



**MORPHO-ANATOMICAL AND PALYNOLOGICAL STUDIES ON ENDEMIC
CRENOSCIADIUM SIIFOLIUM BOISS. & HELDR. FROM TURKEY**

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ABSTRACT

The genus *Crenosciadium* (Apiaceae) is represented by *C. siifolium* which is a monotypic endemic species, in the Flora of Turkey. In the current study, morphological, anatomical and palynological features of *C. siifolium* are described in detail. Expanded descriptions and images of *C. siifolium* are given. Cross-sections of stem, leaf and fruit are examined and a detailed anatomical description is presented and supported by photographs. Mericarps are almost pentagonal in transverse section. Each mericarp has 3-5 vittae on the valliculae and 6-8 vittae on the commissural. The pollen morphology of *Crenosciadium* is studied by SEM. The palynological results confirmed the stenopalynous characteristic of the family Apiaceae, and revealed that the pollen grains of the genus is perprolate shaped. Also fruit surface ornamentation of genus was rugulate-striate at equator and irregular striate at poles.

Keywords: Anatomy, Apiaceae, *Crenosciadium siifolium*, Morphology, Palynology

1. INTRODUCTION

The family Apiaceae is represented in Turkey by 105 genera belonging to 493 species [1]. It is one of the known families of flowering plants due to its inflorescence and fruit characteristics, and the diverse chemistry reflected odour, flavour and even toxicity of many of its members. According to Flora of Turkey [2], *Crenosciadium* Boiss. is a monotypic endemic genus, belonging in Apioideae subfamily. It is a perennial herb endemic to Turkey which is locally known as "Kırkısarak" in the regions where they grow [3]. Also it grows on damp meadows and stream sides in *Pinus nigra* forest at an altitude of 1400–1800 m and endemism category of it is endangered (EN) according to IUCN [4].

According to Bentham & Hooker (1867), it was closely allied to and possibly congeneric with *Opopanax* W. Koch [5]. The genus *Opopanax* Koch (1824) is represented by three species, which are distributed in southern Europe, the Mediterranean region and Western Asia which have been used by local people in folk or traditional herbal medicines [6]. Actually, *Crenosciadium* apparently differs from *Opopanax*, in having convex mericarp, white flower, 1-pinnate basal leaves, these characters which would clearly differentiate the two genera. *Crenosciadium* was described to accommodate *C. siifolium* which was said to differ considerably from *Opopanax* [7]. In 2012, Yusuf Menemen transferred *C. siifolium* to the genus *Opopanax* and identified as *Opopanax siifolius* (Boiss & Heldr.) Menemen. In "Türkiye Bitkileri Listesi" [3], *Crenosciadium* and *Opopanax* were synonymised, the latter name having priority at genus rank. Our findings, together with previous studies and molecular data, support that *Crenosciadium* differs from *Opopanax* considerably.

Anatomy and morphology are taxonomically very important in Apiaceae and nearly all traditional classification systems of the family have relied on especially fruit characteristics. In the previous study, mericarps anatomical structures of *C. siifolium* were studied. However, this paper, besides the internal and external morphological features of *C. siifolium*, is to give detailed information about the morphology

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Received: 20.03.2019 Published: 31.01.2020

of pollen as well as anatomical features of the stem, leaf and fruit. It is expected that it will provide an important information to the taxonomic studies and will contribute for further investigation in the future. Our study aims to resolve the unclear and controversial status of the genera *Crenosciadium* based on their morphological, micromorphological, palynological and anatomical characteristics. Furthermore, our findings will contribute to the systematics of the Apiaceae family. In addition, by comparing with previous studies, the effect of environmental differences and climatic conditions on the botanical characteristics of the plant will be revealed.

2. MATERIAL AND METHODS

2.1. Plant Material

C. siifolium was collected from Kütahya province, Turkey during the flowering period. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University, in Eskişehir, Turkey (ESSE 15000).

2.2. Morphology

Descriptions of species are based on living material. All measurements were made directly from herbarium specimens. Measurements performed on 20 different specimens. General view, flower-fruit in compound umbel, single flower-fruit and petal of specimen had been drawn. Drawing of plant parts had made by drawing tube of a Olympus SZX12 stereomicroscope.

2.3. Anatomy

Living material was stored in 70% ethanol for anatomical studies. All sections of leaves, stems and fruits were made manually. The transverse sections were stained by Sartur reactive [8] and embedded in glycerin-jelatine, then mounted on microscope slides with Canada Balsam. For each structure, at least 30 preparations were observed, and their photographs were taken through a light microscope (Olympus BX51T). The number of stomata and epidermal cells in a unit area was determined and stoma index (SI) as percent was calculated [9].

2.4. Palynology

For scanning electron microscopy (SEM), the pollen grains were mounted directly on stubs by using double-sided adhesive tape and coated with gold. Photographs were taken with a Zeiss Ultra Plus Fesem. The pollen terminology was adopted from Faegri and Iversen and Punt et al [10, 11].

3. RESULTS

3.1 Morphological properties

The plant is perennial herb with a creeping rootstock. Stems erect, simple or branched, 38-68 cm long, terete, striate and glabrous (Figure 1). Leaves mostly basal, oblong in outline, 8-25 x 4-10 cm, 1-pinnate, petiole 4.6-13 cm. Leaflets are 2-3 paired and usually oblong-obovate, rarely linear-elliptic, 7-34 x 6-21 mm, dentate or crenate, apex mucronate, base acute-obtuse, sessile or up to 12 mm. Upper leaves sheath-like, linear-lanceolate, 9-32 x 4-11 mm. Bracts 1-2, linear-lanceolate, apex mucronate, base with ocrea, 5-27 x 2-16 mm. Bracteoles 3, linear, apex acute, base obtuse, 1-5 x 0.5-3 mm. Inflorescence compound umbel, umbels unequally 3-5 rayed, rays 5-13 mm in flower, 6-30 mm in fruit (Figure 2). Flowers 7-15 per umbel. Pedicel 0.5-4 mm in flowers, 2-6 mm in fruits. Corolla consists of five free petals which are white, striated in the middle, 0.8-1.0 x 0.4-0.5 mm. Stamens five, free. Anthers brown, dorsifixed, sometimes versatile. Filaments white, 1.0-1.5 mm. Gynoecium 1.0-1.5 x 0.8-1.2 mm. Stigma bipartite.

Ovary inferior, of 2-united carpels. Fruit is a dry schizocarp which splits down a septum into two one-seeded mericarps, 4-6 x 1.5-2 mm, brown, glabrous and elliptic. Mericarps two, homomorphic, elliptic, subconvex, conspicuous 5-ridges (Figure 1). 8-9 vittae in dorsal, 2-3 vittae in lateral and 8 vittae in commissures. Stylopodium 0.4 x 0.9 mm, conical. Fruit surface is wavy and striped, upper surface is simple, short scales (Figure 2).

Flowering time: July-August

Habitat: Damp meadows and stream sides in *Pinus nigra* forest

Altitude: 1400-1800 m

Red data category: Endangered (EN)

Endemic [2, 4].



Figure 1. *C. stitfolium*, ESSE 15000: a-compound umbel-flower, b-flower- bud, c- flower, d-petal, e-compound umbel- fruit, f-fruit.

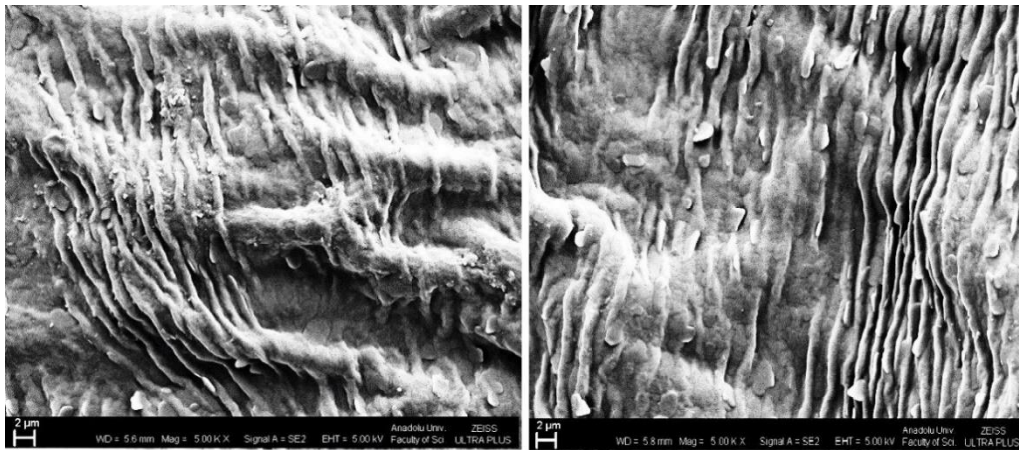


Figure 2. Scanning electron micrographs (SEM) of fruit surface of *C. siifolium*

3.2. Anatomical Properties

3.2.1. Stem

Transverse sections taken from the middle part of the stem were observed as follows (Figure 3): The stem is almost round and ribbed. The epidermis is composed of a single layer of oval, rectangle or square compactly arranged cells. The upper surface is covered with a thin papillose cuticle. The collenchyma tissue is located under the epidermis in ribs. The shape of the collechyma cells is ovoid-quadrangular and 6-9 layers in between the epidermis and secretory canals. Apart from the collenchyma, the cortex tissue consists of 3-5 layered chlorenchyma tissue (=photosynthesizing tissue) which comprises dense parenchyma cells containing chloroplasts and ergastic substance under the epiderma. Secretory canals which are surrounded by 5-8 secretory cells are embedded in continuing collenchyma. The parenchyma tissue is composed 4-9 layered of big ovoid-quadrangular cells, forming a ring. Sclerenchyma tissue is well-developed below the parenchyma, 7-8 layered. Numerous collateral type vascular bundles (13-17) are arranged in a ring. Phloem and xylem partly separated from one another by sclerenchyma and ground tissue. Xylem consists of vessles and tracheids and is embedded in ground tissue. Phloem consists of 5-7 layered and is embedded in sclerenchyma tissue. Rays are multiseriate, usually 7-10 cells wide. Pith consists of large orbicular parenchymatous cells often with abundant intercellular spaces. The centre of pith is ripped (Figure 3).

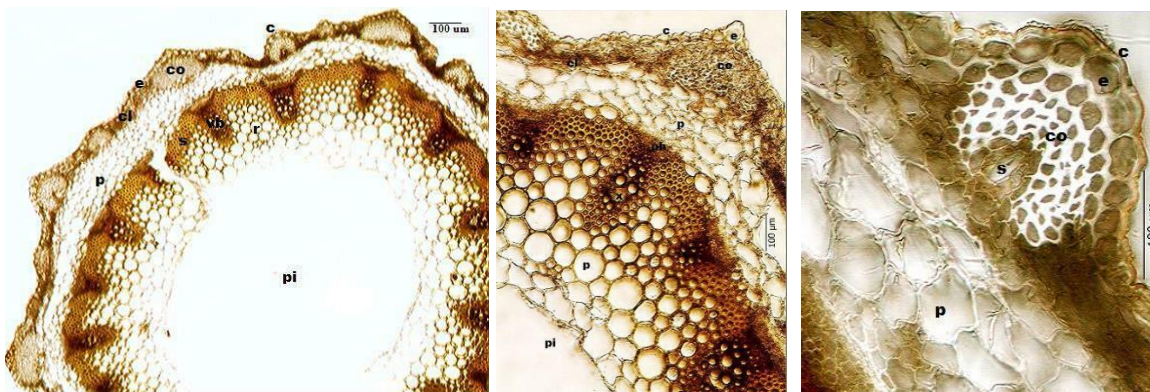


Figure 3. Cross-sections of stem of investigated *C. siifolium*: c- cuticle, e- epidermis, co- collenchyma, cl- chlorenchyma, p- parenchyma, s- sclerenchyma, sc- secretory canal, ph- phloem, x- xylem, vb-vascular bundle, r- rays, pi- pith.

3.2.2. Leaf

Transverse sections of lamina and midrib and surface preparations of both epidermal surfaces revealed the following elements; in transverse sections, upper and lower epidermis comprise uniseriate rectangular and square cells. Upper walls are usually thicker than lower and lateral walls. Cells of the upper epidermis are longer than those of the lower epidermis. Both epidermal surfaces are covered with a cuticle. The different types of stomata (anomocytic, anisocytic and paracytic) are observed on both of leaf surfaces. They are located on the same level with epidermal cells (mezomorphic) (Figure 4). Comparison of epidermis and stomata in upper and lower surfaces of *C. siifolium* leaves was given in Table 1. The stomatal index is 17.30 on upper surface and 18.25 on lower surface. The stomata and epidermis numbers are almost equal in both surfaces. The width and length of stomata are almost same in both surfaces. However, the width of epidermis cells on upper side is smaller than those of the lower and the length of epidermis cells on upper side is more than lower side. The upper anticlinal cell walls are straight or slightly undulate as the lower anticlinal cell walls are deeply undulate. Leaf is bifacial. Mesophyll is differentiated into 1-seriate palisade and 4-6 seriate spongy tissue. Vascular bundle is collateral type and less developed. Xylem faces the upper surface as phloem faces the lower epidermis. Vascular bundles are surrounded by a parenchymatous bundle sheath. Secretory canals are located on upper and lower sides of vascular bundles which are surrounded by of 7-12 layered secretory cells. The collenchymatous cells are found between the epidermis and secretory canals (Figure 4).

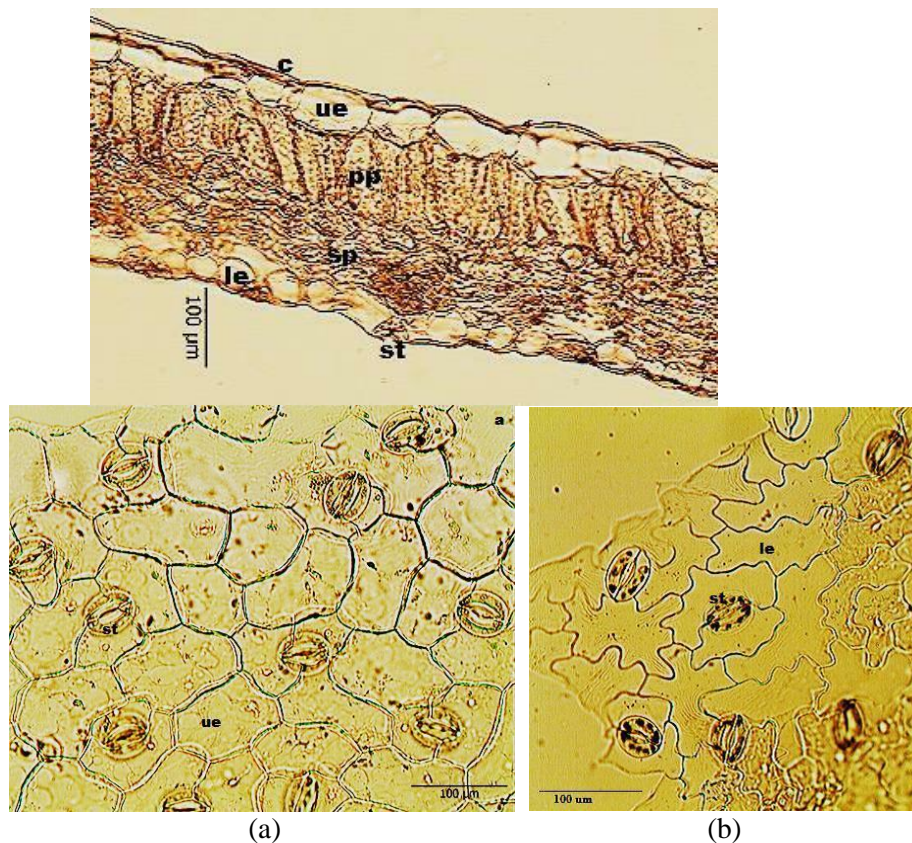


Figure 4. Cross-sections of leaf of investigated *C. siifolium* (a: upper surface, b: lower surface): c-cuticle, ue-upper epidermis, pp-palisade parenchyma, sp-spongy parenchyma, le-lower epidermis, st-stomata.

Table 1. Comparison of epidermis and stomata in upper and lower surfaces of *C. siifolium* leaves.

	Adaxial surface	Abaxial surface
Number of stomata (100 mm ²)	7-11	10-13
Number of epidermis cells (100 mm ²)	34-52	46-57
Stomata index (SI)	17.30	18.25
stomata width (µm)	15-17	13-15
stomata length (µm)	16-22	16-21
width of epidermis cells (µm)	15-35	18-25
length of epidermis cells (µm)	23-55	30-71

3.2.3.Fruit

Transverse sections taken from the middle part of the fruit were observed as follows (Figure 5): Each mericarp is almost pentagonal in transverse section. Exocarp (the outer epidermis) is composed of a single layer of square, rectangularly arranged cells. Upper surface is covered with a papillose cuticle. Epidermal surface is glabrous but has almost short scale in SEM photographs (Figure 2). Fruit ribs are five (include one dorsal rib two lateral ribs and two marginal ribs) and prominent. Mesocarp consists of 3-5 layered, oblong, quadrangular parenchymatous cells with thin walled. Each mericarp has 3-5 vittae on the valleculeae and, 6-8 on the commissural. The oil ducts in the mesocarp are surrounded by polyhedral cells. All vittae usually larger than vascular bundle, sometimes equal to them rarely smaller. Vascular bundles are located on rib. Endocarp (the inner epidermis) consists of a single layer of narrow-long cells with thin walled. Testa is located under endocarp. Endosperm is well-developed (Figure 5).

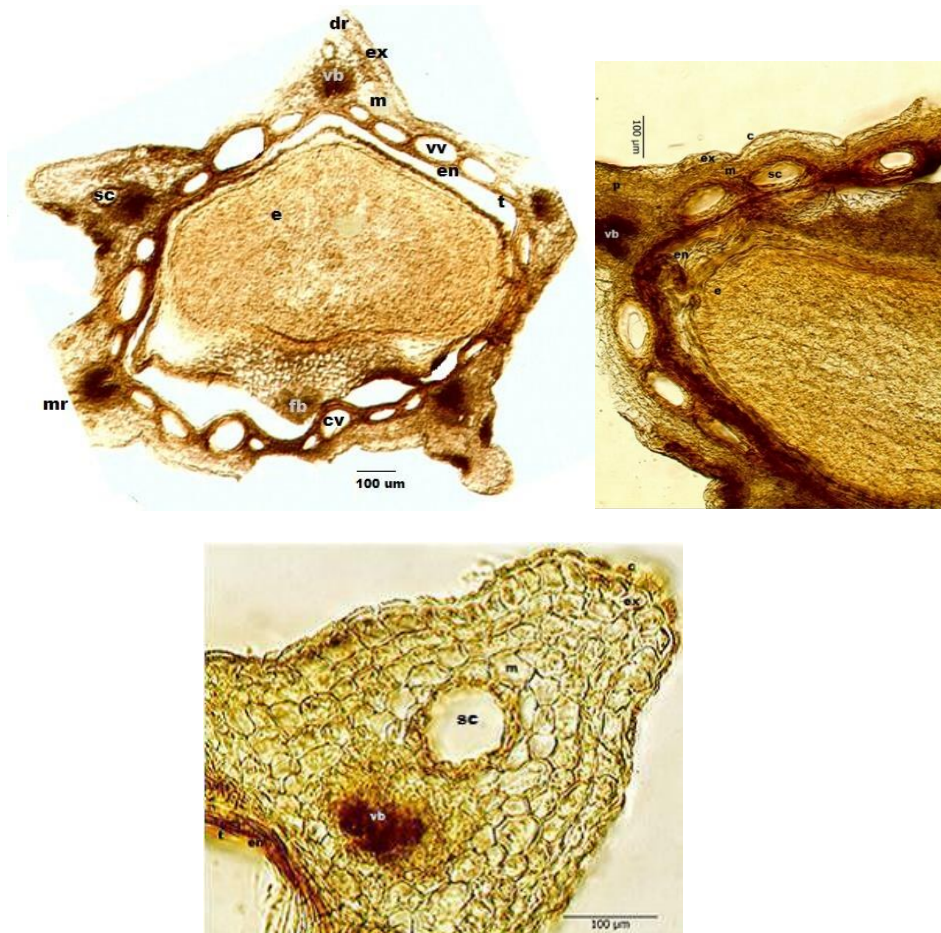


Figure 5. Cross-sections of fruit of investigated *C. siifolium*: c- cuticle, ex- exocarp, m- mesocarp, en- endocarp, vb- vascular bundle, sc- secretory canal, t- testa, e- endosperm, fb- funicular bundle, vv- vallecular vittae, cv- commissural vittae, mr- marginal rib, dr- dorsal rib.

3.3. Palynological Properties

The pollen morphology of *C. siifolium* confirmed the stenopalynous characteristic of Apiaceae, the pollen grains of genus being prolate in shape. The pollen grains of *C. siifolium* are radially symmetrical, isopolar, tricolporate. In SEM, dimensions ranges are: polar axis 25.0-36.8 μm , equatorial axis 11.1-13.6 μm , colpus length 14.4-20.4 μm , colpus width 0.4-0.9 μm , pore length 3-4.6 μm and pore width 2-4.4 μm . The ratio of P/E is 2.25-2.70 μm . The exine sculpturing is rugulate-striate in the equatorial area and irregular striate at the poles (Figure 6).

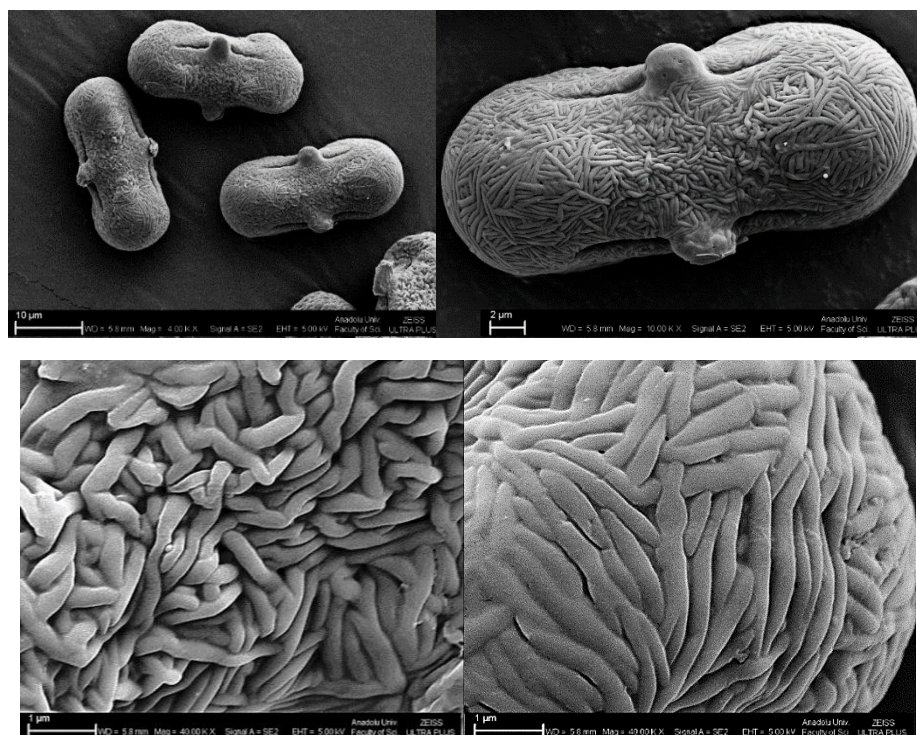


Figure 6. *C. siifolium*, sculpturing of pollen grain in SEM

4. DISCUSSION

Crenosciadium is a monotypic endemic genus, which is distributed in Central Anatolia. Since its description [12], a detailed study about its anatomy on it had never been conducted. In this paper, besides the internal and external morphological features of *C. siifolium*, is to give detailed information about the morphology of pollen as well as anatomical features of the stem, leaf and fruit. We reported the findings of a morphological study of *C. siifolium* so as to improve the present knowledge of morphology for systematic purposes. In addition, the effects of environmental factors on the botanical characteristics of species were compared based on previous studies.

Bentham & Hooker (1867) considered that *Crenosciadium* is closely allied to and possibly congeneric with *Opopanax* [5]. According to Chamberlain, *Crenosciadium* differs from *Opopanax* in having convex, not flattened mericarps, a character that would clearly differentiate both genera. In addition, the flowers of *C. siifolium* are white-coloured as yellow-coloured in *Opopanax* and they are separated by the colour of flower in genera key of Apiaceae in the Flora [2]. Furthermore both genera are separated by presences or absences of fibrous collar, 1 or 2-pinnate basal leaves and number of bract according to Flora. The number of rays ranges from 5–20 in *Opopanax hispidus* (Friv.) Gris., from 7–25 in *O. chironium* (L.) W. Koch, and from 8–14 in *O. persicus* Boiss., whereas it ranges from 3–7 in *Crenosciadium*. While mericarp width of *Opopanax* was reported 3–9 mm, mericarp width of *Crenosciadium* was found approximately 2 mm. In addition the mericarp of *Opopanax* was informed dorsally flattened, although fruit of *Crenosciadium* is laterally

compressed and *Crenosciadium* has five ribs, whereas the genus of *Opopanax* have three dorsal ridges [13]. In 2012, Yusuf Menemen transferred *C. siifolium* to the genus *Opopanax* and identified as *Opopanax siifolius* (Boiss. & Heldr.) Menemen. But, this name is unresolved according to The Plant List [14].

The comprehensive molecular phylogenetic analysis of *Opopanax*, based on ITS sequence [15], showed that *Opopanax* and *Smyrniopsis* Boissier formed a strongly supported clade in a monophyletic “*Opopanax* group”. In addition, Ajani et al. (2008) reported that their genotype was close to that of *Opopanax* but their relationship was not very clear [15]. Cetin and Celik (2018) compared the features of *Crenosciadium* with *Opopanax* and indicated that *Crenosciadium* differs considerably from *Opopanax*, and therefore it should be accepted at genus rank. The leaves are usually 1-pinnate in *Crenosciadium* and 2-pinnate in *Opopanax* [13].

Morphological results have been compared to in previous studies and those published in the Flora of Turkey [2, 13]. While the stem length was reported up to 120 cm, it was indicated 15-25 cm in Flora of Turkey. Although, we measured 38-68 cm in our study. These differences are thought to be caused by geographical and climatic-edaphic factors (time of flowering, altitude, climate). The other sizes of plant parts was reported that similar to previous study. According to result of this study, the variation borders of species in Flora of Turkey was expanded. The other features are consistent with previously published datas (Table 2).

Table 2. Comparison of the morphological characters of *C. Siifolium*

Characters	Our results	Flora of Turkey (1972)	Cetin & Celik (2018)
Stem	38-68 cm	15-25 cm	Up to 120 cm
Stem hairy	glabrous	glabrous	glabrous
Basal leaves	oblong in outline 8-25 x 4-10 cm petiole 4.6-13 cm	oblong in outline 5- 8 cm with petiole	lanceolate-oblong (5-) 15-45 × 3-6 cm (incl. petiole)
Leaflets	7-34 x 6-21 mm, sessile or up to 12 mm oblong-obovate sometimes linear- elliptic	10-18 mm - obovate	-
Upper leaves	9-32 x 4-11 mm Sheat-like, sometimes linear- lanseolate	-	-
Bracts	5-27 x 2-16 mm linear-lanseolate	- linear-lanseolate	3-7 × 0.5-1 mm linear
Bracteoles	3, linear 1-5 x 0.5-3 mm	3-5, linear -	3-5
Ray	3-5 5-13 mm (in flowers) 6-30 mm (in fruits)	3-5 - -	3-7 1-4 cm
Flowers	7-15 per umbellule	5-10 per umbellule	(5-)10-15 (20) per umbellule
Pedicel	0.5-4 mm (in flowers) 2-6 mm (in fruits)	- -	
Petal	White, 0.8-1x 0.4-0.5 mm	white	White, 1-1.3 × 0.4-0.6 mm
Stamen	5, free	-	
Anther	0.4-0.5 x 0.3- 0.5 mm, brown	-	0.5-0.7 mm
Filament	1-1.5 mm, white	-	1.2-2 mm
Gynoecium	1-1.5 x 0.8-1.2 mm	-	-
Fruit	4-6 x 1.5-2 mm, elliptic, brown, glabrous	6 x 2 mm elliptic, glabrous	5-6 × 1.5-2 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 x 0.9 mm, conical	conical	narrow conical

Usual features of Apiaceae anatomy are observed in anatomical studies, e.g. there is always a ring of vascular bundles in the stem, isolated bundles are separated from one another by ground tissue, secretory canals are embedded in paranchyma tissue, the stomata are accompanied by variously orientated subsidiary cells and the different types of stomata (anomocytic, anisocytic and paracytic) are observed on the same preparations [16]. Detailed anatomical description of stem and leaf of *C. siifolium* was presented and supported by photographs in this study. The plant has bifacial type of leaves. Secretory canals are also observed in the stem and leaf. On the basis of the stem and leaf anatomy of *C. siifolium* are almost similar to those previously described in Apiaceae species, namely *Olymposciadium caespitosum* (Sm.) Wolff, *Astrantia* L., *Chaerophyllum* L. and *Kundmannia* Scop. [17-20].

Most systematists agree that data concerning the macro- and microstructure of fruits are very significant for the classification of Angiosperm taxa. The external and internal fruit features of Apiaceae as fruit shape, the number of mericarp ribs, the number of vittae, endosperm shape, the distribution of calcium oxalate crystals, cotyledon shape, aerenchyma and sclerenchyma in the walls of the fruit are more important their taxonomy [21, 22]. According to our study, the fruits of *C. siifolium* are 4-6 x 1.5-2 mm, glabrous and elliptic. Mericarps are subconvex, conspicuous ridges and they are almost pentagonal shaped in transverse section. They have 5 strongly projecting ribs. The results are similar to previous study. Each mericarp has 3-5 vittae on the valleculae and 6-8 on the commissural. Fruit features are almost agree with those reported in the Flora of Turkey [2].

Pollen characteristics have shown great taxonomic significance in Apiaceae species which pollen characters have been mentioned by many authors [10, 23-25]. It is known that Apiaceae is a stenopalynous family [21]. Pollen of the genus *Heptaptera* Marg. and *Reutereptettera* was studied by Yılmaz et al. (2009) and they found the pollen to be perprolate-shaped. In addition, they observed a rugulate-striate sculpturing at equator and irregular striate at poles [26]. Cetin and Celik were reported that the pollen grains of *C. siifolium* are isopolar and the shape is perprolate and the ornamentation is rugulate [13]. Guner et al. (2011) were reported pollen morphology of the *Seseli* L. in Turkey and recognized 3 pollen types in *Seseli* on the basis of exine pattern. Type 1, rugulate at equator, psilate-perforate at pole; Type 2, striate-reticulate at equator, rugulate at pole; and Type 3, rugulate at equator, striate at pole. Our pollen exine pattern is almost similar to Type 3 [27]. According to Perveen and Qaiser (2006), pollen grains usually radially symmetrical, isopolar, prolate to perprolate [25]. Our results confirm the stenopalynous characteristic of the family Apiaceae, and the palynological observations reveal that the pollen grains of all studied members of *Crenosciadium* is perprolate in shape and rugulate rugulate-striate at equator. The pollen morphology of *C. siifolium* is similar to findings of previous studies. In addition, the pollen properties of *C. siifolium* are almost similar to those previously described in other Apiaceae species, namely *Malabaila lasiocarpa* Boiss., *Smyrniium* L. and *Ferulago nodosa* (L.) Boiss. [28-30].

In conclusion, the following characters were found to be important for distinguishing *C. siifolium*: stem, leaf, bract and flower dimensions; the state of secretory canals, number of vascular bundles in the stem; leaf and stoma types and stoma index rates in the leaves; shape, surface ornamentation and number of vittae in the fruit; shape, size and exine sculpture in the pollen. Various factors, both endogenous and exogenous, can affect the morphological characteristics of *C. siifolium*. We believe that the time of flowering, altitude, geographical and climatic-edaphic factors may be very important. The morphological, micromorphological, palynological and anatomical results presented in this study clearly confirm that enough differences exist between *Crenosciadium* and *Opopanax*, which together with reported results by Cetin and Celik (2018) and Ajani et al. (2008), support their treatment as distinct genera.

ACKNOWLEDGEMENTS

The authors are grateful to Scientific Research Projects of Anadolu University (Project No. 1406S314) for financial support and would like to thank Dr. Burcu ARPABAY for her technical support with the SEM.

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