

PALYNOMORPHOLOGICAL AND ANATOMICAL CHARACTERS OF THE SECTION *MESOCENTRON* FROM THE GENUS *CENTAUREA* (ASTERACEAE)

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Abstract

Palynomorphological and anatomical characters of the section *Mesocentron* (Asteraceae) from Türkiye was investigated and compared using microscopically techniques. The studied taxa have radially symmetrical, monad, isopolar, sometimes heteropolar, and generally 3-colporate, occasionally 4-colporate pollen grains. Only prolate pollen shaped pollen grains were observed in all of the studied species. While the colpus and porus morphology of pollen grains were the same in studied taxa the density of spinule on the pollen were changeable. It was observed that the basic anatomical structures of the sections obtained from the trunk anatomy were the same, but it was determined that the cellular sizes differed significantly between species. It has been emphasized that the combined use of pollen and anatomical characters may be effective in distinguishing these species.

Introduction

The genus *Centaurea*, a key member of the tribe Cardueae within the Asteraceae family, includes a diverse range of herbaceous species along with some shrubby forms. These plants are typically recognized by their unarmed leaves (Susanna and Garcia-Jacas, 2007). A distinguishing feature of *Centaurea* L. is its lateral hilum (Dittrich, 1968), along with its unique floral architecture, which consists of prominent sterile peripheral florets that lack staminalodes (Wagenitz and Hellwig, 1996). One of the most significant morphological traits for species identification in this genus is the shape of the scarious bract appendages. While *Centaurea* is predominantly found in the Mediterranean and Irano-Turanian regions, certain species, particularly those belonging to section *Jacea*, also extend into temperate areas of Europe (Hilpold *et al.*, 2014). With approximately 650 species worldwide, *Centaurea* stands as the largest genus within the subtribe Centaureinae (Mabberley, 2008). Türkiye is a major center of diversity for this genus (Wagenitz, 1986), and recent research has documented 247 taxa within the country (İlçim and Demir, 2023; Uysal *et al.*, 2024). Notably, 145 of these taxa are endemic to Türkiye, reflecting an endemism rate of 58.7%. Within Türkiye, the section *Mesocentron* (Cass.) DC. was historically represented by three taxa (Wagenitz, 1975), but with the addition of *Centaurea verutum* L., this number has increased to four (Duran *et al.*, 2014). Among them, two taxa are endemic, resulting in an endemism rate of 50%. According to the *Flora of Turkey*, members of this section are distinguished solely by variations in flower color, spine color, and spine length (Wagenitz, 1975).

The pollen morphological studies are an important tool in taxonomy allowing the separation of the taxonomically complex plant groups through the shape of pollen grains, aperture morphology or others (Fazal *et al.*, 2013). Throughout the decades lots of palynomorphological studies have been used in the family Asteraceae (Compositae) due to the taxonomic importance that pollen has for the taxa of this family (Stix, 1960; Besold, 1971; Skvarla *et al.*, 1977; Keeley and Jones, 1979; Blackmore, 1982; Robinson and Marticorena, 1986; Bremer, 1994). Despite the

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taxonomic importance of pollen grains for Asteraceae as a whole, the type of pollen grains present is more important in exact tribes than in others. The family Asteraceae exhibits distinctive pollen characteristics that have long intrigued researchers due to their systematic significance. Since Wodehouse (1935)'s pioneering work, numerous studies have explored these traits, including those by Wagenitz (1955, 1975), Stix (1960), Pehlivan (1995), Özler *et al.* (2009), Punt and Hoen (2009), and Pınar *et al.* (2016), Wagenitz (1955) classified Asteraceae pollen into eight distinct types based on morphological characteristics, encompassing *Serratula*, *Crupina*, *Centaureum*, *Dealbata*, *Montana*, *Cyanus*, *Scabiosa*, and *Jacea*. Among these, *Serratula* was considered the most primitive, while *Jacea* was regarded as the most evolutionary advanced.

Anatomical researches could be a taxonomic equipment that can aid in the identification of problematic species, and also establish their taxonomical relationships (Metcalf and Chalk, 1957; Janačković *et al.*, 2019a; Janačković *et al.*, 2019b). It is seen that anatomical studies on the genus *Centaurea*, which has a wide distribution in Türkiye, are quite limited (Taşar *et al.*, 2018; Aydın *et al.*, 2019). A significant anatomical contribution was made by Çıtak *et al.* (2021), who studied, described, and characterized the subgenus *Cyanus* Mill. Only a few of these studies contribute to the distinction between species of the genus and are still not sufficient. For this reason, the section *Mesocentron* within *Centaurea* was evaluated anatomically and palynologically in detail. The main aim herein was to: i) investigate the section *Mesocentron* species to identify their anatomical and pollen characteristics, ii) use numerical analysis to elucidate the systematic value of their anatomical and palynological traits, and iii) add to the taxa's systematic position.

Material and Methods

Collecting and storing plant materials

The specimens from the section *Mesocentron* were gathered from different locations, as detailed in Table 1, and preserved in the herbarium of the Biology Department at Selçuk University (KNYA).

Table 1. Localities of the studied *Mesocentron* taxa.

Taxa	Endemic (E)	Locality	Collector number
<i>C. solstitialis</i> L. subsp. <i>solstitialis</i>		Konya: Şelçuklu, roadside, 1212 m, 21 vii 2023	E. Şirin 807
<i>C. solstitialis</i> L. subsp. <i>pyracantha</i> (Boiss.) Wagenitz	E	İçel: Anamur, roadside 356 m, 19 vii 2023	E. Şirin 806
<i>C. solstitialis</i> L. subsp. <i>carneola</i> (Boiss.) Wagenitz	E	İçel: Mut, roadside, 187 m, 19 vii 2023	E. Şirin 805
<i>C. verutum</i> L.		Gaziantep: Gaziantep–Kilis road, interior of red lentil field, 703 m, 07 vi 2023	E. Şirin 795

The microscopical studies

For optical microscopical studies, the pollen samples were prepared according to the Wodehouse method (1935). All microscope slides were stored in the pollen and anatomy collection of the Plant Anatomy and Palynology Laboratory of Department of Biology, University of Selçuk, Türkiye. For light micrographs of pollen grains were acquired using a camera attached to a Leica DM 1000 microscope equipped with a 100× objective lens.

For the analysis of scanning electron microscopy (SEM), the anthers were dissected and pollen grains were dusted on a glass slide and they were controlled with light microscope, then they were swept up to carbon tape. These tapes with pollen grains were covered with a pure gold film. Subsequently, pollen samples were analyzed using a Zeiss Evo LS 10 scanning electron microscope.

At least five fully-grown of the stems the middle parts were used in the anatomical analysis. Cross sections of the stem were obtained manually using a razor. The anatomical tissues were stained with phloroglucin-HCL. The vascularization patterns of the stems, parenchyma pattern, and arrangement of the midrib vascular system were analyzed.

Measurement of pollen grains

Polar and equatorial views were used to measure the pollen grains. The morphometric study comprised 12 measurements of the equatorial diameter in polar view, 10 measurements of the apocolpium side, 12 measurements of aperture length and width in equatorial view, and 30 randomly selected measurements of the polar and equatorial diameters in equatorial view. Fifteen exine (sexine and nexine) thickness measurements were also made in the median region of the mesocolpium in polar view. The arithmetic mean (\bar{x}), sample standard deviation (s), mean standard deviation ($s\bar{x}$), coefficient of variation, and 95% confidence interval were calculated using statistical analysis of data from variables with 25 measurements. To guarantee sample standardization, at least five permanent slides of non-acetolysed pollen grains were investigated for every specimen (Çıtak *et al.*, 2019). Only the arithmetic mean was computed for variables with ten measurements.

Terminology

The pollen terminology adopted are those of Punt *et al.* (2007), Punt and Hoen (2009), Halbritter *et al.* (2018), and Çıtak and Kabalcı (2024) for pollen size, shape, aperture number, and exine ornamentation pattern.

Multivariate analysis

Microsoft Excel was used to create a matrix with quantifiable pollen and anatomical characters of reference specimens. The variables including in matrix are polar and equatorial diameter, apocolpium, colpus width, colpus length, exine and intine thickness, porus length and porus width, the width of cortex parenchyma, presence/absence of stem protrusions, the width of sclerenchymatic caps. Four taxa were clustered using Gower's (1971) general coefficient similarity and a dendrogram was created using the clustering analysis method, i.e. the unweighted pair group method with arithmetic mean (UPGMA), in MVSP 3.22 software. Principle coordinate analysis (PCA) was performed in the same program according to the method of Çıtak *et al.* (2019).

Results and Discussion

Palynological results

The investigated taxa were quantitatively and qualitatively summarized in Tables 2-3 and illustrated in Figs 1-2. All the pollen grains in studied species were determined as monad, radially symmetrical and isopolar. They were elliptic and not compressed at the poles in equatorial view, while they were subcircular or slightly triangular with obtuse angles in polar view.

Size, symmetry and shape

The polar axis ranging from 27.83 to 52.02 μm and equatorial axis ranging from 20.18 to 36.18 μm in studied species. Although *C. verutum* had subprolate shaped pollen grains while the

other ones had prolate. The dimensions were smaller in *C. solstitialis* subsp. *carneola* (and larger in *C. verutum* (Table 2, Fig. 2). Apocolpium is smallest in *C. solstitialis* subsp. *solstitialis* (5.47 μm) and is largest in *C. verutum* (9.28 μm).

Sculptural elements and sporoderm

The intine was very thin and the intine thickness varied from 0.75 μm to 0.85 μm . The scabrate-perforate sculpturing was determined in the studied species and the spinule length was 0.46–0.66 μm (Fig. 2). All of the taxa had a costae thickness ranging from 1.42 μm to 1.68 μm (Table 2, Fig. 2).

All of the specimens of the subgenus examined generally had 3-zonocolporate pollen grains. Heteromorphic pollen production was determined in section *Mesocentron*. Some of pollen grains in studied species were smaller than the other ones. The colpus in the observed species was sunken (Fig. 2). The colpus was long (25.54–34.96 μm) and narrow (6.17–8.77 μm). The highest values were seen in *C. verutum* and *C. solstitialis* subsp. *carneola*, which had the largest measured colpus. Margins were distinct, straight, and the ends were acute to slightly obtuse in all of the investigated taxa. The colpus membrane was granulose in all of the taxa. The porus length changes from 8.12 μm to 10.42 μm and the porus width also changes from 9.83 μm to 11.76 μm .

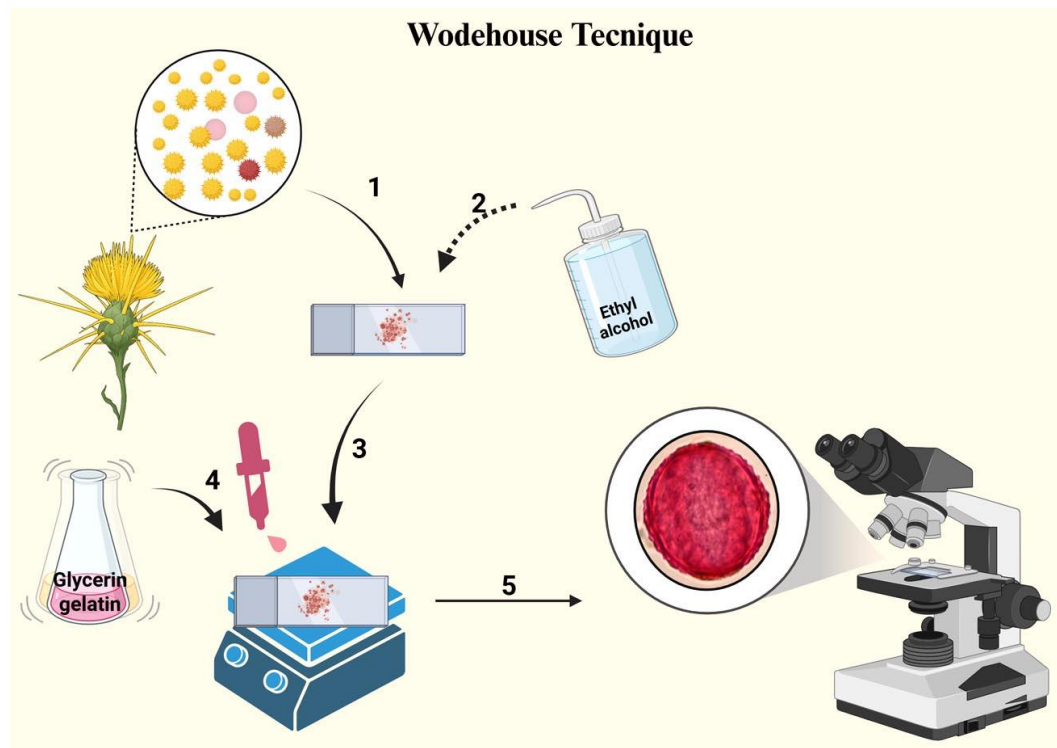


Fig 1. The Wodehouse technique (1935) used to obtain pollen grains for light microscopy.

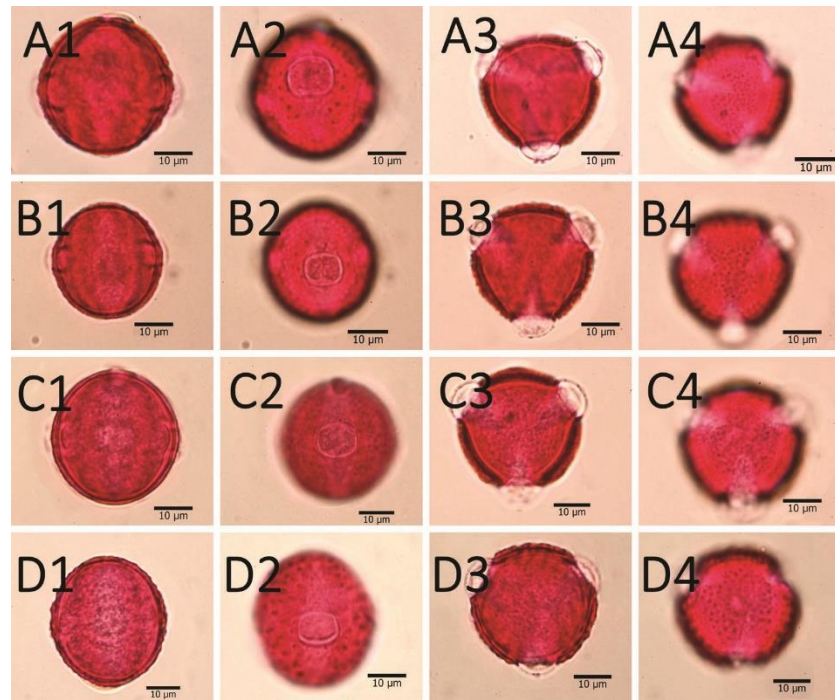


Fig. 2. The light microscope microphotographs of the members of *Mesocentron*. A1-A4: *Centaurea solstitialis* subsp. *solstitialis* B1-B4: *Centaurea solstitialis* subsp. *pyracantha* C1-C4: *Centaurea solstitialis* subsp. *carneola* D1-D4: *C. verutum*.

Anatomical characters

Stem anatomy

Leaf extensions were detected in cross-sections taken from the stems of species in the section of *Mesocentron* (Figs 3-4). The stem size varied among the species (Table 4). The oval shaped stems with densely covered by unicellular trichomes (Figs 3-4). Stem cross sections showed collenchyma at protrusion sites. Two types parenchyma cell were detected that the first one was cylindrical-shaped with abundant chlorophyll, the second one was oval-shaped with less chlorophyll. In most species' cortical parenchyma, the sclerenchyma was positioned above the phloem. The vascular bundles were collateral type in all investigated taxa. In all of the species examined, vascular bundles were collateral type, forming either continuous or discontinuous rings, arcs, or rings with arcs inside (Figs 3-4). Sclerenchymatic caps were found around the vascular bundles in the examined species, except in *C. tchihatcheffii*, *C. woronowii*, and *C. thirkei* (Figs 3-4). Vascular bundles were observed near the abaxial surface of the stems and varied in number among the species.

Table 2. Pollen characteristics of the section *Mesocentron* in Türkiye.

Species/Characters	Chromosome number (Şirin 2025)	Polar axis (μ)		Equatorial axis (μ)		P/E	Pollen shape	Exine at poles (μ)	Exine at equator (μ)	Intine (μ)
		Min-Max	Mean	Min-Max	Mean					
<i>C. solstitialis</i> L. subsp. <i>carneola</i> (Boiss.) Wagenitz	2n=18	32.34-45.02	36.61±3.88	20.18-31.99	27.27±4.11	1.34	Prolate	2.26	2.04	0.79
<i>C. solstitialis</i> L. subsp. <i>pyracantha</i> (Boiss.) Wagenitz	2n=16	27.83-48.01	40.22±7.88	23-29.02	26.17±1.78	1.53	Prolate	2.26	2.04	0.85
<i>C. solstitialis</i> L. subsp. <i>solstitialis</i>	2n=16	28.17-52.02	38.76±9.73	21.5-31.01	28.23±2.01	1.37	Prolate	1.98	2.07	0.76
<i>C. verutum</i> L.	2n=16	34.81-48.05	41.34±5.22	29.58-36.18	32.54±2.45	1.27	Subprolate	2.32	2.87	0.75

Table 3. Additional pollen characteristics of section *Mesocentron*.

Species/Characters	Costa	t (μ)	The length of colpus (μ)	The length of colpus (μ)	The width of colpus (μ)	The length of porus (μ)	The width of porus (μ)	The length of spine	The width of base	Distance between spines
<i>C. solstitialis</i> L. subsp. <i>carneola</i> (Boiss.) Wagenitz	1.50	6.04	34.96	34.96	6.17	8.97	10.68	0.60	1.79	0.53
<i>C. solstitialis</i> L. subsp. <i>pyracantha</i> (Boiss.) Wagenitz	1.59	5.52	25.54	25.54	8.11	8.4	9.83	0.66	1.99	0.61
<i>C. solstitialis</i> L. subsp. <i>solstitialis</i>	1.42	5.47	32.35	32.35	7.95	8.12	9.86	0.65	1.76	0.63
<i>C. verutum</i> L.	1.68	9.28	34.9	34.9	8.77	10.42	11.76	0.46	1.59	0.51

Numerical analysis of pollen and anatomical characters states

The derived dendrogram based on 21 variables given in Tables 2-4 is presented in Fig. 5. This dendrogram reflected the similarities among the investigated taxa and revealed 2 main groups: group A (with 30% similarity), which comprised 3 species in group B (with 56% similarity). According to the UPGMA dendrogram, *Centaurea solstitialis* subsp. *solstitialis* and *C. solstitialis* subsp. *carneola* form a clade with a similarity ratio of 63%. *C. solstitialis* subsp. *pyracantha* is placed just outside this clade with a similarity ratio of 58%. *C. verutum*, on the other hand, is located at the outermost position in the dendrogram with a similarity ratio of 30%.

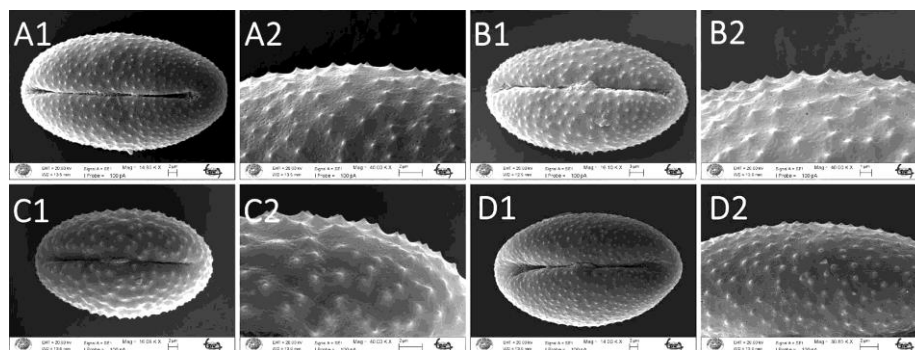


Fig 3. The scanning electron microscope microphotographs of the members of *Mesocentron*. A1-A2: *Centaurea solstitialis* subsp. *solstitialis* B1-B2: *Centaurea solstitialis* subsp. *pyracantha* C1-C2: *Centaurea solstitialis* subsp. *carneola* D1-D2: *C. verutum*

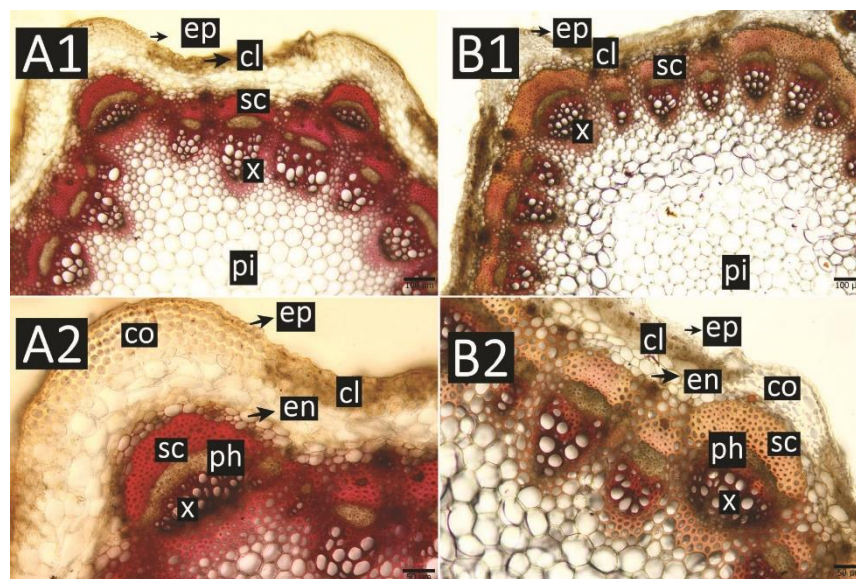


Fig 4. The stem anatomical microphotographs of A1-A2: *Centaurea solstitialis* subsp. *solstitialis* B1-B2: *Centaurea solstitialis* subsp. *pyracantha* ep: epidermis, co: cortex, cl: clenchyma, sc: sclerenchyma, x: xylem, pi: pith region, en: endodermis, le: leaf, ph: phloem.

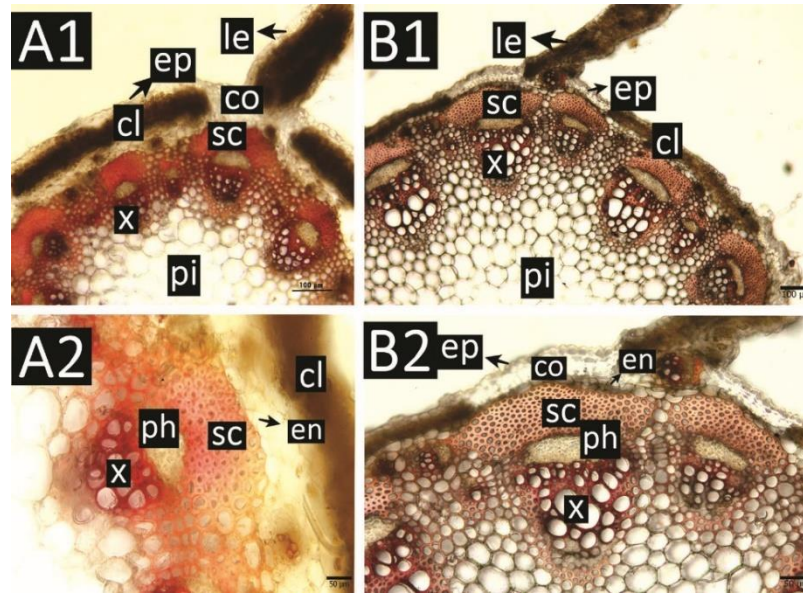


Fig 5. The stem anatomical characters A1-A2: *Centaurea solstitialis* subsp. *carneola*, B1-B2: *C. verutum* ep: epidermis, co: cortex, cl: clenchyma, sc: sclerenchyma, x: xylem, pi: pith region, en: endodermis, ph: phloem.

Table 4. The measurements of anatomical characters of the section *Mesocentron*.

Taxa/anatomical characters	A1	A2	A3	A4	A5	A6	A7
<i>C. solstitialis</i> L. subsp. <i>solstitialis</i>	18	59.00	61.76	14.41	14.61	17.08	22.03
<i>C. solstitialis</i> L. subsp. <i>pyracantha</i> (Boiss.) Wagenitz	19	60.82	30.32	11.99	10.24	12.56	35.78
<i>C. solstitialis</i> L. subsp. <i>carneola</i> (Boiss.) Wagenitz	26	61.78	69.59	8.82	13.55	13.38	21.44
<i>C. verutum</i> L.	27	39.59	47.51	16.46	15.9	16.29	21.84

Note: A1 Number of vessels, A2 Diameter of pith cells, A3 Width of collenchyma, A4 Width of sclerenchyma, A5 Length of epidermis, A6 Width of epidermis, A7 Width of trachea.

Nowadays, pollen morphological studies lead to a better understanding of delimitation of plant species and therefore using in plant systematics. Because of unique feature of pollen grains specific to species can be employed to clarify systematic confusion. Accordingly, by emphasizing very similar pollen characters in closely related groups, the results obtained here for species of the *Mesocentron* stand out. The species exhibit similarities in the ornamentation types as scabrate however some differences were stood out. Different researchers performed on the palynology of Asteraceae (Çeter *et al.*, 2013; Çitak *et al.*, 2019; Çitak *et al.*, 2021) showed that the number of aperture was a useful tool for identification of members of the family. When many pollen grain morphs with varying aperture numbers are produced by the same plant, this is known as pollen grain heteromorphy (Till-Bottraud *et al.*, 1995). Aperture heteromorphism may occur due to hybridization or meiotic defects (Matsuda, 1927; Aytuğ *et al.*, 1971). Additionally, Borsch and

Wilde (2000) stated that varying levels of ploidy were typically associated with changes in the type of pollen aperture. In current study, heteromorphy was seen in *C. verutum* which is a different species in *Mesocentron*.

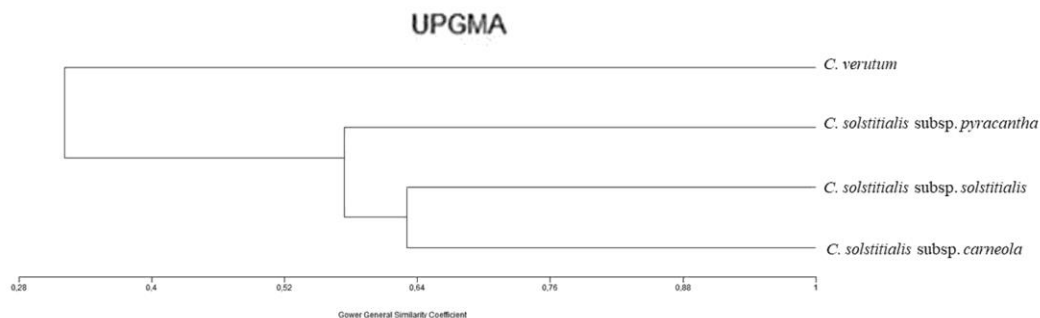


Fig 6. The derived dendrogram based on pollen and anatomical characters.

The anatomical characteristics of the stem were found to differ between species in *Mesocentron* but no patterns that would allow for species differentiation were found. The vascularization patterns of the stems of *C. tchihatcheffii* and *C. depressa* differed, according to Çakırlar *et al.* (2005). These variances included differences in the kind of vascular bundle as well as the quantity and location of accessory bundles. The stem cross-sections in the Asteraceae family were rounded, semi-rounded, rectangular, round, or irregular in shape (Aydın *et al.*, 2013; Aydın *et al.*, 2019; Çıtak *et al.*, 2021) also, in plant communities, the location and quantity of layers of the collenchyma tissue are crucial (Özcan, 2018). The present investigation found that different rows of collenchymatic tissue (3-5 rows) were localized beneath the epidermis. The vascular bundles in the stem were normally grouped in two rings, with those under the collenchymatic area being larger than the others (Çıtak *et al.*, 2021) like in *Mesocentron*. However, Çelik *et al.* (2005, 2008) and Kaya *et al.* (2010) reported that the vascular bundles were scattered in a circular manner as one ring in the stem of some species of *Centaurea*. In all of the investigated species, the stem cortex was made up of parenchymatic oval cells with thin walls, ranging from two to four rows.

The findings from the statistical analysis employed here demonstrated the significance of similarity among the many variable types examined in order to classify species with comparable traits. This phenetic tree enabled us to identify two groups of species mostly based on the form and exine of the pollen grains. The most distinct species of *Mesocentron*, *Centaurea verutum*, differs from *C. solstitialis* in stem indumentum, spine color, involucre, and achene measurements (Duran *et al.*, 2014) according to morphology and this difference has been proved in derived phenetic tree. *C. solstitialis* subsp. *pyracantha* is the second different species in the group and differs from *Centaurea solstitialis* subsp. *solstitialis* in the color of the anther tube, as well as in the color and length of the spines. This two subspecies can be separated pollen and anatomical characters that the length of colpus, width of collenchyma, epidermis, and trachea. The other subspecies, *C. solstitialis* subsp. *carneola*, on the other hand, is distinguished from these two subspecies by its flower color and achene characteristics (Wagenitz, 1975) based on the width of colpus, and epidermis and number of vessels.

Conclusion

In conclusion, the anatomical and pollen characters of the examined species, such as colpus length, collenchyma width, and vascular bundle pattern, are micro characteristics that can be used to distinguish species and will form a database for future phylogenetic studies.

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