

A New Distribution Area of *Angelica archangelica* L. (Apiaceae) Around Uludağ (Bursa) and Its Micromorphological, Anatomical, Palynological Properties

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ABSTRACT

Angelica archangelica L. is an important medicinal plant that spreads from south to north in the world. This study aims to give details on the species' morphological, anatomical, and palynological properties, and to present the second locality of *A. archangelica*, which is known from a single locality in Turkey. Plant materials were collected from Bursa (Uludağ). Micromorphological properties, fruit cross-section, palynological and anatomical properties were examined with SEM and Light Microscope. In the fruit cross-section, rib oil duct, vallecular vittae, and commissural vittae were observed. The stem is covered by a single-layered epidermis. The corner collenchyma cells and secretory ducts were observed in the stem cross-section. An open collateral type of vascular system was observed in the stem, consisting of sclerenchyma cells, phloem, xylem, and vascular cambium cells. The leaf is bifacial and has a single-layered epidermis. Anomocytic stomata were located on both leaf surfaces and denser on the lower surface than on the upper surface. Hypodermis was observed under the epidermis layer. Collenchyma tissue was located between hypodermis cells. Tricolporate pollen grains were observed. Pollens are perprolate in shape. The exine ornamentation is rugulate.

Keywords: *Angelica archangelica* L., New locality, Anatomy, Palynology, SEM, Turkey

INTRODUCTION

The family Apiaceae contains 466 genera and about 3820 species that are distributed mainly in temperate Eurasia and North America (Plunkett et al. 2018). The Apiaceae family is characterized by hollow stems, schizocarp fruits, umbel inflorescence, and epigynous flowers (Kalsoom et al. 2019). Apiaceae comprises 105 genera and about 493 species in Turkey (Cetin et al. 2020). *Angelica* L. is one of the largest genera within Apiaceae. *Angelica* includes 120 species that are spread in Europe, Asia, N Africa, and N America (Plunkett et al. 2018). *Angelica* species grow in fens, riverbanks, moist grasses, and woods (Nahar and Sarker 2004). The members of this genus are recognized by their large inflorescence and dorsally compressed mericarps including vittae (Liao et al. 2021). *Angelica* species are an important group, especially their traditional medicinal uses (Nahar and Sarker 2004). They have been used for the treatment of colds, flu, influenza, hepatitis, arthritis, indigestion, coughs, bronchitis, pleurisy, typhoid, headaches, fever, rheumatism, and as an anti-inflammatory, diuretic, expectorant, and diaphoretic (Nahar and Sarker 2004).

Angelica archangelica L. is one of the species of genus *Angelica* that grows naturally in Turkey. The native origin of *A. archangelica* is northern Europe, Eastern Europe, northern Asia, and Greenland (Wahlin and Blixt 1994, Kylin 2010). *A. archangelica* is an interesting medicinal plant due to its wide distribution from the north to the south (Kylin 2010). Until now, the distribution of *A. archangelica* in Turkey was known from one locality in Uludağ, Bursa (Daşkın and Kaynak 2012).

Morphologically, it is hard to distinguish *Angelica* species from other large members of Apiaceae without mature fruits (Liao et al. 2021). Correct identification of taxa is crucial especially in medicinal plants due to their biological properties (Forycka and Morozowska 2020). The micromorphological characters and anatomical structure are also important sources of information for the identification of the family that *Angelica* belongs to (Forycka and Morozowska 2020).

Palynological studies support to resolve of taxonomic problems at variety, species, genus, and family level (Gul et al. 2021, Birjees et al. 2022). Especially exine ornamentation and apertures are important for genera and species (Gul et al. 2021). Pollens of the Apiaceae family have characteristic features and these palynological characters have been used by several researchers in pollen keys (Erdtman 1952, Pehlivan et al. 2009). Some

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investigation on the pollen characteristics of *Angelica* species has been studied before (Chen et al. 2007, Lan et al. 1997). These studies reported that the pollen grains of *Angelica* species vary in shape.

The aims of this study are: (a) to present the second locality of *A. archangelica* detected in Uludağ (Bursa/Turkey); (b) to investigate its micromorphological, anatomical, and palynological features to provide a useful source for future studies on the identification of other *Angelica* species.

MATERIALS AND METHODS

Plant materials were collected from Uludağ (Bursa/Turkey) in June 2017 and 2021. Herbarium materials were stored in the Herbarium of Faculty of Science, Bursa Uludağ University, Turkey (BULU). For morphological observations, deposited specimens from the Bursa Uludağ University Faculty of Science Herbarium (BULU) were studied. Micromorphological structure of the stem, leaf (upper and lower surface), fruit (surface and cross-section), and pollen were analyzed on the scanning electron microscope (SEM). Fruit specimens were passed through alcohol-acetone series and prepared for the SEM. Samples were coated with gold-palladium for 2 min in a BALTEC SCD 005 sputter-coater. The micrographs were obtained using an EVO 40 microscope at 20 kV. For anatomical investigations fresh stem and leaf specimens were kept in 70% alcohol. All cross-sections were taken by hand using commercial razor blades. Afterwards they were mounted on slides using glycerine-jelly. Slides were photographed with a Leica DM4000 light microscope. Pollen grains were obtained from herbarium specimens. Pollen grains were treated following the method of Wodehouse (1935). Measurements were made using a Leica DM4000 light microscope. The polar length, equatorial length, colpus length, colpus width, distance between colpus, pore length, pore width, distance between pores, exine and intine thickness of pollen grains were measured using the light microscope ($\times 1000$) and P/E ratios were calculated.

RESULTS AND DISCUSSION

Morphological characteristics

Angelica archangelica L., Sp. Pl. 250 (1753).

Syn: *Angelica officinalis* Moench, Methodus 81 (1794). *Angelica officinalis* Bernh., Syst. Verz. 170 (1800).

Lectotype: Habitat in Alpihus Lapponiae, ad rivulos. 354.1 (LINN) “designated by J.-P. Reduron in Lectotypification of Linnaean names for Flora Nordica (Brassicaceae - Apiaceae), Nordic Journal of Botany, 22(1): 83, 2002.”

Perennial. Stems with ridges and grooves, up to 2 m. Basal leaves bipinnate, very large and glabrous. Primary leaflets, ovate to elliptic, serrate, acuminate, irregular in outline and incised. Terminal leaf segments with 3 acuminate to obtuse and distinctly decurrent lobes. Cauline leaves bipinnate, petioles sheathed at the base. Umbels globose. Peduncles 2 - 25 cm, glabrous. Bracts absent. Umbellules 15 – 55 flowered. Pedicels 0.3 – 2.7 cm, papillose. Bracteoles linear. Sepals 0.5 – 2.5 mm. Petals 2 - 3 \times 1.0 - 1.5 mm, green to cream, ovate, cordate at base. Fruits oblong, dorsiventrally flattened, glabrous (Figs 1, 2).

Examined specimens: Bursa: Uludağ, above Pınarcık, 1369 m, 14. 07. 2017, Ö. Yılmaz 2294, B. Uçur, C. Aktürk & M. Özerkan (BULU)! Uludağ, Alaçam, Alaçam-Kilimli Lake, 1765 m, 29. 07. 2021, G. Kaynak, Ö. Yılmaz 3345 & A. Yılmaz (BULU)!

Known Distribution: Bursa: Uludağ, above Pınarcık, 1369 m (first locality).

A new locality: Bursa: Uludağ, Alaçam, Alaçam-Kilimli Lake, 1765 m, 40°05'08'' N, 29°16'53'' E (Figure 3). It's 10 km far from the first locality, Pınarcık and covers an area of about 100 km². Habitat: Streamside, water meadow.



Figure 1. A General view of *Angelica archangelica* L., B Close-up view of the inflorescence (First locality), C General view from second locality.



Figure 2. Fruit view of *A. archangelica*.

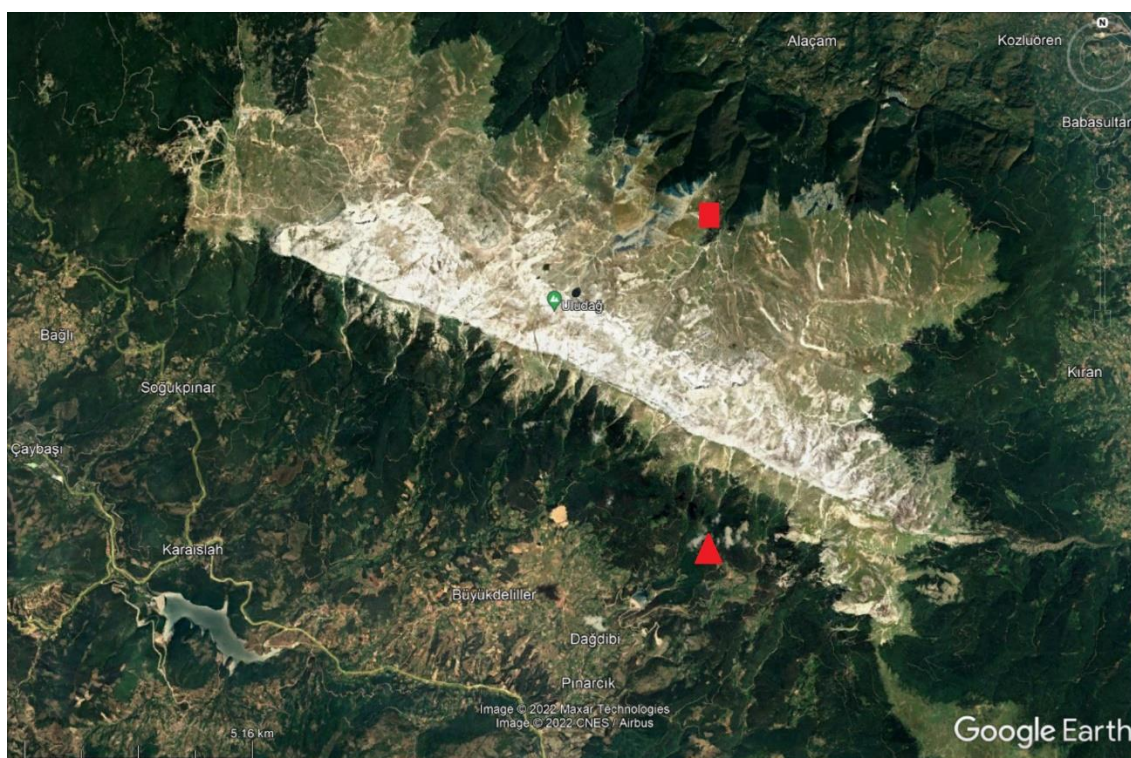


Figure 3. Distribution map of *Angelica archangelica* in Turkey: First locality where the species was detected (red triangle) and the new locality (red square).

Micromorphological properties

The stem surface of *A. archangelica* has striations and many stomata (Fig. 4, A). The surface of leaves is undulating at many places (Fig. 4, B and C). The density of stomata on the lower and upper surfaces of the leaves is variable. The stoma is denser on the lower surface than on the upper surface of the leaf (Fig. 4, B and C). The stomata are surrounded with strongly striated cuticle (Fig. 4, D). In the fruit, there are three costae and two valliculae on the surface (Fig. 4, E). The stomata are located on the dorsal surface of the fruits (Fig. 4, F). There are no trichomes on the fruits. Schizocarp-type fruits in *A. archangelica* consist of two mericarps. The commissural side of the mericarp is flat, while the dorsal side is convex. The exocarp and the mesocarp layers are visible in the cross-section of the mericarp. The exocarp layer is composed of rectangular epidermal cells while the mesocarp layer is composed of parenchyma cells. There is an air-filled cavity between the mesocarp and endosperm. The dorsal side of the endosperm is undulate and 3-lobed. Mericarps have three ribs on the dorsal surface and two marginal ribs that are named wings. There are the rib oil duct, vallicular vittae, and commissural vittae in the cross-section of mericarps (Fig. 5).

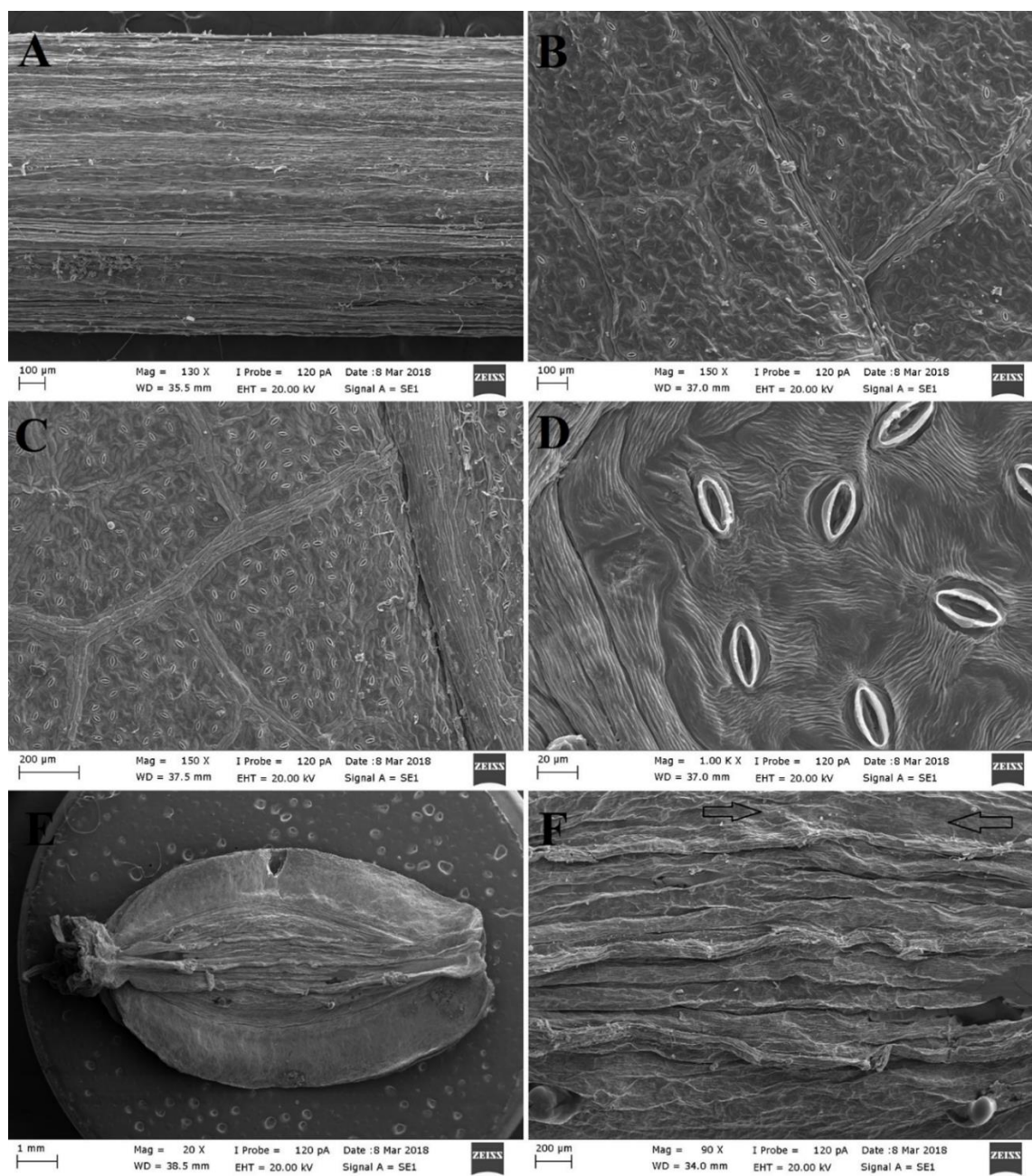


Figure 4. Micromorphology of *A. archangelica*. A Stem surface, B Upper surface of the leaf, C Lower surface of the leaf, D Close-up view of stoma, E General view of fruit, F Close-up view of fruit surface and stoma.

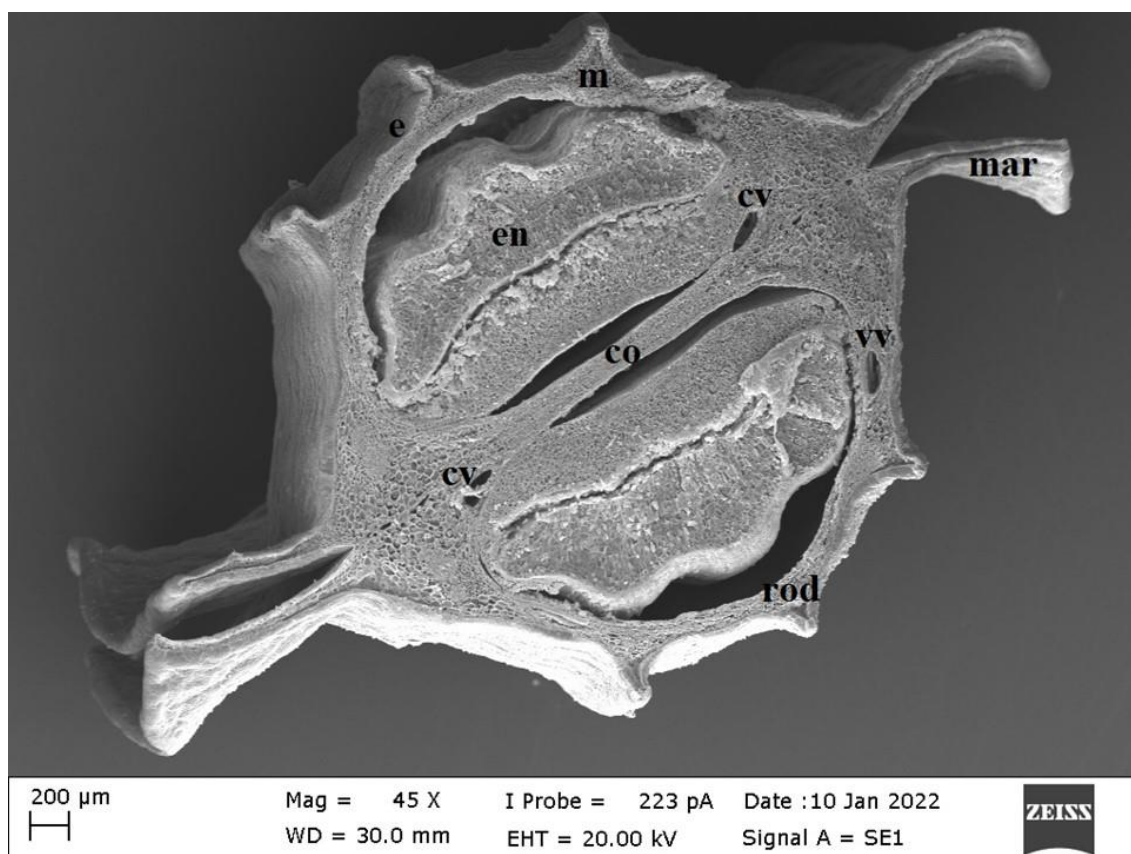


Figure 5. Fruit cross section of *A. archangelica* (e: exocarp, en: endosperm, m: mesocarp, cv: commissural vitta, co: commissure, vv: vallecular vitta, rod: rib oil duct, mar: marginal rib).

Anatomical characteristics

The epidermis is single-layered and surrounded by a cuticle layer in the stem anatomy of *A. archangelica*. Cortex consists of 12-15 rows of parenchyma cells. Bundles of corner collenchyma cells are located under the epidermis layer. Secretory channels are found between collenchyma tissue and vascular bundles. The open collateral type of vascular bundles consists of sclerenchyma cells, phloem, xylem and vascular cambium. Vascular bundles are surrounded by endodermis. The pith region is in the middle and composed of relatively large parenchyma cells (Fig. 6).

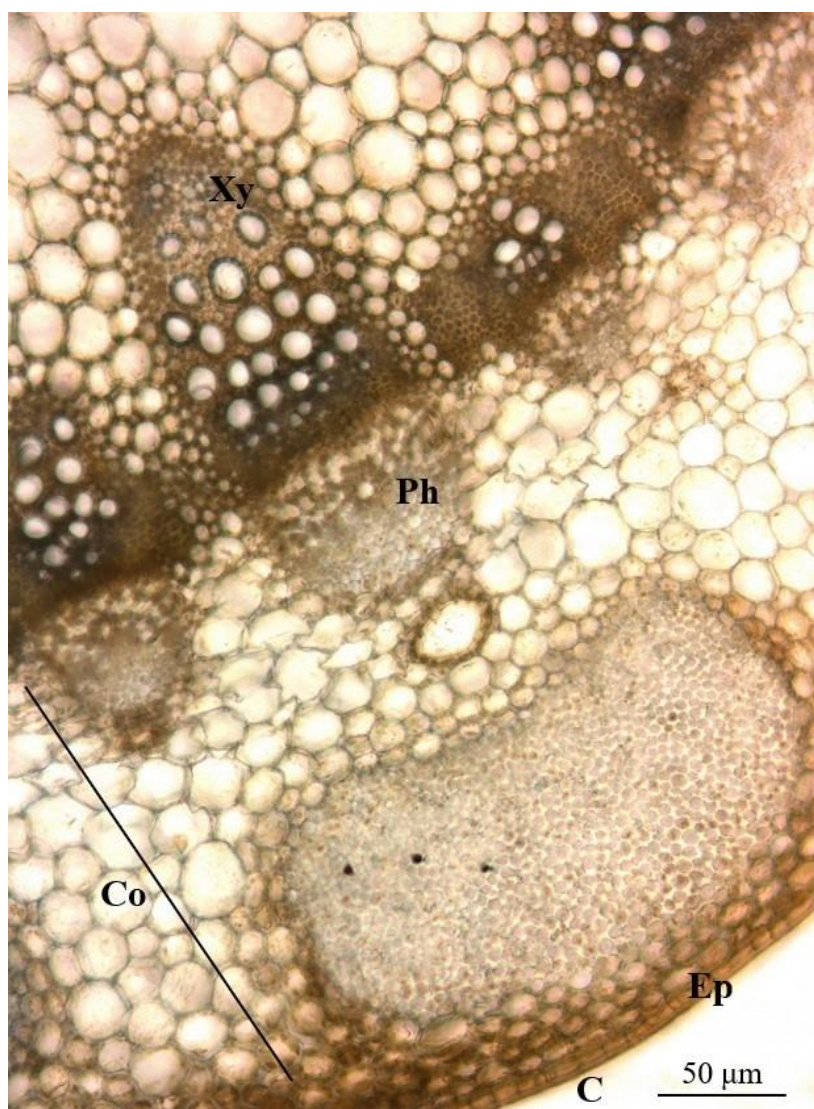


Figure 6. Cross sections from the stem of *A. archangelica* (Xy: Xylem, Ph: Phloem, Co: Cortex, Ep: Epidermis, C: Cuticle).

In the cross-section of the leaf, single-layered epidermal cells are covered by a thick cuticle layer. 2-7 rows of hypodermis cells occur under the epidermis layer. Hypodermal collenchyma take place between the hypodermis cells. Each collenchyma having next to it one or two secretory ducts (Fig. 7, A and C). In the flanks region of leaf, epidermal cells on both the upper and lower surfaces are covered by a thick cuticle layer. The mesophyll is composed of palisade parenchyma with an elongated regularly arranged cells and spongy parenchyma with an irregularly arranged elliptical cells (Fig. 7, B and D). The type of leaf is bifacial. In the superficial section of the leaves, stomata are located on both leaf surfaces and denser on the lower surface than on the upper surface. Stomata are anomocytic and surrounded by 3-4 epidermal cells (Fig. 7, E and F).

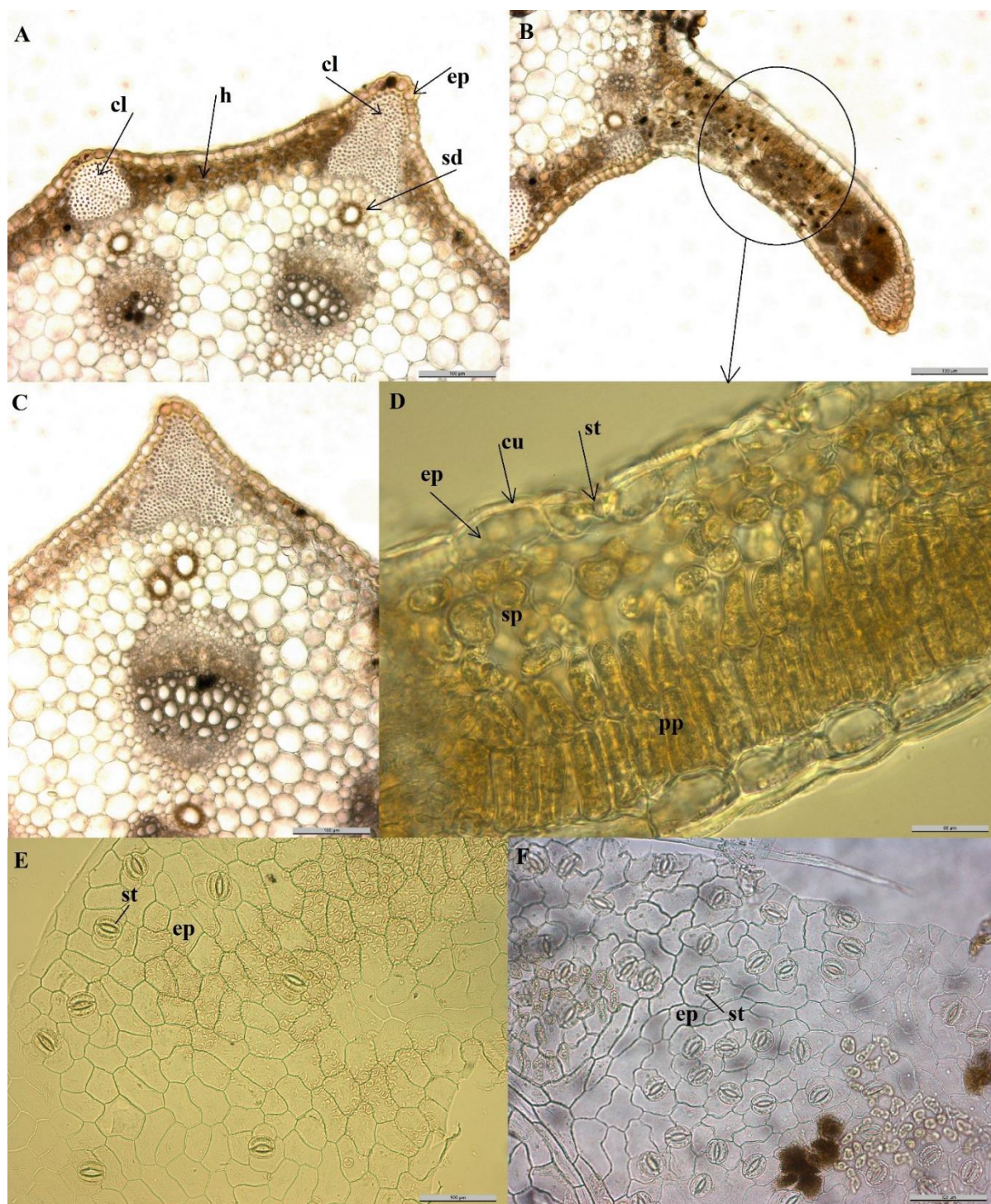


Figure 7. A and C Cross sections the leaf of *A. archangelica*, B and D Mesophyll layer, E Upper surface of the leaf, F Lower surface of the leaf (cl: collenchyma, h: hypodermis, ep: epidermis, sd: secretory duct, cu: cuticle, st: stoma, sp: sponge parenchyma, pp: palisade parenchyma).

Palynological characteristics

A. archangelica has tricolporate pollen grains. Polar axis (P) is 28.34 - 31.87 μm and equatorial axis (E) is 11.88 - 14.59 μm . The ratio of P/E is 2.24 μm . The pollen grains are perprolate in shape. Colpus length is $24.1 \pm 1.1 \mu\text{m}$. Colpus width is $2.12 \pm 0.25 \mu\text{m}$. The distance between the colpus is 3.33 - 7.88 μm . Pore length is $2.9 \pm 0.3 \mu\text{m}$.

Pore width is $3.5 \pm 0.6 \mu\text{m}$. The distance between the pores are $2.63 - 6.4 \mu\text{m}$. The exine thickness is $0.95 \pm 0.2 \mu\text{m}$ and intine thickness is $0.75 \pm 0.13 \mu\text{m}$. The exine ornamentation is rugulate (Fig. 8).

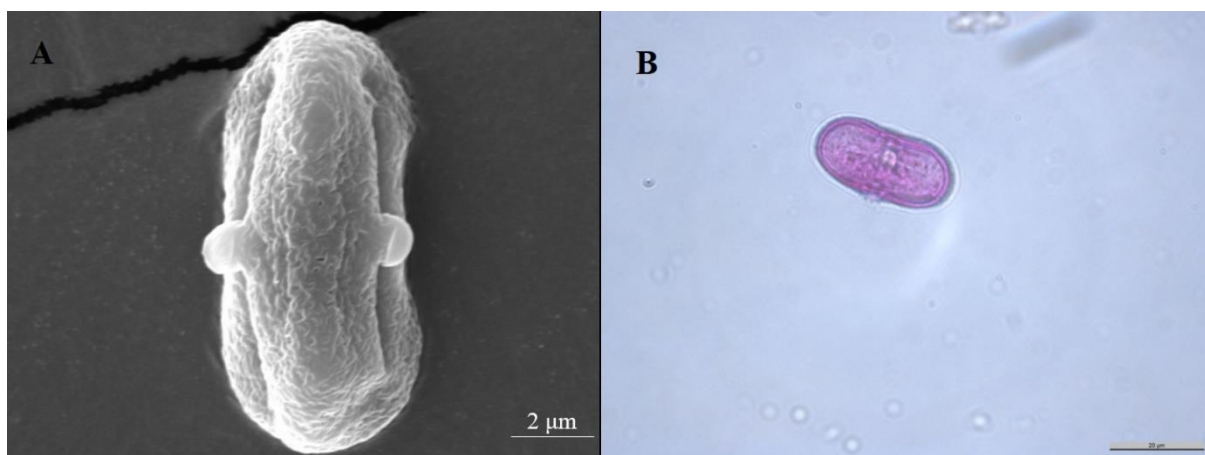


Figure 8. Pollen grains of *A. archangelica* A Scanning electron micrograph, B Light microscopy micrograph.

In this study, morphological, anatomical, and palynological characteristics of *A. archangelica* distributed in Bursa (Uludağ) were analyzed. According to the micromorphological results of the present study, trichomes were not observed on the surface of specimens. Anomocytic stomata were observed on the surface of stem, leaf, and fruit. Stomata were located more densely on the lower surface than the upper surface of the leaf. Previous studies show that fruit micromorphological properties of Apiaceae family is important for the taxonomic classification of this family (Forycka and Morozowska 2020, Ostroumova 2018). According to the information on two subspecies of *A. archangelica* by Forycka and Morozowska (2020); SEM examination of fruit micromorphological and anatomical characters are valuable source of information about the intraspecific differentiation of *A. archangelica*. Microsculpture characteristics of the mericarps such as the presence of hairs and excrescences, and the occurrence of cuticular striation are significant for describing this species and some of these properties exhibit differentiation within the species (Forycka and Morozowska 2020). In another study (Liao et al. 2021), the fruits of North American *Angelica* species are divided into five types and three subtypes. *A. archangelica* fruits are categorized into the subtype 1 because of its unequal ribs and vascular bundles located in mesocarp (Liao et al. 2021). According to the SEM results of fruit characteristics in the present study, *A. archangelica* fruits show variation in terms of the existence of the hairs on the surface. However, the fruit type and the striation of the cuticle layer are compatible with the literature.

The primary structure was observed in the stem of *A. archangelica*. Single-layered epidermis is covered by a thick cuticle. The cortex layer is composed of 12-15 rows of parenchyma cells. There are corner collenchyma cells under the epidermis layer. Secretory channels are located between collenchyma and vascular bundles. The open collateral type of vascular bundles comprise of sclerenchyma cells, phloem, xylem and vascular cambium. Endodermis is located around the vascular bundles. The pith region in the middle is composed of large parenchyma cells. A study on comparative anatomy of *Angelica* taxa that include *A. archangelica* in Romania, focused on some shared and different features (root, stem, rachis and foliole anatomy features) in all the three taxa (Lobiuc et al. 2012). According to this study, these kind of features could be used to distinguish these species. The authors reported that the stem anatomy of *A. angelica* has air cavities within the parenchymal cortex and these cavities sometimes can be observed in the region of the secondary phloem. In accordance with the literature, air cavities are observed within the cortex in the present study.

In the present study, pollen morphology features of *A. archangelica* were determined by using a Light microscope and a Scanning electron microscope. The pollen grains of *A. archangelica* are isopolar and have tricolporate apertures. Considering the P/E ratio of the current study, the pollen form is perprolate. P/E ratio is significant to understand the taxonomic problem of Apiaceae (Birjees et al. 2022). Pollen types of Apiaceae was divided into five types by Cerceau-Larrival (1962) on the basis of P/E ratio. According to this classification, type I (P/E: 1–

1.5) subrhomboidal, type II (P/E: 1–1.5) subcircular, type III (P/E: 1.5–2) oval, type IV (P/E: 2) subrectangular, and type V (P/E: above 2) equatorially constricted type have been determined (Birjees et al. 2022, Cerceau-Larrival 1962). The results of present study show that P/E ratio is 2.24 μm and *A. archangelica* pollens can classify as type V (equatorially constricted). The study of Baczyński et al. (2021) show that there are three types of general view (rhombic, elliptic, bone-shaped) and exine outline (concave, straight, convex) in Apiaceae. General view of *A. archangelica* pollens are bone-shaped and exine outline is straight. Polar caps of *A. archangelica* pollen grains are obtuse. Polar view of Apiaceae is important for the differentiation of pollen types (Cerceau-Larrival 1962, Cerceau 1959, Punt 1984, Baldemir et al. 2018). Baczyński et al. reported that the polar view of *A. archangelica* pollen grains are triangular (Baczyński et al. 2021). The report of Baczyński et al. (2021) is compatible with the results of present study. The exine ornamentation is important due to its use in distinguishing plant taxa (Gabr 2018). Three types of exine pattern (striate, irregularly rugulate, cerebriform) were reported in Apiaceae by Punt (1984). The exine ornamentation of *A. archangelica* pollen is rugulate.

CONCLUSIONS

In this study, a second locality from Turkey was presented and the micromorphological, anatomical, and palynological characteristics of *Angelica archangelica* were revealed. The fruit is composed of two mericarps. There are three dorsal ribs and two marginal ribs on the mericarp. The rib oil duct, vallecular vittae, and commissural vittae is located in the cross-section. There is no trichome on the fruit surface. The stem has secretory channels located between collenchyma and vascular bundles. The leaf has hypodermal collenchyma very close to the secretory ducts. The leaf is bifacial. The mesophyll consists of regularly arranged elongated palisade parenchyma cells and irregularly arranged spongy parenchyma cells. Stomata are anomocytic type. The tricolporate pollen grains are prolate in shape. The exine ornamentation is rugulate.

As an important medicinal plant, *A. archangelica* is spread from south to north. This study focused on the specimen of this species in Turkey. The present results will contribute to determine the taxonomic delimitation of this species, and the understanding of the biological characteristics of *A. archangelica*, which has a wide distribution area in the world.

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