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Morphological, Micromorphological, and Anatomical Investigations on the Genus *Physospermum* (Apiaceae) from Turkey

Türkiye Physospermum (Apiaceae) Cinsi Üzerine Morfolojik, Mikromorfolojik ve Anatomik İncelemeler

Mustafa Çelik[1*], Özlem Çetin[2]

[1]Selçuk University, Advanced Technology Research and Application Center, Konya, Turkey
[2]Selçuk University, Science Faculty, Department of Biotechnology, Konya, Turkey

Abstract

Physospermum Cusson ex Juss. is a small genus of Apiaceae that is represented in Turkey by only one species, *P. cornubiense* (L.) DC. In this study, the morphological, micromorphological, and anatomical properties of *P. cornubiense* were investigated and the morphological findings were compared with the description in *Flora of Turkey and the East Aegean Islands* (Chamberlain 1972). Some differences between our study and the description in *Flora of Turkey and the East Aegean Islands* (Chamberlain 1972) are presented, as well as flowering time, a distribution map, and the photos of natural habitat. Also, the pollen characteristics and the mericarp surface of *P. cornubiense* were examined using a Scanning Electron Microscope (SEM). In addition, its fruit anatomy was studied for the first time in detail.

Keywords: Fruit anatomy, Palynology, *Physospermum*, SEM, Umbelliferae

Öz

Physospermum Cusson ex Juss., Apiaceae familyasının küçük bir cinsi olup Türkiye'de sadece *P. cornubiense* (L.) DC. türü ile temsil edilmektedir. Bu çalışmada, *P. cornubiense* (L.) DC. türünün morfolojik, mikromorfolojik ve anatomik özellikleri incelenmiş ve morfolojik bulgular *Flora of Turkey and the East Aegean Islands* (Chamberlain 1972) isimli eserde yer alan betim ile karşılaştırılmıştır. Arazi fotoğrafları, yayılış haritası, çiçeklenme zamanının yanı sıra çalışmamız ile *Flora of Turkey and the East Aegean Islands* (Chamberlain 1972) isimli eserde yer alan betim arasındaki bazı farklılıklar verilmiştir. Ayrıca *P. cornubiense* türünün polen özellikleri ve meyve yüzeyi Taramalı Elektron Mikroskobu ile incelenmiştir. Ek olarak meyve anatomisi detaylı olarak ilk kez çalışılmıştır.

Anahtar Kelimeler: Meyve anatomisi, Palinoloji, *Physospermum,* SEM, Umbelliferae

1. Introduction

The flowering plant family Apiaceae Lind. (Umbelliferae Juss.) comprises approximately 450 genera and 3700 species (Pimenov and Leonov 1993, Amiri and Joharchi 2016). It is widely distributed in the temperate zones of both the northern and southern hemispheres and is highly diverse in Central Asia (Liu et al. 2014). Apiaceae comprises 105 genera and approximately 495 species in Turkey and includes the following three monotypic endemic genera; *Crenosciadium* Boiss. & Heldr., *Aegokeras* Raf. and *Postiella* Kljuykov. The numbers of endemic taxa are 191 species belongs to 45 genera. The endemism ratio in Turkey is 38% (Güner et al. 2012, Lyskov et al. 2017).

*Corresponding author: mstfclk.54@gmail.com

Mustafa Çelik orcid.org/0000-0002-2708-9944
Özlem Çetin orcid.org/0000-0003-2886-3409

Physospermum belongs to the Apiaceae family and is distributed in Europe, the Mediterranean region, and Western Asia (Meikle 1977). The genus is represented by only one species in *Flora Iranica* (Rechinger 1987) and *Flora of Cyprus* (Meikle 1977), and by two species in *Flora Europaea* (Tutin 1968). There is only one species, *P. cornubiense* (L.) DC., in Turkey (Menemen 2012).

Physospermum verticillatum Vis. and *P. cornubiense* are used as flavorings and sweeteners (Quezel 1956, Quezel and Santa 1963). *Physospermum cornubiense* is used to prepare liqueurs and to flavor different types of cake make (Pardo-de-Santayana et al. 2006).

There are many studies on the antioxidant, antihemolytic, anticancer, and anti-inflammatory properties of *Physospermum*. Ebrahimzadeh et al. 2009 evaluated the antioxidative and antihemolytic potential of hydroalcoholic from *P. cornu-*

biense extract, Bencheraiet et al. (2012) isolated flavonoids from *P. acteaefolium*, and Boulacel et al. (2017) evaluated secondary metabolites and antibacterial activity of *P. verticillatum*, but there are no detailed morphological, anatomical, and palynological studies.

The aim of our study is to investigate the morphological, palynological, micromorphological and anatomical characteristics of the *Physospermum cornubiense*, and we think that our findings will contribute to the systematics of the genus *Physospermum*.

2. Material and Methods

Morphological study: Specimens belonging to *Physospermum* were collected from different localities in Turkey (Appendix 1) and plant samples were identified according to Tutin 1968, Chamberlain 1972, and Rechinger 1987. In the morphological description below, each numerical value represents the average of 10 measurements from different specimens. The abbreviations were verified from *Authors of Plant Names* (Brummitt and Powell 1992).

Micromorphological study: Pollen slides were prepared according to Wodehouse 1935. Measurements were taken with a Leica microscope are based on 20 samples. For Scanning Electron Microscope (SEM) investigations, fruit and pollen grains were mounted directly onto the prepared stubs and coated with gold. Using a Zeiss LS-10 after coating with a Polaron SC7620 sputter coater, photographs of the specimens were taken and the terminologies of Erdtman 1952 and Faegri and Iversen 1975 were used for the SEM aspects.

Anatomical study: Mature mericarps of *Physospermum cornubiense* were kept in 70 % ethanol for using anatomical studies. Each mericarp was rehydrated and placed formalin-acetic acid-alcohol (1:1:8) for a minimum of 24 h. Rehydrated materials were embedded into paraffin blocks following the traditional paraffin section method. Transverse sections cut about 10 µm thickness using with a microtome, and stained safranin solution. Micrographs were taken using a light microscope.

3. Results

Physospermum cornubiense (L.) DC., Prodr. 4: 246 (1830). Turkish name: kızbara. Figure 1-4.

Syn.: *Ligusticum cornubiense* L., Cent. PI. 2: 13 (1756). *Coriandrum aquilegiifolium* All., Auct. Syn. Meth. Stirp. Hort. RegiiTaur.: 81 (1773). *Smyrnium nudicaule* M.Bieb., FI. Taur. Cauc.1. 238 (1808). *Physospermum aquilegifolium* (All.) W.D.J.Koch, Nova Acta Phys.-Med. Acad. Caes.

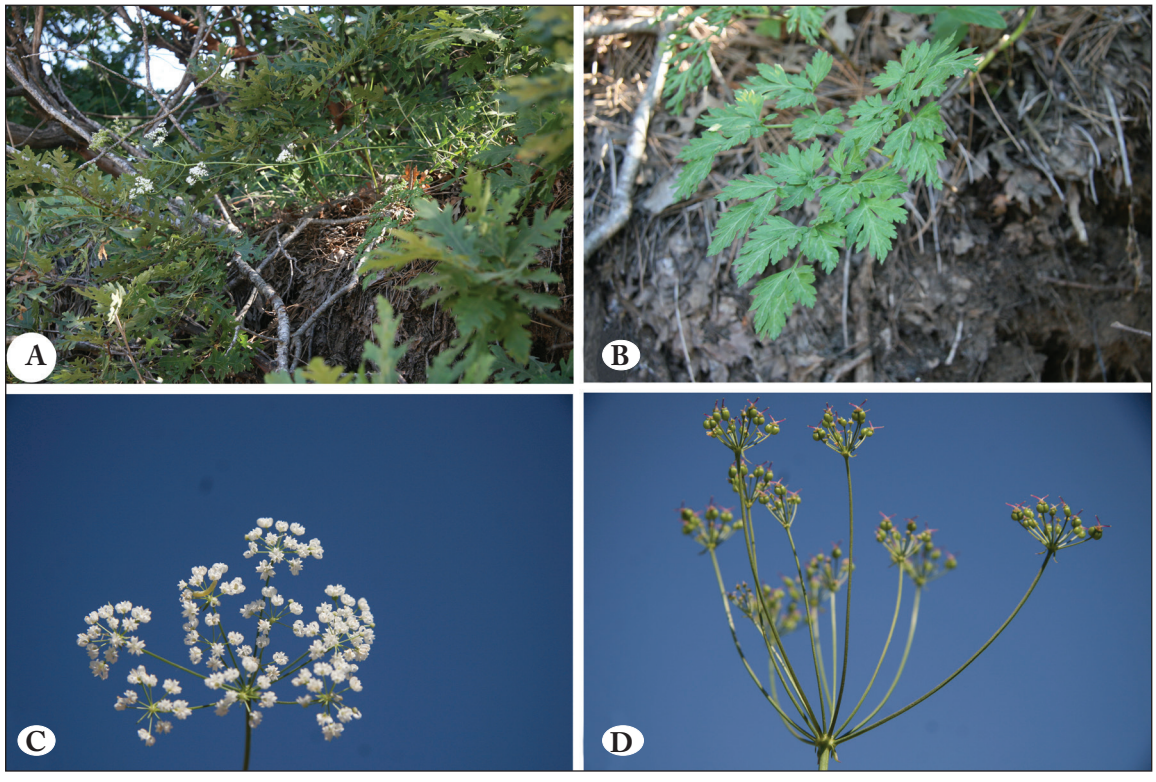

Figure 1. *Physospermum cornubiense.* **A)** general view of habitat, **B)** leaf, **C)** flowers, **D)** umbel.

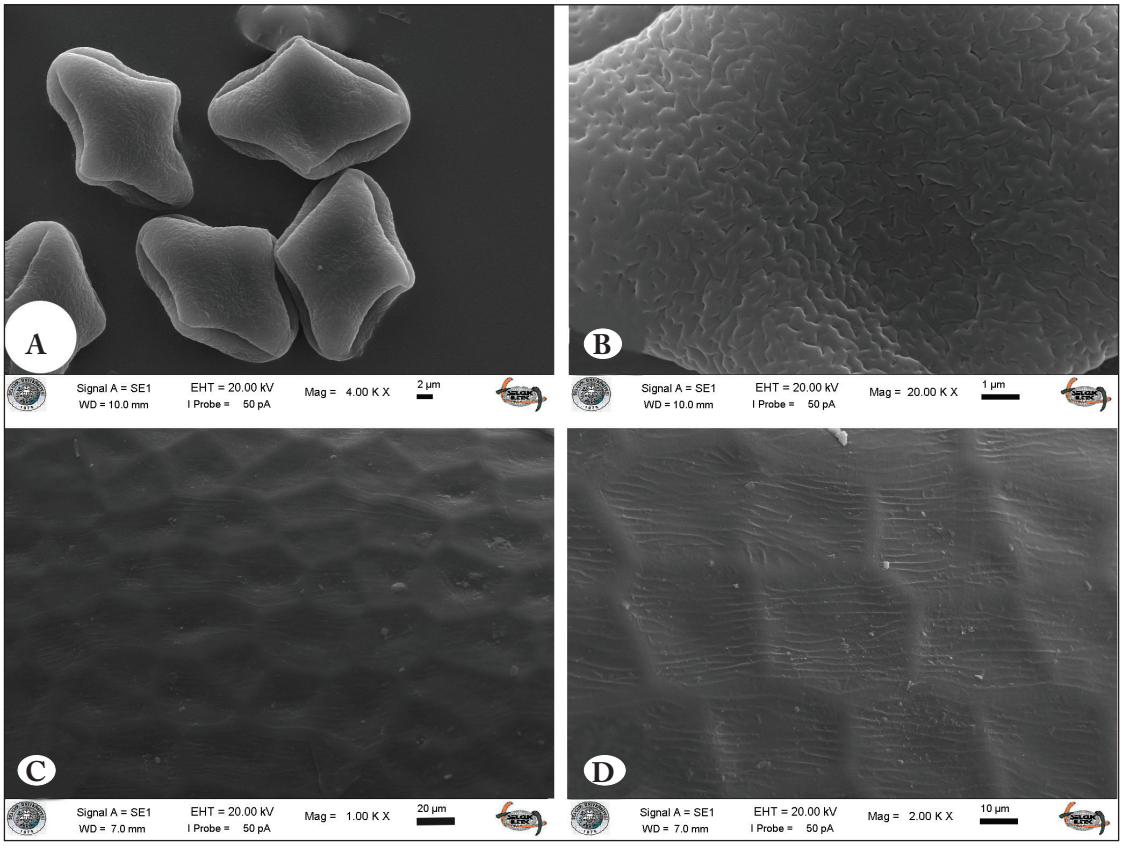

Figure 2. SEM photos of *Physospermum cornubiense* (Coll. no: M.Çelik 482 & Ö.Çetin) pollen and fruit.
A) general shape of pollen,
B) surface details of pollen,
C) surface ornamentation of fruit,
D) surface details of fruit.

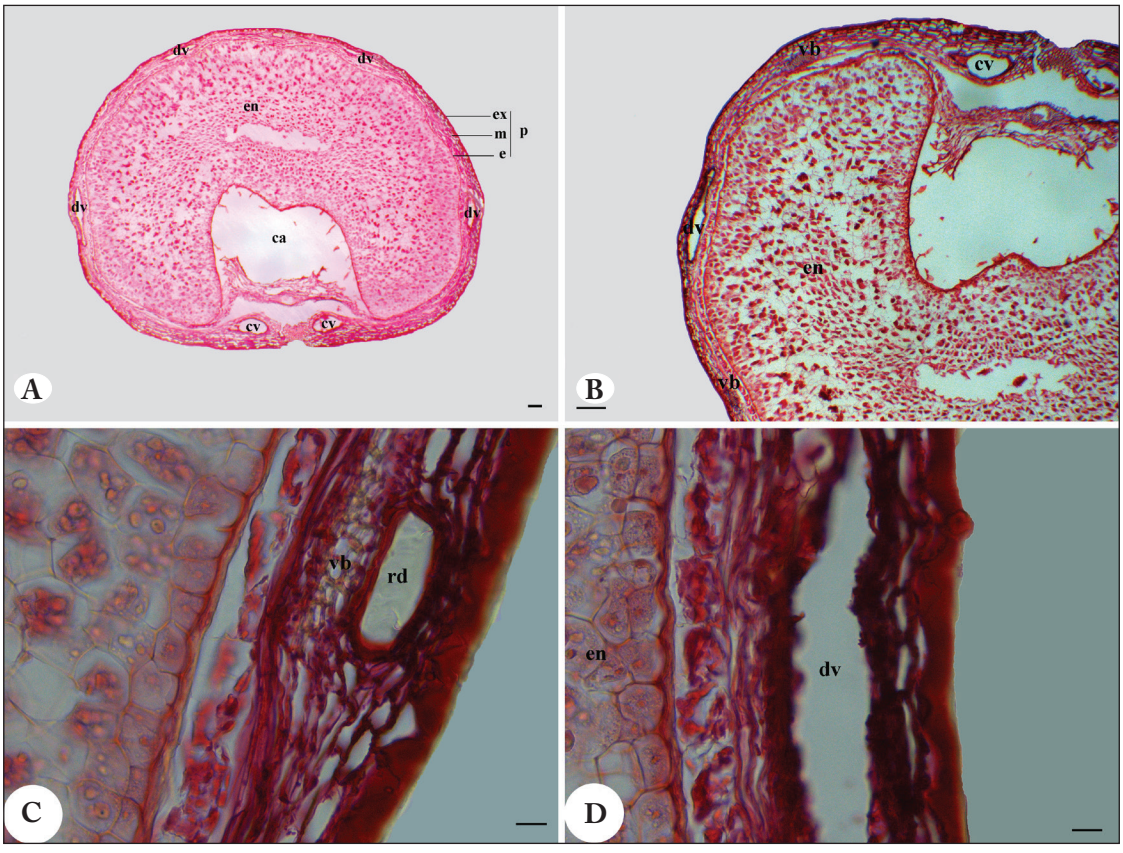

Figure 3. Fruit anatomy of *Physospermum cornubiense* (Coll. no: M.Çelik 412 & F.Altınordu).
A) general view of mericarp,
B) close view of mericarp,
C) view of the rib duct and vascular bundle,
D) view of the dorsal vittae. **ca**- cavity, **cv**- commissural vitta, **dv**-dorsal vitta, **e**-endocarp, **en**-endosperm, **ex**-exocarp, **m**-mesocarp, **p**-pericarp, **rd**-rib duct, **vb**- vascular bundle. Scale bar: 0.1 mm (a and b), 0.01 mm (c and d).

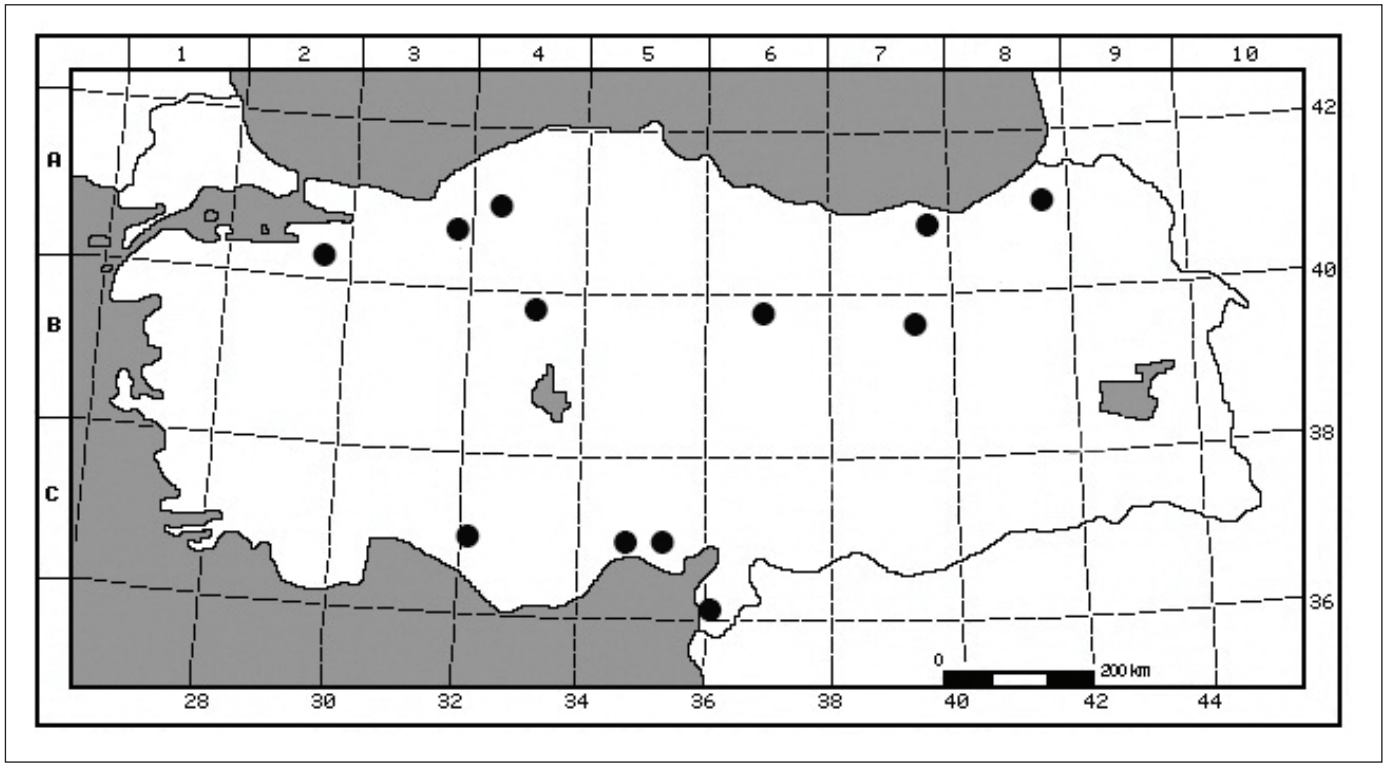

Figure 4. Distribution map of *Physospermum cornubiense*.

Leop.-Carol. Nat. Cur. 12(1): 134 (1824). *P. nudicaule* (M.Bieb.) Grossh., Opred. Rast. Kavkaza 3: 220 (1949).

Erect, perennial herbs, 30-130 cm tall. Stems usually unbranched except near apex, glabrous or asperulous, shallowly striate. Basal leaves broadly deltoid in outline, 15-30 cm long (incl. petiole), 12-25 cm wide, 2-3 pinnate or ternate, the ultimate segments deeply and acutely pinnatisect, broadly ovate, 1.5-6 x 1-5 cm, glabrous, often rather distinctly reticulate veined with narrowly revolute, scabridulous margins. Petiole 14-25 cm, glabrous, canaliculate above. Stem leaves usually absent or reduced to short lanceolate-subulate sheaths, 1-3 cm long, occasionally developed and resembling the basal leaves, but smaller. Peduncles are 6-16 cm long. Bracts 2-8, narrowly oblong-lanceolate, 5-8 x 1-2 mm. Umbels 5-20 rayed, glabrous, rays unequal to subequal, 1-6 cm, slender, ascending to suberect. Bracteoles 2-6, oblong-linear or lanceolate, 2-5 x 1 mm. Umbellule 5-20 flowered. Pedicels filiform, 4-9 mm long. Sepals deltoid, mucronate, c. 0.5 mm long and as wide or rather wider. Petals white, oblong-obovate, c. 1.5 x 1 mm, apex strongly incurved-emarginate, filaments c. 1.5 mm long, anthers oblong, yellow, 0.8 x 0.6 mm, stylopodium conical, styles up to 2 mm long at anthesis, at first erect, becoming strongly reflexed in fruit, stigmas slightly capitate. Fruits conspicuously didymous, 3-5 x 3-6 mm, mericarps separating readily at maturity, hemispherical or ovoid, somewhat laterally compressed with a very narrow commissure, dorsal ridges obscure, filiform, carpophore bipartite, vallecula 1-vittate, commissure 2-vittate, endosperm conduplicate.

Flowering time: June-August

Habitat: In woodland or on shaded hillsides under trees or bushes, 500-1500 m.

Distribution: S. & W. Europe, Turkey, Cyprus, Crimea, S. Russia, Caucasia, N.W. Iran.

The micromorphological results show that pollen of *P. cornubiense* has radial symmetry and is isopolar, and 3-zonocolporate. The pollen ranges from 23.15 to 29.57 µm long, and from 13.97 to 18.93 µm wide. The mean length (P) of pollen is 25.70 µm (standard deviation 1.84), the mean width (E) is 16.63 µm (standard deviation 1.57), and the P/E ratio is 1.55. The pollen grain is prolate and the ornamentation is rugulate. The exine is 0.69-1.04 µm thick and the intine is 0.32-0.49 µm thick. The colpus length (Clg) of pollen grains ranges from 19.40 to 24.29 µm, colpus width (Clt) ranges from 0.88 to 1.26 µm. The porus width (Plt) ranges from 4.23-5.89 µm and porus length

(Plg) ranges from 2.92-4.12 µm. The fruit of *Physospermum cornubiense* comprises two homomorphic mericarps that are 3-5 x 3-6 mm, hemispherical or ovoid, and not significantly compressed either dorsally or laterally. The mericarp coat surface consists of pentagonal or hexagonal cells that are slightly striate and glabrous (Figure 2).

The transverse section of the mericarps of *P. cornubiense* is semicircular. Each mericarp has five indistinct filiform ribs and consisting of pericarp and endosperm. The exocarp is formed by a single layer of epidermal cells that are rectangular with thick outer walls. The mesocarp comprises 4-10 layers of parenchymatous cells. Vascular bundles in the mesocarp are located below each rib and surrounded by parenchyma cells. Rarely, there is a small oil duct near the vascular bundle. *Physospermum cornubiense* has four dorsal vittae (one in each furrow) and two commissural vittae. The endosperm is curved or horse shoe shaped on the commissural side (Figure 3).

In this study, the morphology, pollen morphology and fruit anatomy and micromorphology of *Physospermum cornubiense* were studied. Some differences between our study and the description in Flora of Turkey and the East Aegean Islands are presented in Table 1: ultimate segments 1.5-6 cm (not 2-3 cm), umbel 5-20 rays (not 5-12 rays), bracts 2-8 (not 2-5). Some additional morphological characteristics of *P. cornubiense* are also given in description.

The majority of taxonomists have regarded *Physospermum* and *Pleurospermum* Hoffm sensu lato (including *Aulacospermum* Ledeb., *Eleutherospermum* K. Koch, and several other genera) as related (Downie et al. 2000). Sprengel (1820) treated both in his tribe *Smyrnieae*, and most authors have followed this example with some adjustment of rank. The results of phylogenetics analyses of Downie et al (2010), the genus *Physospermum* is placed in tribus *Pleurospermeae* with the genera *Pleurospermum*, *Aulacospermum*, *Eleutherospermum*, *Eremodaucus* Bunge, *Hymenidium* Lindl., *Korshinskya* Lipsky, two species of *Physospermopsis* C. Norman, *Pseudotrachydium* (Kljuykov, Pimenov & V.N. Tikhom.) Pimenov & Kljuykov, *Trachydium* Lindl.

A fair number of palynological studies of the family Apiaceae date back to the mid-1950s (Mačukanović-Jocić et al. 2017). The first reports regarding the pollen morphology of some species were published by Erdtman (1952). Then many Apiaceae species, originating from different regions or countries of almost all the continents, have already been palynomorphologically described by Cerceau-Larrival 1959, Ting 1961, Cerceau-Larrival 1962, 1965, Nilsson et al. 1977, Van Zeist et al. 1977, Punt 1984, Chester and Raine 2001, Doğan-Güner et al. 2011, Ecevit-Genç 2014, Mačukanović-Jocić et al. 2017. However pollen morphology of this family has been studied by various authors, there is not enough palynological study on the genus *Physospermum*.

Table 1. The comparison of morphological characters of *P. cornubiense* based on our study and the description in *Flora of Turkey and the East Aegean Islands*.

Characters	Flora of Turkey	Our study
Stem	erect, glabrous	erect, glabrous or asperulous
Stem	30-120 cm	30-130 cm
Basal leaves	2-3 pinnate or ternate	2-3 pinnate or ternate
Basal leaves	triangular in outline	broadly deltoid in outline
Basal leaves	-	15-30 cm long (incl. petiole), 12-25 cm wide
Ultimate segment	ovate, pinnatifid	deeply and acutely pinnatisect, broadly ovate
Ultimate segment	2-3 cm	1.5-6 x 1-5 cm,
Umbels	5-12 rayed	5-20 rayed
Umbellule	5-15 flowered	5-20 flowered
Bracts	2-5, linear-lanceolate	2-8, narrowly oblong-lanceolate
Bracts	-	5-8 x 1-2 mm
Petal	-	oblong-obovate, c. 1.5 x 1 mm
Fruit	3-4 x 3-5 mm	3-5 x 3-6 mm
Mericarp	hemispherical	hemispherical or ovoid

According to Erdtman 1952, Umbelliferae is a stenopalynous family. Perveen and Qaiser 2006 studied pollen grains of 50 species representing 27 genera of the family Umbelliferae from Pakistan and their result indicates that pollen grains of Umbelliferae usually radially symmetrical, isopolar, prolate to per-prolate, tricolporate, colpi generally with costae, colpal membrane psilate to sparsely or densely granulated, ora la-longate, sexine as thick as nexine, or slightly thicker or thinner than nexine. Tectal surface commonly striate-rugulate or rugulate-striate rarely simply striate. Perveen and Qaiser 2006 divided Apiaceae into three groups based on exine pattern; *Bupleurum gilessii*-type, *Pleurospermum hookeri*-type, *Trachyspermum ammi*-type. *Pleurospermum hookeri* Clarke and *Trachydium* are placed in *Pleurospermum hookeri*-type group. Pollen grains of this group is 3 -colporate, 3-zonocolporate, ectoaperture-colpus long, sunken, narrow, end acute, endoaperture circular, rugulate-striate. In another study, pollen morphological characteristics of thirteen species in *Pleurospermum* were observed by Zhang et al. (2013). The results show that pollen of thirteen species is divided into five types including subrhomboidal, subcircular, ellipse, subrectangular and super-rectangular shapes. Exine ornamentation of equatorial region is dense and diversified forms, and may be divided into three types of brevistriate crisped cerebroid, granulate and finely reticulate, in which, that of only *P. rupestre* (Popov) K. T. Fu et Y. C. Ho possesses finely reticulate (Zhang et al. 2013). In our study, pollen grains of *Physospermum cornubiense* has radial symmetry and is isopolar, and 3-zonocolporate, the P/E ratio is 1.55 µm. The pollen grain is prolate and the ornamentation of pollen grains is rugulate.

Ever since Morison's 1672 Plantarum umbelliferarum, fruit morphology and anatomy have been regarded as essential to the taxonomy of Apiaceae (Drude 1898, Constance 1971, Spalik et al. 2001). Despite their general similarity, umbellifer fruits vary with respect to their external and internal features, and nearly all classification systems of the family are based on these characters (Spalik et al. 2001).

Fruits morphology of *Aulacospermum* dorsally not compressed, mericarps dorsally convex, all ribs approximately equal winged or keeled, exocarp consisting of cells with convex outer walls, not separating from the mesocarp, vittae 1-3, more rarely numerous, rib secretory ducts obsolete. Fruits morphology of *Pseudotrachydium* dorsally not compressed, mericarps dorsally convex; all ribs approximately equal, narrow-winged or keeled, exocarp consisting of cells with convex outer walls, not separating from mesocarp; vittae 1-3 or numerous, rib secretory ducts obsolete (Pimenov et al. 2000).

Fruits of *Pleurospermum* oblong to broad-ovoid, slightly flattened laterally, glabrous, often with numerous, shining tubercules, ribs prominent and acute, sometimes undulate, cristate or narrowly winged, vittae 1(–3) in each furrow, 2(or 4 or 6) on commissure, seed face concave, carpophore 2-parted (Zehui and Watson 2005). In our study, *Physospermum cornubiense* comprises two homomorphic mericarps that are 3-5 x 3-6 mm, hemispherical or ovoid, and not significantly compressed either dorsally or laterally. The mericarp coat surface consists of pentagonal or hexagonal cells that are slightly striate and glabrous.

Appendix 1. Examined samples: C5 Adana: Pozantı, between Pozantı-Akçatekir, 890 m, 08.07.2015, under forest, UTM 36 S 664770 E, 4141530 N, M.Çelik 412 & F.Altınordu; **C6 Hatay:** Belen, Atik, 1018 m, 18.06.2017, open *Pinus* forest, UTM 37S 0251856E, 4044095N, M.Çelik 481 & Ö.Çetin; Hatay: Belen, between Tahtaköprü-Çerçikaya, RES road, 1050 m, 18.06.2017, under *Pinus* forest, UTM 37S 241795E, 4033249 N, M.Çelik 482 & Ö.Çetin.

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