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Original Article

Morpho-anatomical and palynological studies on *Glaucosciadium cordifolium* (Boiss.) B.L. Burtt & P.H. Davis (Apiaceae) from Turkey

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ABSTRACT

Background and Aims: The aim of the present study was to determine the morphological, micromorphological, anatomical and palynological characteristics of *Glaucosciadium cordifolium* (Boiss.) B.L. Burtt & P.H. Davis
Methods: Measurements were performed on approximately 30 different samples for morphological study. The fruit micromorphologies and pollen properties were examined using scanning electron microscopy. For anatomical studies, all sections prepared from stem, leaf and fruit were taken by hand.
Results: Cross-sections of stem, leaf and fruit are examined and a detailed anatomical description is presented and supported by photographs. It is a glaucous perennial herb with a height of 34-180 cm which has an acrid smell when bruised. The fruit is a dry schizocarp, glabrous and oblong-elliptic. The mericarp is elliptic in the transverse section which is winged in the lateral. The fruit surface ornamentation of the genus is occasionally wavy and striped and the upper surface simple, short and scaly. The stomata is observed.
Conclusion: In this study, morphological, anatomical and palynological properties of the *Glaucosciadium cordifolium* are described and illustrated for the first time. In addition, the palynological results confirmed the stenopalynous characteristic of the family Apiaceae, and revealed that the pollen grains of the genus are perprolate in shape.

Keywords: Anatomy, Apiaceae, *Glaucosciadium cordifolium*, morphology, palynology

INTRODUCTION

Apiaceae, a family known for its characteristic flower structure, includes 464 genera and 3780 species (Calviño, Teruel & Downie, 2016). Moreover, this family, which is capable of being the eighth largest family,consists of about 455 species occurring in 33% of Turkey's endemic (Davis, Mill & Tan, 1988; Duman, Guner, Ozhatay, Ekim & Baser, 2000).

Anatomy and morphology are taxonomically very important in Apiaceae and nearly all traditional classification systems of the family have relied on fruit characteristics. Apiaceae species have secretory cavities (vittae) in roots, petioles, stems, leaves and fruits, which carry schizogenic fatty ducts containing resin, oil or mucilage (Duman et al., 2000; Metcalfe & Chalk, 1950). Although anatomical characters are used to distinguish between closely related species and genera in the Apiaceae family, they are not always as useful as morphological characters (Akalın Uruşak & Kızılarslan, 2013).

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It is also a solution for the taxonomic problems that can be encountered in species determination to reveal and evaluate all the characters of the plant together.

According to the Flora of Turkey, the genus *Glaucosciadium* B.L.Burtt & P.H.Davis is represented by one taxon in Turkey and two taxa in the world (Hedge & Lamond, 1982). *G. cordifolium* was published by Burtt and Davis in Kew Bulletin in 1949. *G. cordifolium*, which grows on the stony river banks, slopes and chalk slopes of Southern and Central Anatolia, is a monotypic species and is known as "Çağşır otu" and "Sakar otu" in the regions where it grows. The plant, which has an acrid smell when crushed, is used as an aphrodisiac in Turkish folk medicine, mixed with honey (Güner, 2012; Özhatay & Koçak, 2011; Hedge & Lamond, 1982). The aim of this paper is to give a detailed description of the morphological and anatomical features and pollen characteristics of *G. cordifolium*. Our findings will contribute to the systematics of the Apiaceae family. Although there are a limited number of studies on the chemical content of *G. cordifolium*, the fact that there are no studies on the morphology, anatomy and pollen properties makes the study important.

MATERIALS AND METHODS

Plant material

G. cordifolium was collected from the provinces of Kütahya (B2: Kütahya, Uşak-Gediz road, 3. km after the Uşak junction, 658 m., ESSE 15001) and Karaman (C4: Karaman, Ermenek, Ermenek-Balkusan road 4. km, 1650 m., ESSE 15500) in Turkey during flowering time in July 2014. Herbarium specimens, prepared by pressing and drying of the plant, were stored in the Herbarium of the Faculty of Pharmacy of Anadolu University, in Eskisehir, Turkey (ESSE).

Morphology

All measurements regarding the morphological characters of the plant were made on living samples. Measurements were performed on approximately 30 different samples, and land notes were used for some morphological characteristics. General view, flower-fruit in compound umbel, single flower-fruit and petal of specimen were drawn. The flower and fruit parts of the plant were drawn using the drawing tube of the Olympus SZX12 stereo microscope.

Anatomy

Some of the plant samples were preserved in 70% ethanol to be used in anatomical studies. All sections prepared from stem, leaf and fruit were taken by hand, after staining with Sartur reactive (Çelebioğlu & Baytop, 1949), the sections were taken into glycerin-gelatine and the microscope slides were made permanent with Canada Balsam. At least 30 preparations were examined for each structure and photographs of the plant parts were taken with a light microscope (Olympus BX51T). The width and length of the epidermal cells, as well as the number of stomata and epidermal cells per unit area were determined for both leaf surfaces and the stomatal index (SI) as a percentage was calculated (Meidner & Mansfield, 1968).

Palynology

The pollen of the plant, taken on double-sided adhesive tape,was mounted on SEM stubs, covered with gold and photographed with Zeiss Ultra Plus Fesem. In pollen terminology, characteristic structures were adopted from Faegri & Iversen (1975) and Punt,Hoen, Blackmore, Nilsson & Le Thomas (2007).

RESULTS AND DISCUSSION

Morphological properties

Glaucosciadium is a monotypic genus, which is distributed in South and Central Anatolia. Since its description (Burtt & Davis, 1949), a detailed study about its anatomy, morphology and pollen properties on it had never been conducted before.

Description

Creeping, perennial, glaucous, erect, 34-180 cm long and 3-10 mm diameter, glabrous plant. Stem branched and pronounced stripes, with an acrid smell when bruised. Basal leaves 1-2 pinnate, ovate-triangular, 9-53 × 4-26 cm. Basal leaflets, widely ovate, rarely reniform, 10-35 × 8-90 mm, apex mucronate, base cordate - truncate, reticulate veined, sessile or up to 75 mm handle. Upper leaves sheath-like, elliptic-oblong, 5-25 × 10 mm, apex acute-obtuse, divided 1-3 segments at the top, base ocrea. Bracts 3-6, linear-lanceolate to elliptic-oblong, 4-27 × 2-16 mm. Bracteoles absent. Umbells compound, wide spread, unequal 4-10 rayed, rays 5-45 mm

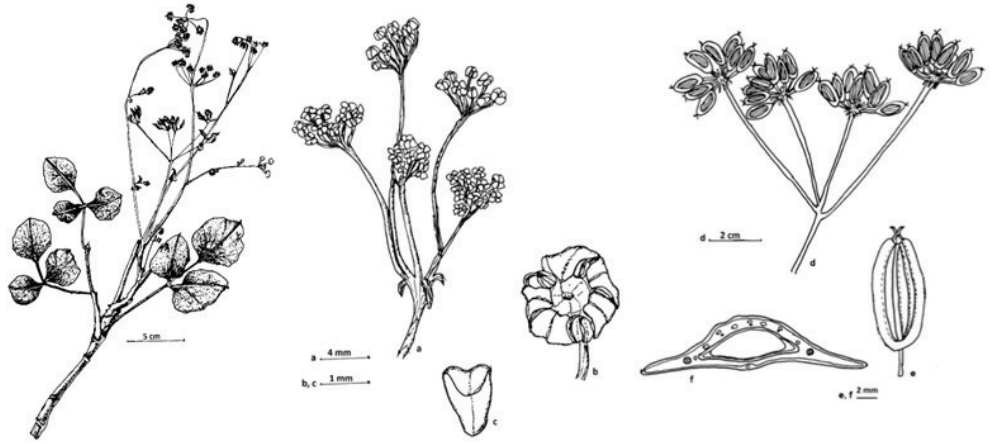

Figure 1. *G.cordifolium* ESSE 15001, Kütahya; a-compound umbel-flower, b-flower, c- petal, d-compound umbel- fruit, e-fruit; f- fruit cross section (Saltan, 2015).

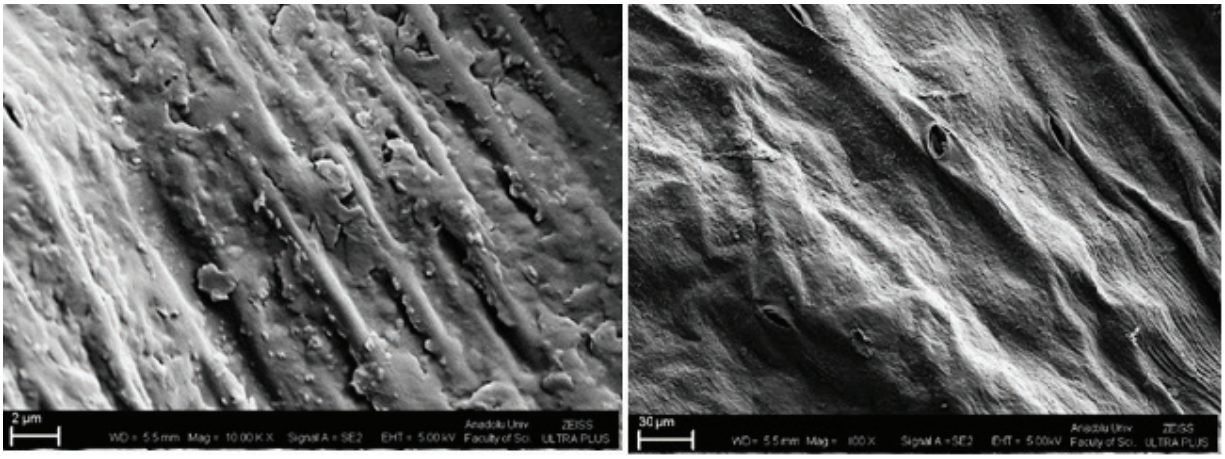

Figure 2. Scanning electron micrographs (SEM) of fruit surface of *G. cordifolium* (Saltan, 2015).

in flower, 17-100 mm in fruit. Flowers 7-23, orbicular, actinomorphic. Outer flowers of umbel hermaphrodite, 4-10, pedicels 4-5 mm. Sepals absent. Petals creamy white, 5, equal, free, 0.7-1.5 x 0.8-1 mm, significantly internal curved. Pedicels with glandular hairs. Stamens 5, free, cream, 0.4-0.5 × 0.2-0.4 mm, versatile sometimes basifixed. Filaments white and 0.5-1 mm. Ginekeum 0.8-1 × 1.2-2 mm, stylopodium 0.7 × 1 mm. Ovary inferior, of 2- united carpels. Fruit is a dry schizocarp, glabrous, oblong-elliptic, 9-19 × 3-6 mm. Ridges conspicuous 1-2 mm, 6-12 on dorsale, 2 on side (Figure 1). The fruit surface is occasionally wavy and striped, upper surface simple, short scaly. The stomata is observed (Figure 2).

Morphological features of *G. cordifolium* specimens collected from two different localities were compared with the description in Flora of Turkey, and some morphological features were found to be more variable than previously reported. When the plant samples collected from Kütahya and Karaman were evaluated morphologically, it was observed that they were highly similar. However, the leaves in the plant collected from Karaman were larger than those collected from Kütahya, and the pedicel and ray lengths were also higher. In addition, measurements related to many characters not in the Flora of Turkey were given in Table 1. While the stem length was reported to be up to 180 cm, it was indicated as 40-150 cm in Flora of Turkey. These differences are thought to be caused by geographical and climatic-edaphic factors (time of flowering, altitude, climate). When the findings were evaluated, it was determined that the dimensions of the plant parts were compatible with the Flora of Turkey data, in addition, the limits of variation of the species in the Flora of Turkey were expanded (Table 1). According to Burtt & Davis (1949), *Glaucosciadium*, which is distributed in the Old World, especially in Eurasia and Africa, resembles *Peucedanum*, a heterogeneous genus of 100-120 species, but the absence of commissural vittae shows that *Glaucosciadium* can certainly be isolated from *Peucedanum* (Pimenov & Leonov, 1993). The fruits of *G. cordifolium* are 9-14 × 5-6 mm and elliptic. Mericarp is elliptic in the transverse section which is winged in the lateral. Oil ducts were embedded in mesocarp. While 6-12 vittae were seen onthe dorsal side, there were no oil ducts on the commissural side. Fruit features almost agree with those reported in the Flora of Turkey (Hedge & Lamond, 1982). Fruit surface is occasionally wavy and striped, the upper surface is simple, short and scaly. The stomata is observed. Similarly, Saltan & Kaya (2020) reported that the fruit surface of *C. siifolium* Boiss. & Heldr is wavy and striped in places, simple, short and scaly.

In 2014, Zhou et al. examined the epidermal characters of 21 species of *Peucedanum* in the leaf using light microscopy and scanning electron microscopy, and found that stomata in all species were anisocytic, anomocytic or staurocytic in the abaxial epidermis, and in 12 species, there were stomata on both sides (Zhou, Wang, Gong & Liu, 2014). Zhang & He (2009) described six *Peucedanum* species growing in Southwest China and reported that *P. praeruptorum* had stomata only in the lower epidermis (Xuemei & Xingjin, 2009). On the other hand, it was reported that the leaves of *Ferula* (Karakaya, Bostanlık & Kılıç, 2018), *Prangos* (Ahmed, 2008) and *Seseli* (Güner & Duman, 2013) were monofacial as *G. cordifolium*.

The observation of different types of stomata (anomocytic, anisocytic and parasitic) on both leaf surfaces and the fact that the leaf is monofacial and mesomorphic show that our findings are similar to previous studies with close genera.

Anatomical properties

Stem anatomy

Transverse sections taken from the middle part of the stem were observed as follows. Stem were nearly round and ripped. The epidermis is composed of a single layer of oval, square, oblong to rectangular. A regular arrangement of cells in the epidermis is observed. Upper surface is covered with a thin cuticle. The cortex tissue consists of 5-8 layered chlorenchyma tissue (= photosynthesizing tissue) which comprises dense parenchyma cells containing chloroplasts and an ergastic substance located below the epidermis. Several rows of lightly crushed parenchyma cells and 3-4 layered parenchyma cells which contain an ergastic substance were located under this layer. Sclerenchyma tissue is well-developed below the parenchyma and located in groups with 10-13 layers. The collenchyma tissue, which consists of 12-15 layered ovoid or triangular

Table 1. Comparison of the morphological characters of *G. cordifolium.*

Characters	Kütahya province	Karaman province	Flora of Turkey (Hedge & Lamond, 1982)
Stem	34-180 cm	35-175 cm	40-150 cm
Basal leaves	Ovate-triangular 9-53 × 4-15 cm petiole 35-80 mm	Ovate-triangular 10-30 × 6-26cm petiole 50-80 mm	Ovate-triangular 10-40 × 7-25 cm
Leaflets	10-65 × 8-70 mm, sessile or up to 65 mm ovate sometimes reniform	35-63 × 30-90 mm sessile or up to 75 mm ovate sometimes orbiculate	1.8-8 × 1.9-9 cm ovate
Upper leaves	5-25 × 9 mm Sheat-like, sometimes elliptic-oblong	7-25 × 10 mm Sheat-like, sometimes elliptic-oblong	-
Bracts	4-25 × 2-10 mm linear-lanceolate	5-27 × 2-16 mm Lanceolate	-
Bracteoles	3, linear 1-5 × 0.5-3 mm	3-5, linear 1-5 × 0.5-3.5 mm	3-5
Ray	4-10 5-35 mm (in flowers) 17-100 mm (in fruits)	4-10 5-45 mm (in flowers) 19-110 mm (in fruits)	5-13 10-75 mm (in flowers)
Flowers	7-23 per umbellule	9-22 per umbellule	15- 25 per umbellule
Pedicel	0.5-5 mm (in flowers) 1-6 mm (in fruits)	1.5-7 mm (in flowers) 2-8 mm (in fruits)	-
Petal	White, 1-1.5 × 0.8-1 mm mm	White, 0,7-1 × 0,9-1 mm	-
Stamen **Anter**	5, free, versatile, bazifiks 0.3 × 0.5 mm, brown 0.5-1 mm, white	5 free, versatile, bazifiks 0.2 × 0.5 mm, brown 1.5-2 mm, white	-
Ginekeum	0.8-1 × 1.2-2 mm	0.8-1 × 1.5-2 mm	
Fruit	9-14 × 5-6 mm oblong elliptic, brown	9-19 × 3-6 mm oblong elliptic, brown	10-12 x 5-6.5 mm
Mericarps	subconvex 5 ridges 8-9 vittae in dorsal, 2-3 vittae in lateral commissures 8 vittae	subconvex 5 ridges valleculae 3-4 vittae commissures 6-8 vittae	oblong–elliptic 5 ridges
Stylopodium	0.4 × 0.9 mm, conical	0.5 × 0.9 mm, conical	-

cells, is located between epidermis, secretory canals and vascular bundles. The secretory canals, which surround 8-12 secretory cells, are embedded below the parenchyma cells. Vascular bundles are numerous (17-24) and arranged in a ring. Phloem and xylem are partly separated from one another by parenchyma and sclerenchyma tissue. Phloem consists of flattened, irregular cells, 8-10 layers. The pith located in the center of the stem is completely filled with large polygonal parenchymatic cells (Figure 3).

Leaf anatomy

Anatomical features have been revealed by examining the transverse and superficial sections of the leaf. In transverse sections, upper and lower epidermis comprise uniseriate rectangular to quadrangular cells. The upper walls are generally thicker than the lower and side walls, with both epidermal surfaces covered by a cuticle. Mid-rib was swollen and included a larger vascular bundle compared to the other parts of the mesophyll. Different types of stomata (anomocytic, anisocytic and parasitic) were observed on both surfaces of the leaf, which were located at the same level as the epidermal cells (mesomorphic) (Figure 5). While the leaf stomata index was 15.38 on the upper side, the lower side of the leaf was found to be 15.06. The parameters of stomata and epidermis were given in Table 2. According to Table 2, percent lower leaf epidermis and stomata number is similar for both faces. Leaf is monofacial. Mesophyll comprises 2-seriate palisade parenchyma cells which contain chloroplasts. 2-4 seriate spongy tissue is located between palisade tissue. 6-8 layers of collenchyma are located below lower epidermis. Secretory canals were surrounded by 8-12 layers of secretory cells. Vascular bundles were located in the parenchymatic tissue. Sclerenchyma is located between phloem and xylem (Figure 4).

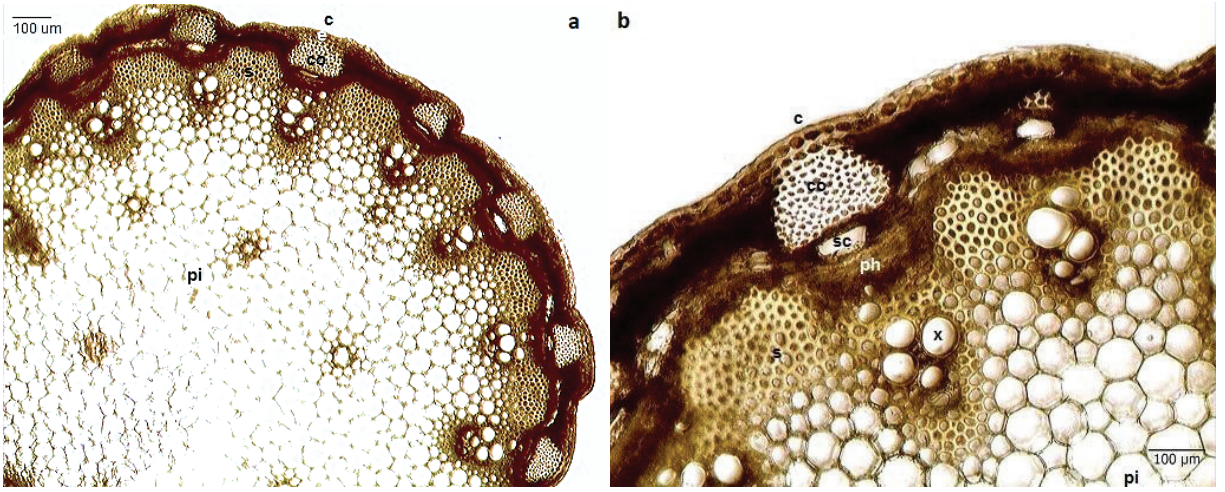

Figure 3. Cross-sections of the stem of *G. cordifolium* (a-Kütahya, b-Karaman): c- cuticle, e- epidermis, co- collenchyma, sc- secretory canal, s- sclerenchyma, ph- phloem, x-xylem, pi- pith.

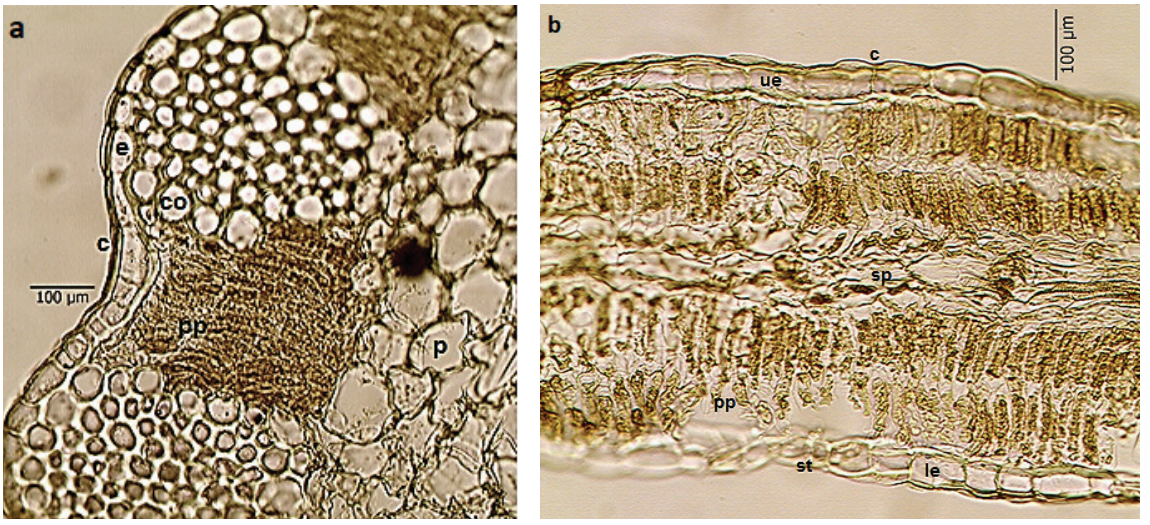

Figure 4. Cross-sections of the leaf of *G. cordifolium* (a- Kütahya, b- Karaman): c- cuticle, e- epidermis, co- collenchyma, p- parenchyma, pp- palisade parenchyma, sp- spongy parenchyma, le-lower epidermis, st- stomata, vb- vascular bundle, ue- upper epidermis.

Table 2. Comparison of epidermis and stomata in upper and lower surfaces of *G. cordifolium* leaves.

	Adaxial surface	Abaxial surface
Number of stomata (mm²)	12 (±2)	11 (±2)
Number of epidermis cells (mm²)	66 (±5)	62 (±5)
Stomata index (SI)	15.38	15.06
Stomata width (µm)	12-20	12-16
Stomata length (µm)	15-24	13-20
Width of epidermis cells (µm)	10-35	12-34
Length of epidermis cells (µm	19-49	14-55

Fruit anatomy

In the cross sections taken from the middle part of the fruit, it was seen that the fruit was a schizocarp with two mericarps. In addition, the mericarp is elliptic in the transverse section and is winged in the lateral. Exocarp (the outer epidermis) is composed of a single layer of square and rectangularly arranged cells.The upper surface is covered with a cuticle. Mesocarp consists of 4-6 layered, oblong-ovoid, polygonal parenchymatic cells with thin walls. Oil ducts (vittae) were embedded in the mesocarp. 6-12 vitae were seen on the dorsal side and there were no oil ducts on the commissural side. There was a vascular bundle in the wings. Collenchyma was located near the secretory canals and is small in clusters. The endocarp (the inner epidermis) consists of a single layer of narrow, long cells with thin walls. The testa was located under endocarp. The endosperm was well developed. The embryo was sunk into the endosperm (Figure 6).

There are less anatomical studies on vegetative organs compared to those on generative organs in the family Apiaceae. In this study, it was observed that the leaves of *G. cordifolium* were equifacial, while the leaves of *Chaerophyllum astrantiae* Boiss. & Bal. and *C. aureum* L. were reported to be bifacial by

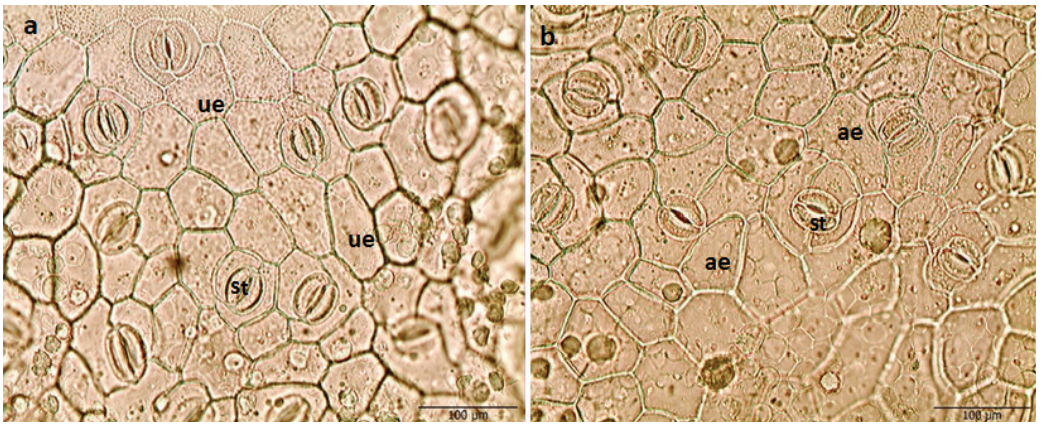

Figure 5. Surface-sections of the leaf of *G. cordifolium*: le-lower epidermis, st- stomata, ue- upper epidermis (a: upper surface, Kütahya, b: lower surface, Karaman).

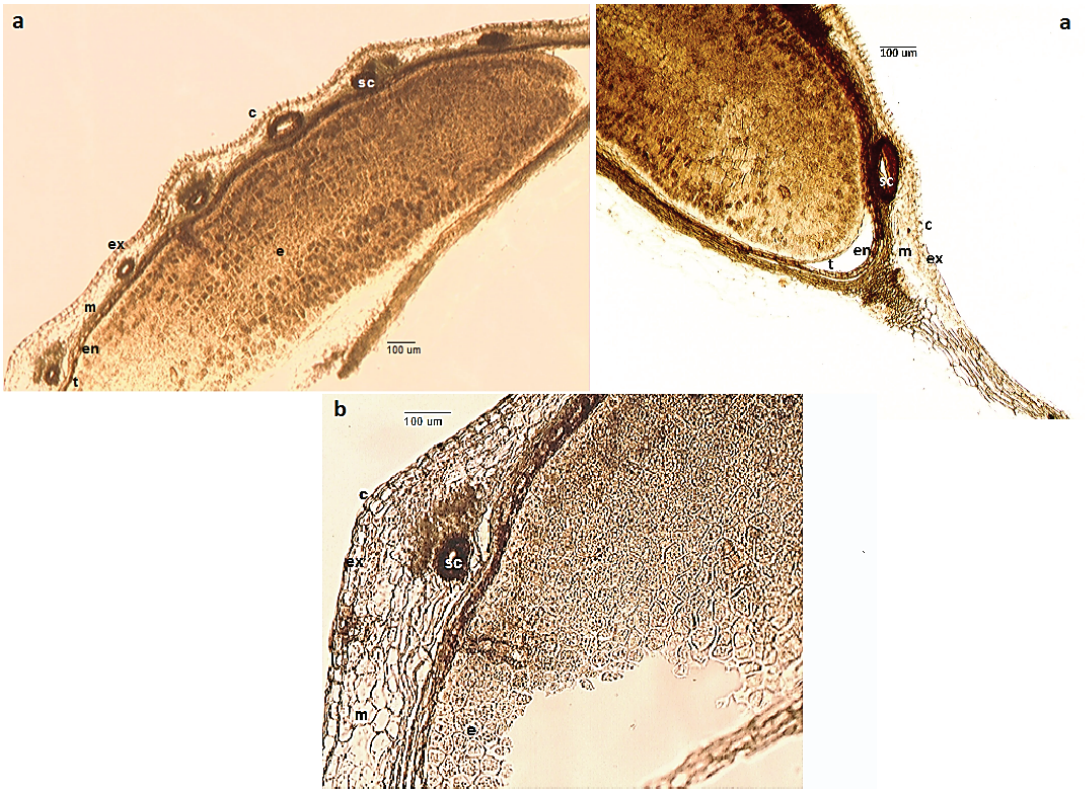

Figure 6. Cross-sections of the fruit of *G. cordifolium* (a-Kütahya, b-Karaman): c- cuticle, ex- exocarp, m- mesocarp, en- endocarp, sc- secretory canal, t- testa, e- endosperm.

Yılmaz & Tekin (2013). In a different study, it has been shown that the leaf of *C. siifolium*, which is another monotypic species in the Apiaceae family, is bifacial and that the epidermis cells only have wavy walls on the lower part of the leaf (Saltan & Kaya 2020). The results show that while mesophyll anatomy is not useful for interspecific classification, it has taxonomic importance in generic classification in the family Apiaceae.

While it has been reported that the collateral type vascular bundles are 13-17 in *C. siifolium*, and the pith consists of 7-10 cell rows, in *G. cordifolium* there are 17-24 vascular bundles and the piths are composed of 4-8 cell rows. In addition, it was stated that the parenchyma cells were fragmented and the pith was empty in *C. siifolium* (Saltan & Kaya 2020). However, in *G. cordifolium* the pith region is well developed and there are secretory channels in places.

There are 4 dorsal vallecular and 2 ventral commissural ducts in the mericarps of *Peucedanum* and *Chaerophyllum* species (Parolly & Nordt, 2005; Yılmaz & Tekin, 2013), 2-10 dorsal vallecular ducts and 2-6 commissural vittae in those of *Pimpinella* species in Iran (Khajepiri, Ghahremaninejad & Mozaffarian, 2010), but only 4 dorsal vitae (oil ducts) and no commissural vittae in the mericarps of *G. cordifolium*. The occurrence of sclerenchymatic

tissue in the mesocarp shows differences among the species in the family Apiaceae. In contrast to some *Peucedanum*, *Chaerophyllum* and *Angelica* species (Parolly & Nordt, 2005; Yankova, 2004; Yilmaz & Tekin, 2013) sclerenchyma is completely lacking in mericarp in the *Pimpinella* species in Iran (Khajepiri et al., 2010) as seen in *G. cordifolium*. Vascular bundles occur in the commissural or dorsal face of mesocarp in Apiaceae. While there are commissural vascular bundles in fruits of *Pimpinella* species (Khajepiri et al., 2010), commissural face of mesocarp lacks vascular bundles in *Peucedanum longibracteolatum* (Parolly & Nordt, 2005) as seen in *G. cordifolium*. In addition, both *Peucedanum longibracteolatum* and *G. cordifolium* include rudimentary oil ducts associated with the vascular bundles in their mericarps.

Palynological properties

The pollen grains of *G. cordifolium* were radially symmetrical, isopolar, and tricolporate. Their shape is euprolate-perprolate. In SEM, dimensions ranges were: polar axis 20.8-31.1 µm, equatorial axis 11.5-15.1 µm, colpus length 17.6-25.4 µm, colpus width 0.4-0.8 µm, pore length 0.8-1.0 µm and pore width 3.5-4.0 µm. The ratio of P/E (Polar axis/Equatorial axis) is 1.80-2.05 µm. The exine sculpturing is rugulate (Figure 7).

Identifying the pollen exine is useful for detecting relationships between species. Apiaceae is a stenopalynous family. The pollen grains and fruit surface belonging to species were firstly photographed via scanning electron microscope (SEM). Except for *G. cordifolium*, the pollen properties of the taxa studied were here described for the first time. Pollen morphology of the examined specimen exhibits some variation in size, shape and sculpture. Saltan & Kaya (2020) determined that the shape of *C. siifolium* pollen was perprolate and the ornamentation was rugulate-striat in the intermediate region and striate in the poles. Perveen & Qaiser (2006), who defined 3 types of pollen in the Apiaceae (Umbelliferae) family according to the exine pattern, determined that the pollen grains were generally tricolporate, the shape of the grains expanded to the prolate-perprolate and the P/E ratio varied between 1.2 and 2.6. P/E ratio is similar toour results (Perveen & Qaiser, 2006). Cerceau-Larrival (1962) identified the pollen of Umbelliferae with 5 types according to the P/E ratio. These types are as follows: subrhomboidal (type 1, P/E: 1-1.5), subcircular (type 2, P/E: 1-1.5), oval (type 3, P/E: 1.5-2), subrectangular (type 4, P/E: 2), and equatorially constricted (type 5, P/E: over 2). When our data is compared with previous studies with close genera, the pollen of *Glaucosciadium* is similar to type 3 with respect to the P/E ratio.

In conclusion, the morphological features of *G. cordifolium* were compared with those in two different localities and Flora of Turkey. Similar morphological features were observed in plants collected from both localities. When the characteristics of the plants collected from different localities were compared, the effect of environmental differences and climatic conditions on the botanical characteristics of the plant were revealed. In addition to the morphological description given in the book Flora of Turkey, the measurements of the new diagnostic char-

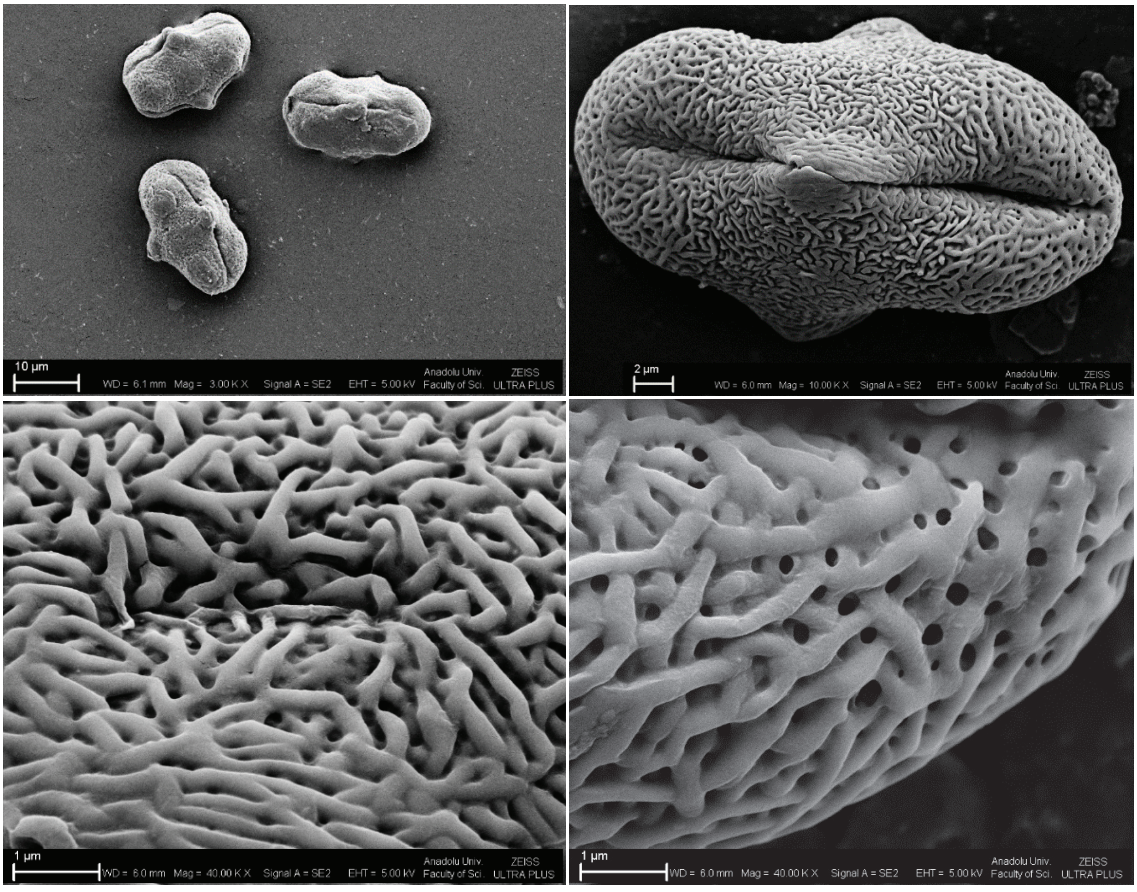

Figure 7. *G. cordifolium*, sculpturing of pollen grain in SEM (Saltan, 2015).

acters of *G. cordifolium* were revealed in this study. Moreover, the description of the studied samples were enlarged with this research. More clearly, the following characters were found to be important for distinguishing *G. cordifolium*: stem, leaf, bract and flower dimensions; the state of secretory canals, number of vascular bundles in the stem; leaf and stoma types and stomata index rates in the leaves; shape, surface ornemantation and number of vittae in the fruit; shape, size and exine sculpture in the pollen. We believe that the time of flowering, altitude, geographical and climatic-edaphic factors may be important. Various factors, both endogenous and exogenous, can affect the morphological characteristics of *G. cordifolium*. Also, the results of this study would support classification based on morphology.

CONCLUSION

Although studies with other Apiaceae species are frequently encountered in the literature, there is very little information about *G. cordifolium*. To our knowledge, this is the first comparative report on the morpho-anatomical and palynological features of *G. cordifolium*.

In this study, in addition to internal and external morphological features of *G. cordifolium*, detailed information is given about the pollen morphology and anatomical features of stem, leaf and fruit. Moreover, the descriptions of the species are expanded and the drawings of the morphological characters of the species, as well as photographs of their anatomical parts and pollen structures are presented for the first time in this study.

We believe that the obtained morphological findings will improve the morphology information of *G. cordifolium* for systematic purposes and the other findings can form a basis for future studies.

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Ethics Committee Approval: There are no ethical issues after the publication of this manuscript.

Author Contributions: Conception/Design of Study- N.S., M.D., S.D.; Data Acquisition- N.S.; Data Analysis/Interpretation- N.S., A.K.; Drafting Manuscript- N.S.; Critical Revision of Manuscript- N.S., A.K., M.D., S.D.; Final Approval and Accountability- N.S., A.K., M.D., S.D.

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

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