2), 1-56.

Cassini, H. (1827). Saussurée, Saussurea. In: Cuvier, F. (Ed.) Dictionnaire des sciences naturelles, vol. 47. F.G.Levrault, Strasbourg, pp. 498-513.

Djamali, M., Baumel, A., Brewer, S., Jackson, S.T., Kadereit, J.W., Lopez-Vinyallonga S, Mehregan, I., 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 of Palaeobotany and Palynology*, *172*, 10-20.

Huber-Morath, A. (1975). *Cousinia Cass*. In Davis P.H.(ed.) *Flora of Turkey and the East Aegean Islands* (Vol. 5, pp. 329-353). UK: Edinburgh University Press.

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

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*, 153-171.

Mehregan, I., & Kadereit, J. W. (2008). Taxonomic revision of *Cousinia* sect. *Cynaroideae* (Asteraceae, Cardueae). *Wildenowia*, *38*, 293-362.

R Core Team. (2021). R 4.1.2.R *Foundation for Statistical Computing*. Vienna, Austria.

Rechinger, K.H. (1972). Compositae–Cynareae I: *Cousinia*. In: Rechinger, K.H. (Ed.) *Flora Iranica*, Vol. 90, pp. 1-329) Graz: Akademische Druck- und Verlagsanstalt.

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.

Stearn, W.T. (1983). *Botanical Latin*. London David & Charles.

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*, 411-418.

Tscherneva, O. (1962). *Cousinia* Cass. In: Schischkin, B.K. (Ed.) *Flora of the USSR* (Vol. 27, pp. 108–357). Leningrad: Akademiya Nauk.

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

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*, 259-269.

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

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

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.

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.

Ulukuş, D. (2019). Anatomical, palynological and achene micromorphological characteristics of *Cousinia boissieri* Buhse (Sect. Leiocaules, Asteraceae) growing in Turkey. Biodicon, *12 (2)*, 119-125.

Winkler, C. (1892). Synopsis specierum generic *Cousinia* Cass. Trudy Imperatorskago S.-Peterburgskago Botaniceskago. *Sada*, 12, 181-286.

Winkler, C. (1897). Mantissa synopsis specierum generic *Cousinia* Cass. Trudy Imperatorskago S.-Peterburgskago Botaniceskago. *Sada*, 14, 187-243.

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