

Comparative Anatomy, Pollen and Seed Morphology of Two *Verbascum* Varieties (Scrophulariaceae) and Their Taxonomic Significance

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Abstract

In this study, the anatomical, palynological, and seed micromorphological traits of two *Verbascum* varieties found in the Southeastern Anatolia Region were investigated. These variations are *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* Murb. and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* L. Cross-sections from the taxa's roots, stems, and leaves were viewed under a light microscope for anatomical study. The xylem components play a significant role in the taxonomic root portions. The pith area is a broad region in stem sections, and the upper portion of the epidermis cells is encircled by a distinct indented cuticle layer. The leaves' primary vein is represented by open collateral bundles. Pollen grains in *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* are tricolporate-tricolpate, prolate-spheroidal, and have reticulate exine ornamentation. Similarly, pollen grains in *V. sinuatum* subsp. *sinuatum* var. *sinuatum* are tricolporate-tricolpate, prolate, and have reticulate exine ornamentation. The brown seeds of *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* have an alveolate, deep and wide posterior region, a truncated beak, and a prismatic-oblong shape. The ornamentation of the seed coat is made up of irregular polygonal cells that have noticeable and dense vesicles. The importance of a few traits in the *Verbascum* variety delimitation has been investigated in this study. For the purpose of supporting ancestral traits in the infrageneric classification and helping with genus systematics, some pollen and seed characters which are typically connected to pollen and seed micromorphological and anatomical patterns can be applied.

Keywords: anatomy, pollen, seed, SEM, Southeastern Anatolia, Turkey *Verbascum*

1. Introduction

Verbascum L. contains approximately 360 species in the World [1]. Although it is extensively dispersed throughout Eurasia and North Africa, a sizable region that includes Anatolia, the southern Balkans, the Middle East, the Caucasus, and northwest Iran has the highest diversity of species [2]. It has been split into 13 artificial groups and is represented by 257 species and 132 additional hybrids and with 202 endemic species (80% of the total), the genus has an extremely high endemism ratio in Flora of Turkey [3-6]. The genus *Verbascum* presents taxonomic challenges, and its infrageneric categorization is somewhat contrived and informal. Moreover, the challenges of producing a practical key may make taxonomic identification tough as well. Many scientists in Turkey and around the world are studying

the morphology and systematics of *Verbascum* taxa in order to achieve this goal [7].

Many SEM investigations [7-15]. are based on the pollen morphology of some *Verbascum* species taxa. Juan et al. [8], Attar et al. [9], Kheiri et al. [16], Karaveliogullari et al. [11], Kılıç [14], Mungankılıç & Kılıç [15], Cabi et al. [17], and Duman et al. [18] have studied the seed micromorphology of the genus *Verbascum*. The genus has had very few anatomical research [12-16, 19-22], and there are still unexplored taxa in Turkey. The anatomical features for *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* Murb. and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* L. (group H) are absent, as is the palynological and seed micromorphology. The purpose of this study was to determine the relationships between two *Verbascum* varieties that are found throughout the Southeastern Anatolia Region. This information was obtained

through comparative analysis, which helped to highlight the significance of anatomical characteristics as well as the fine structure of the surfaces of the pollen and seeds. The information gathered should be used to categorize the group and genus in further research or to offer strong support for its assessment.

2. Materials and Methods

A number of locations in Diyarbakır, Mardin, and Şanlıurfa were used to gather the specimens of *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* (Figure 1). The voucher samples were kept in the herbarium of the Kızıltepe Vocational School, Mardin Artuklu University, Turkey's Department of Plants and Animal Production. The collection locations, the collector's number, and the habitat of the samples that were examined for their micromorphological characteristics list in the table 1. The plant's taxonomic description was established in accordance with Davis et al. [4] and Karaveliogulları [5].

For use in anatomical research, collected specimens were stored in falcon tubes with 70% alcohol. Using a razor, sections were cut from the plant's root, stem, and leaf sections. They were then prepared by staining them with safranin-fast green, viewed under a light microscope, and taken pictures of [23]. The anatomy uses terms that correspond to those of Metcalfe and Chalk [24].

All of the pollen grains were subjected to light (LM) and (SEM) palynological studies using the conventional techniques outlined by Erdtman [25]. In accordance

with Wodehouse's [26] standard protocol, pollen grains were prepared for LM examination. They were examined under an Isolab standard microscope in glycerin-water. For the purposes of the palynological analysis, thirty pollen grains per specimen were considered sufficient [16-17, 26]. Pollen washed with distilled water, allowed to air dry, then immediately mounted on stubs using double-sided tape and covered in gold for SEM. A ZEISS EVO 50 scanning electron microscope was used to capture the photomicrographs. Under a light microscope, the measurements of P (Polar axis length), E (Equatorial diameter), Clg (Colpus length), Clt (Colpus width), Plg (Porus length), Plt (Porus width), Ex (Exine thickness), and In (Intine thickness) for thirty pollen grains were made. The P/E ratio was also computed. The nomenclature used for the pollen is in accordance with Punt et al. [27]. As seen in Table 2, the data are given as the minimum, maximum, and mean.

Using an Isolab stereomicroscope, the seeds were initially inspected to make sure they were developed and of a reasonable size. Thirty mature seeds were measured in order to get the average seed sizes. After treating the seeds with distilled water to remove any dirt, the air-dried seeds were placed on stubs and covered for SEM. The ZEISS EVO 50 scanning electron microscope was used to take the photomicrographs. The terms used to describe the morphological traits of the seeds are based on those found in Juan et al. [8], and Attar et al. [28]. The capsule uses terminology that is consistent with Attar et al. [28].



Figure 1. General and flower close-up view of plants belonging to *Verbascum* varieties from the area, (A) *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum*, (B) *V. sinuatum* subsp. *sinuatum* var. *sinuatum*.

Table 1. *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* species used for anatomy and morphology studies and collected localities.

Species	Collection areas and habitat	Collector number	
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>adenosepalum</i>	Mardin: Artuklu, road side, 37°21'47"N 40°44'11"E, 893 m.	M.Kılıç 270	
	Mardin: Artuklu, road side, creek edge, 37°21'37"N 40°40'26"E, 930 m.	M.Kılıç 305	
	Mardin: Midyat, road side, 37°27'55"N 41°10'53"E, 985 m.	M.Kılıç 310	
	Mardin: Yeşilli, creek edge, 37°17'45"N 40°50'37"E, 694 m.	M.Kılıç 329	
	Mardin: Savur, road side, stony area, 37°32'47"N 40°53'35"E, 833 m.	M.Kılıç 339-2	
	Diyarbakır: Çınar, road side, 37°42'08"N 40°26'02"E, 716 m.	M.Kılıç 347-1	
	Diyarbakır: Yenişehir, road side, damp and wet area, 747 m.	M.Kılıç 348	
	Mardin: Artuklu, road side, next to water channel, 37°11'44"N 40°57'27"E, 607 m.	M.Kılıç 360-1	
	Mardin: Artuklu, road side, rocky slope, 37°13'02"N 40°57'58"E, 674 m.	M.Kılıç 362	
	Mardin: Artuklu, road side, creek edge, 37°22'11"N 40°41'10"E, 983 m.	M.Kılıç 367	
	Mardin: Yeşilli, road side, cultivated area, 37°18'37"N 40°49'49"E, 737 m.	M.Kılıç 368	
	<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	Mardin: Yeşilli, road side, rocky slope, 37°18'31"N 40°49'54"E, 733 m.	M.Kılıç 328-2
		Mardin: Savur, road side, 829 m.	M.Kılıç 337-2
		Mardin: Savur, road side, 37°32'32"N 40°51'19"E, 834 m.	M.Kılıç 338-2
Diyarbakır: Çınar, road side, 37°42'08"N 40°26'02"E, 716 m.		M.Kılıç 347-2	
Mardin: Artuklu, road side, 37°20'49"N 40°39'03"E, 932 m.		M.Kılıç 366	
Şanlıurfa: Karaköprü, road side, stony area, 37°19'54"N 38°48'00"E, 676 m.		M.Kılıç 389	
Mardin: Savur, road side, 37°32'32"N 40°51'19"E, 834 m.		M.Kılıç 408-2	

3. Results and Discussion

Several anatomical, pollen, and seed structural characteristics of *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* are reported in this work. The root, stem, and leaf tissues and cells' biometric measurements are listed in Table 2 and displayed in Figures 2, 3, and 4. Table 3 and Figure 5 present a summary of the properties of pollen grains. Table 4 summarizes the morphological properties of the seed grains, which include their size, shape, color, and surface features. Figure 6 illustrates these properties. Table 5 summarizes the morphological properties of the capsule grains,

which include their size, shape, and color features. Figure 7 illustrates these properties.

3.1. Anatomy Characteristics

3.1.1. Root anatomy

The periderm layer on the outermost surface of *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* is irregularly shaped and measures 13.49-43.56 x 9.14-34.97 µm (micrometer) in cross-sections taken from the root. A multi-layered parenchyma measuring 6.88-40.95 x 8.14-20.29 µm is located beneath the periderm. Phloem cells with 3-5

layers and sizes of 5.80-17.00 x 5.90-12.87 μm are seen beneath the parenchyma. There is unclear cambium. The center of the root is filled with xylem, which occupies a greater area. Trachea cells measure between 20.11 and 94.49 x 22.50 and 89.61 μm and are larger than tracheid cells. They are also sporadically placed. The xylem has a wider coverage area than the phloem. The two to six rows of rectangular cells that make up its pith rays. Polygonal or circular parenchymatous cells make up the pith (Figure 2, Table 2).

V. sinuatum subsp. *sinuatum* var. *sinuatum* cross-sections show that the periderm layer on the outermost surface of the root has irregularly shaped cells and measures 9.83-40.80 x 7.64-21.01 μm . A multi-layered parenchyma measuring 8.47-52.69 x 8.71-40.60 μm is located beneath the periderm. Phloem cells with three to four layers and dimensions of 5.79 to 15.14 x 4.06 to 15.73 μm are found beneath the parenchyma. There is unclear cambium. The center of the root is filled with xylem, which occupies a greater area. Trachea cells range in size from 14.86-65.96 x 25.63-56.19 μm , are larger than tracheid cells, and are sporadically distributed. The xylem has a wider coverage area than the phloem. Its pith rays are made up of two to four rows of rectangular cells. Polygonal or circular parenchymatous cells make up the pith (Figure 2, Table 2).

3.1.2. Stem anatomy

The stem of *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* has cross-sections measuring 4.88-9.57 μm that show a single-layered epidermis coated in an undulate cuticle. Oval or rectangular cells measuring 6.34-19.99 x 6.03-12.47 μm make up the epidermis. The epidermis contains branch multicellular hairs. Three to five rows of collenchyma cells measuring 7.16 to 25.19 x 7.49 to 20.41 μm are located beneath the epidermis. Ovular and orbicular parenchymatous cells arranged in 7-10 rows and measuring 10.10-37.83 x 8.86-27.09 μm make up parenchyma tissue. Underneath the parenchyma are 4-8 rows of sclerenchyma layers. There is a thin cambium. Phloem measures 2.53-7.87 x 2.41-5.14 μm , but xylem is greater than phloem and measures 12.98-44.61 x 15.09-59.81 μm . The parenchymatous cells that make up the pith are hexagonal or circular, and their intercellular gaps have dimensions of 10.33-26.46 x 9.72-29.28 μm (Figure 3, Table 2).

V. sinuatum subsp. *sinuatum* var. *sinuatum* cross-sections measuring 6.14-10.49 μm showed a single-layered epidermis coated in an undulating cuticle. Oval or rectangular cells measuring 9.95-30.91 x 5.37-17.22 μm make up the epidermis. The epidermis contains complicated multicellular hairs. Three to four rows of 5.25-21.63 x 5.18-23.93 μm collenchyma cells lie beneath the epidermis. Oval orbicular parenchymatous

cells arranged in 5-7 rows and measuring 11.25-43.77 x 7.98-23.34 μm are the constituents of parenchyma tissue. Underneath the parenchyma are 2-4 rows of sclerenchyma layers. There is no difference in the cambium. Size-wise, phloem measures 4.56-14.26 x 3.99-9.40 μm , whereas xylem is larger than phloem and measures 7.35-23.55 x 11.38-35.29 μm . The parenchymatous cells that make up the pith are hexagonal or circular, and their intercellular gaps range in size from 17.20 to 65.10 x 16.94 to 64.43 μm (Figure 3, Table 2).

3.1.3. Leaf anatomy

V. sinuatum subsp. *sinuatum* var. *adenosepalum*'s lamina of the adaxial and abaxial epidermis cross-sections revealed that both epidermises have a single row of rectangular or oval cells, are covered in branched, glandular, and eglandular multicellular hairs, and have a cuticle that is 3.50-5.40 μm thick. There are open collateral vascular bundles. Parenchymal cells encircle the bent vascular bundle. A single layer is formed by the radial arrangement of the xylem components. The midrib is fully grown. A sizable portion of the vascular bundle's surrounding parenchyma layer is covered. Parenchymal tissue cells are organized firmly and in polygons. The diameters of the cells in the lower epidermis are 5.57-25.84 x 7.59-26.56 μm , whereas the cells in the upper epidermis are 12.89-21.96 x 13.03-23.98 μm . The epidermis on top is bigger than that on the bottom. The hypodermis is located beneath the top and lower epidermis. Palisade and spongy parenchyma make up the 144.38-242.77 μm thick mesophyll tissue, which is composed of 1-2 layers of palisade parenchyma above the lower epidermis and 2-3 layers below the upper epidermis. Palisade parenchyma cells are long, uneven, cylindrical or quadrangular, and measure 11.85-19.68 x 18.98-43.66 μm . Spongy parenchyma cells, which are made up of two to three rows of cells, are located between the lower and upper palisade parenchyma cells. The oval or polygonal cells of spongy parenchyma measure 11.24-20.70 x 11.36-31.60 μm and have greater intercellular spacing. Additionally, idioblasts have been found in the leaf mesophyll tissue (Figure 4, Table 2).

In *V. sinuatum* subsp. *sinuatum* var. *sinuatum*, cross-sections of the lamina of the adaxial and abaxial epidermis revealed that both epidermises have a single row of rectangular or oval cells, are covered in multicellular hairs that are glandular, eglandular, and branched, and have a cuticle that is 4.69-9.19 μm thick. There are open collateral vascular bundles. Parenchymal cells encircle the bent vascular bundle. A single layer is formed by the radial arrangement of the xylem components. The midrib is fully grown. A sizable portion of the vascular bundle's surrounding parenchyma layer is covered. Parenchymal tissue cells are organized firmly and in polygons. The cell

diameters of the upper and lower epidermis are 6.89-21.23 x 7.76-16.92 μm and 8.12-21.27 x 3.63-18.41 μm , respectively. The epidermis on top is bigger than that on the bottom. The hypodermis is located beneath the top and lower epidermis. Mesophyll tissue has a thickness of 178.27–270.57 μm and is composed of 1-2 layers of palisade parenchyma above the lower epidermis and 2-3 layers of spongy parenchyma below the upper epidermis. Palisade parenchyma cells are irregular,

long, cylindrical or quadrangular, and measure 9.86-19.49 x 29.45-48.46 μm . Spongy parenchyma cells, which are made up of two to three rows of cells, are located between the lower and upper palisade parenchyma cells. The oval or polygonal cells of spongy parenchyma measure 12.25–24.00 x 16.36–33.43 μm and have greater intercellular spacing. Additionally, idioblasts have been found in the leaf mesophyll tissue (Figure 4, Table 2).

Table 2. The anatomical measurements of varieties *Verbascum* (μm).

Species / Tissues	Width			Length		
	Min.	Mak.	Mean \pm S.	Min.	Mak.	Mean \pm S.
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>adenosepalum</i>						
Root						
Peridermis cell	13.49	43.56	26.67 \pm 8.91	9.14	34.97	19.65 \pm 6.17
Parenchyma cell	6.88	40.95	21.91 \pm 9.36	8.14	20.29	14.87 \pm 3.14
Phloem cell	5.80	17.00	9.83 \pm 2.94	5.90	12.87	9.26 \pm 1.87
Trachea cell	20.11	94.49	54.11 \pm 19.70	22.50	89.61	56.84 \pm 19.38
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>						
Peridermis cell	9.83	40.80	19.27 \pm 7.76	7.64	21.01	14.22 \pm 3.92
Parenchyma cell	8.47	52.69	29.65 \pm 12.11	8.71	40.60	22.11 \pm 8.07
Phloem cell	5.79	15.14	9.48 \pm 2.50	4.06	15.73	6.61 \pm 2.52
Trachea cell	14.86	65.96	41.45 \pm 16.39	25.63	56.19	40.38 \pm 10.60
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>						
Stem						
Cuticle	-	-	-	4.88	9.57	7.46 \pm 1.43
Epidermis cell	6.34	19.99	12.85 \pm 3.59	6.03	12.47	9.08 \pm 1.68
Collenchyma cell	7.16	25.19	14.69 \pm 4.45	7.49	20.41	12.82 \pm 3.44
Parenchyma cell	10.10	37.83	20.49 \pm 8.03	8.86	27.09	16.26 \pm 5.69
Phloem cell	2.53	7.87	4.80 \pm 1.32	2.41	5.14	3.62 \pm 0.81
Trachea cell	12.98	44.61	30.89 \pm 8.04	15.09	59.81	40.12 \pm 12.40
Pith cell	10.33	26.46	18.53 \pm 4.62	9.72	29.28	18.13 \pm 4.84
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>						
Cuticle	-	-	-	6.14	10.49	8.28 \pm 1.33
Epidermis cell	9.95	30.91	17.19 \pm 4.82	5.37	17.22	10.64 \pm 3.62
Collenchyma cell	5.25	21.63	13.12 \pm 3.77	5.18	23.93	11.59 \pm 4.19
Parenchyma cell	11.25	43.77	24.45 \pm 8.38	7.98	23.34	14.00 \pm 4.00
Phloem cell	4.56	14.26	8.79 \pm 2.34	3.99	9.40	6.79 \pm 1.52
Trachea cell	7.35	23.55	16.42 \pm 4.81	11.38	35.29	22.37 \pm 6.35
Pith cell	17.20	65.10	38.76 \pm 14.39	16.94	64.43	38.07 \pm 14.53
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>						
Leaf						
Cuticle	-	-	-	3.50	5.40	4.31 \pm 0.60
Upper epidermis cell	12.89	21.96	16.50 \pm 2.92	13.03	23.98	16.63 \pm 4.00
Palisade parenchyma	11.85	19.68	15.05 \pm 2.19	18.98	43.66	30.23 \pm 5.61
Spongy parenchyma	11.24	20.70	15.60 \pm 2.33	11.36	31.60	24.20 \pm 5.01
Mesophyll layer	-	-	-	144.38	242.77	181.71 \pm 20.68
Lower epidermis cell	5.57	25.84	12.66 \pm 4.69	7.59	26.56	12.78 \pm 4.13
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>						
Cuticle	-	-	-	4.69	9.19	6.29 \pm 1.12
Upper epidermis cell	6.89	21.23	14.72 \pm 4.16	7.76	16.92	11.54 \pm 3.07
Palisade parenchyma	9.86	19.49	14.20 \pm 2.48	29.45	48.46	40.82 \pm 4.94
Spongy parenchyma	12.25	24.00	17.34 \pm 3.01	16.36	33.43	23.74 \pm 4.13
Mesophyll layer	-	-	-	178.27	270.57	205.50 \pm 20.93
Lower epidermis cell	8.12	21.27	12.40 \pm 3.36	3.63	18.41	9.71 \pm 3.06

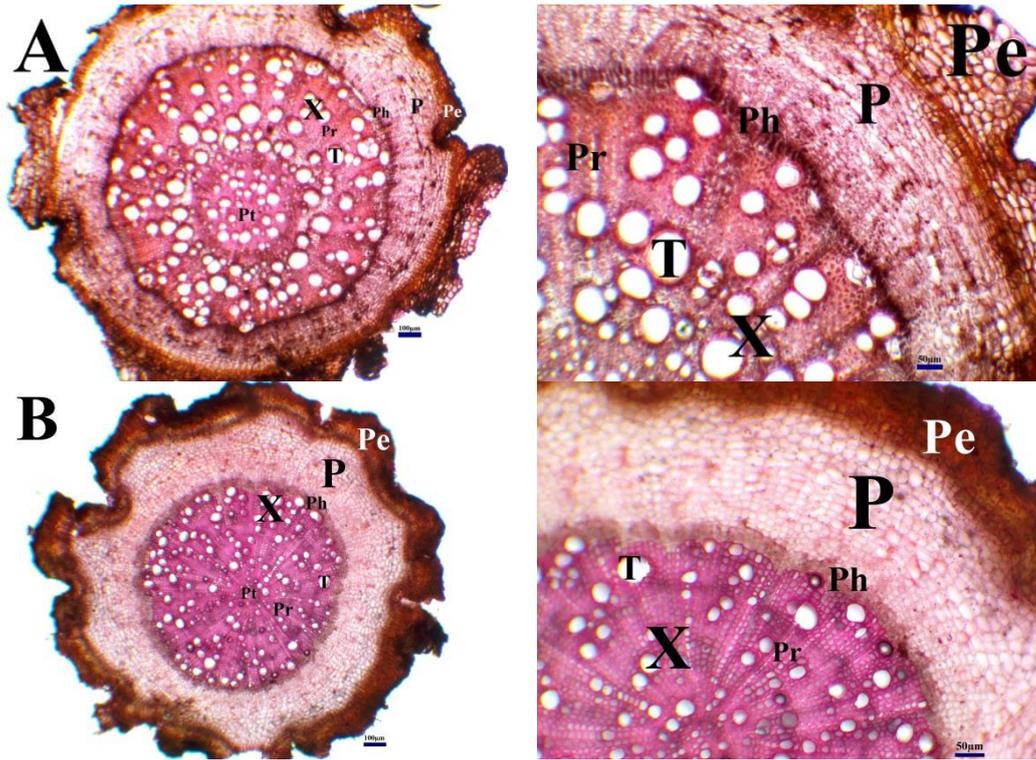


Figure 2. Cross-section of the root of A: *V. sinuatum* subsp. *sinuatum* var. *adensepalum*, B: *V. sinuatum* subsp. *sinuatum* var. *sinuatum*. Pe: Periderm, P: Parenchyma, Ph: Phloem, X: Xylem, Pr: Pith ray, T: Trachea, Pt: Pith region.

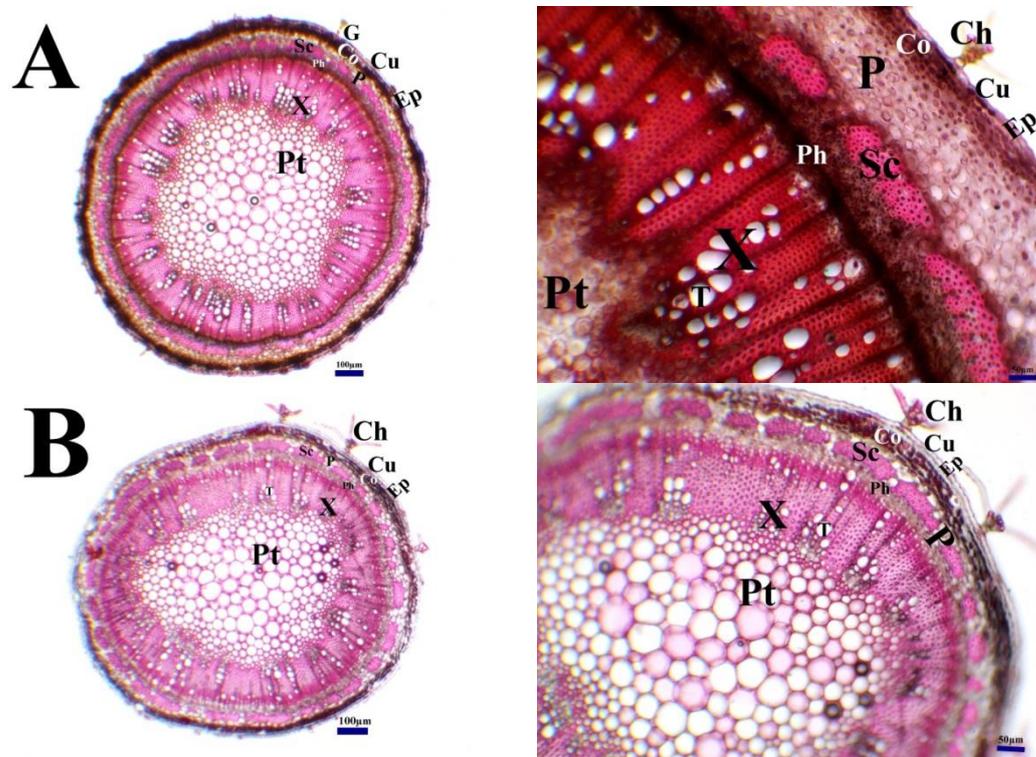


Figure 3. Cross-section of the stem of A: *V. sinuatum* subsp. *sinuatum* var. *adensepalum*, B: *V. sinuatum* subsp. *sinuatum* var. *sinuatum*. G: Glandular hair, Ch: Candelabra hair, Cu: Cuticle, Ep: Epidermis, Co: Collenchyma, P: Parenchyma, Sc: Sclerenchyma, Ph: Phloem, X: Xylem, T: Trachea, Pt: Pith region.

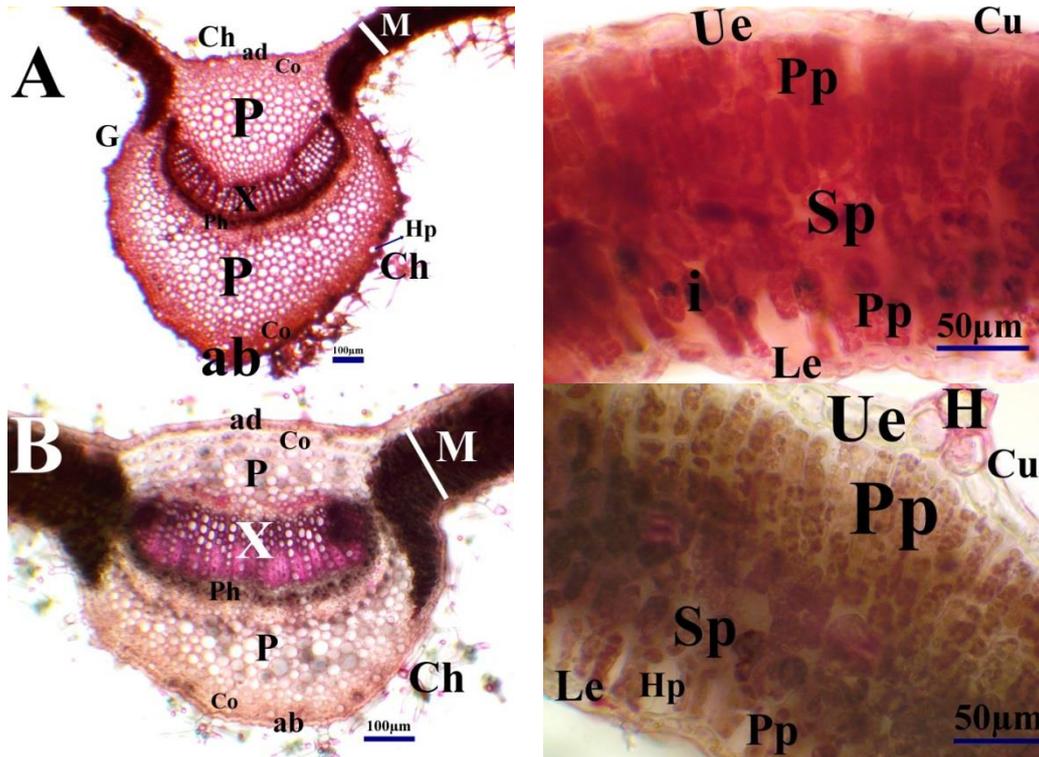


Figure 4. Cross-section of the leaves of A: *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, B: *V. sinuatum* subsp. *sinuatum* var. *sinuatum*. G: Glandular hair, H: Hair, Ch: Candelabra hair, M: Mesophyll layer, ad: Adaxial surface, Co: Collenchyma, P: Parenchyma, X: Xylem, Ph: Phloem, ab: Abaxial surface, Ue: Upper epidermis, Le: Lower epidermis, Pp: Palisade parenchyma, Sp: Spongy parenchyma, Hp: Hypodermis, i: idioblast.

3.2. Pollen morphology

3.2.1. Size, symmetry and Shape

Verbascum pollen grains have radial symmetry and are isopolar. The prolate-spheroidal and prolate pollens of the variations have an equatorial diameter of 4.41-18.11 µm and a polar axis of 10.18-16.61 µm. According to Table 3 and Figure 5, they are larger in *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* and smaller in *V. sinuatum* subsp. *sinuatum* var. *sinuatum*.

3.2.2. Apertures

Verbascum pollen grains are tricolporate (eighty-three percent of *V. sinuatum* subsp. *sinuatum* var. *sinuatum* and seven percent of *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* are tricolporate). The dimensions of the colpus are length (7.58-14.78 µm) and breadth (1.74-4.61 µm), with sharp edges and defined, regular margins. The porus measures 1.19–5.59 µm in width and 2.15–6.31 µm in length. (Figure 5, Table 3).

3.2.3. Exine, intine and ornamentation

The thickness of the exine varies between 0.44 and 1.23 µm. Intine thickness ranges from 0.20 to 0.83 µm (Table 3). In the two examined types, exine sculpturing is reticulate (Table 3, Figure 5).

3.3. Seed morphology

3.3.1. Seed size

The measurements show that the dimensions range from 0.57 to 1.11 mm in length and 0.31 to 0.68 mm in width for each species. According to Table 4 and Figure 6, they are larger in *V. sinuatum* subsp. *sinuatum* var. *sinuatum* and smaller in *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*.

3.3.2. Seed Shape

Within the genus *Verbascum*, species and infra-specific taxa can be distinguished by their seed morphology. Among the examined species, the morphologies of the seeds were found to be prismatic-oblong with ±shallow alveolate and alveolate (Table 4, Figure 6).

3.3.3. Apex of the seeds

In *V. sinuatum* subsp. *sinuatum* var. *sinuatum* and *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, the seeds have large beaks and a deep-broad back (Table 4, Figure 6).

3.3.4. Seed color

Two distinct seed colors were detected in this study: light brown and brown (Table 4, Figure 6).

3.3.5. Seed ornamentation

The seed coat has an alveolate or longitudinally ridged appearance due to the irregular polygonal cells with distinct and tightly packed vesicles (Table 4, Figure 6).

3.4. Capsule morphology

The measurements show that the length and width of *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* range from 3.33 to 4.75 mm and 2.48 to 3.46 mm, respectively, while the dimensions of *V. sinuatum* subsp. *sinuatum* var. *sinuatum* range from 1.76 to 2.52 mm and 2.11 to 2.21 mm, respectively.

While the capsules of *V. sinuatum* subsp. *sinuatum* var. *sinuatum* are spheroidal and ovate, those of *V. sinuatum* subsp. *sinuatum* var. *adenosepalum* are ovate and rectangular in shape. Brown-light brown capsules belong to *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, while light brown capsules belong to *V. sinuatum* subsp. *sinuatum* var. *sinuatum*. Stellate and glandular hairs cover *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, whereas densely stellate and glandular hairs cover *V. sinuatum* subsp. *sinuatum* var. *sinuatum* (Table 5, Figure 7).

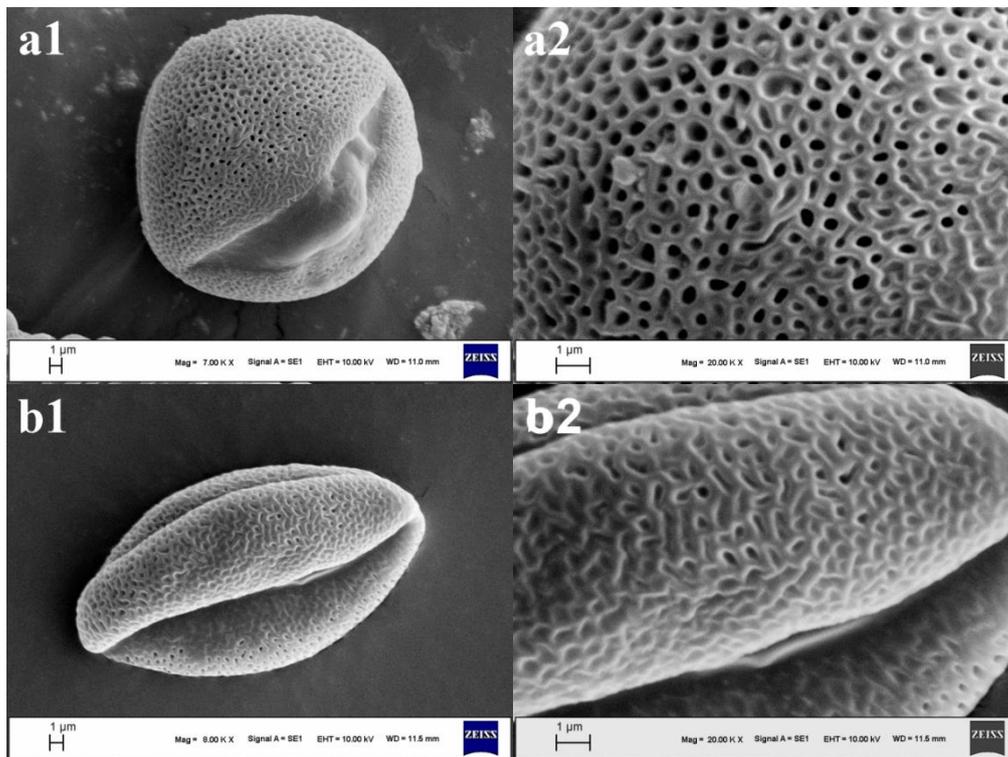


Figure 5. Pollen photographs of *Verbascum* varieties (SEM). 1- Equatorial view, 2- Exine sculpturing. (a1-a2) *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, (b1-b2) *V. sinuatum* subsp. *sinuatum* var. *sinuatum*.

Table 3. Pollen morphological characters of *Verbascum* varieties (μm) (min (mean) max).

Species	P	E	P/E	Shape	Clg	Clt	Plg	Plt	Ex	In	Apt	Or
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>adenosepalum</i>	11.68 (14.25)	12.39 (13.97)	1.02	Prolate-spheroidal	9.24 (11.73)	2.31 (3.22)	3.30 (4.86)	2.49 (3.84)	0.59 (0.88)	0.32 (0.52)	93 % Tricolporate	Reticulate
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	16.61	18.11			14.78	4.61	6.31	5.59	1.23	0.83	7 % Tricolporate	
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	10.18 (11.38)	4.41 (7.12)	1.59	Prolate	7.58 (8.85)	1.74 (2.59)	2.15 (2.61)	1.19 (1.74)	0.44 (0.63)	0.20 (0.43)	17 % Tricolporate	Reticulate
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	13.46	9.46			10.83	3.76	3.55	2.25	0.88	0.54	83 % Tricolporate	

Table 4. Seed morphological characters in varieties of *Verbascum* (mm) (min (mean) max).

Species	Group ^a	Length	Width	Color	Shape	Seed surface
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>adenosepalum</i>	H	0.57 (0.87) 1.08	0.32 (0.52) 0.68	Brown	Prismatic-oblong, deep and broad back, broad beaks	alveolate, broad Irregular, polygonal and rectangular cells with densely and distinct vesicles. Inside the cells are 3-6 transverse lines
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	H	0.61 (0.83) 1.11	0.31 (0.52) 0.68	Brown	Prismatic-oblong, shallow alveolate, deep and broad back, broad beaks	± ovate, Irregular, polygonal and long rectangular cells with densely and distinct vesicles. Inside the cells are 4-8 transverse lines

Table 5. Capsules morphological characters -of *Verbascum* varieties (mm).

Species	Group ^a	Length	Width	Color	Shape	Hair
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>adenosepalum</i>	H	3.33 (3.97) 4.75	2.98 (3.06) 3.46	Brown-light brown	Ovate, oblong	Stellate, glandular
<i>V. sinuatum</i> subsp. <i>sinuatum</i> var. <i>sinuatum</i>	H	1.76 (2.14) 2.52	2.11 (2.16) 2.21	Light brown	Spheroidal, ovate	Densely stellate, glandular

^a According to Huber-Morath (1978)

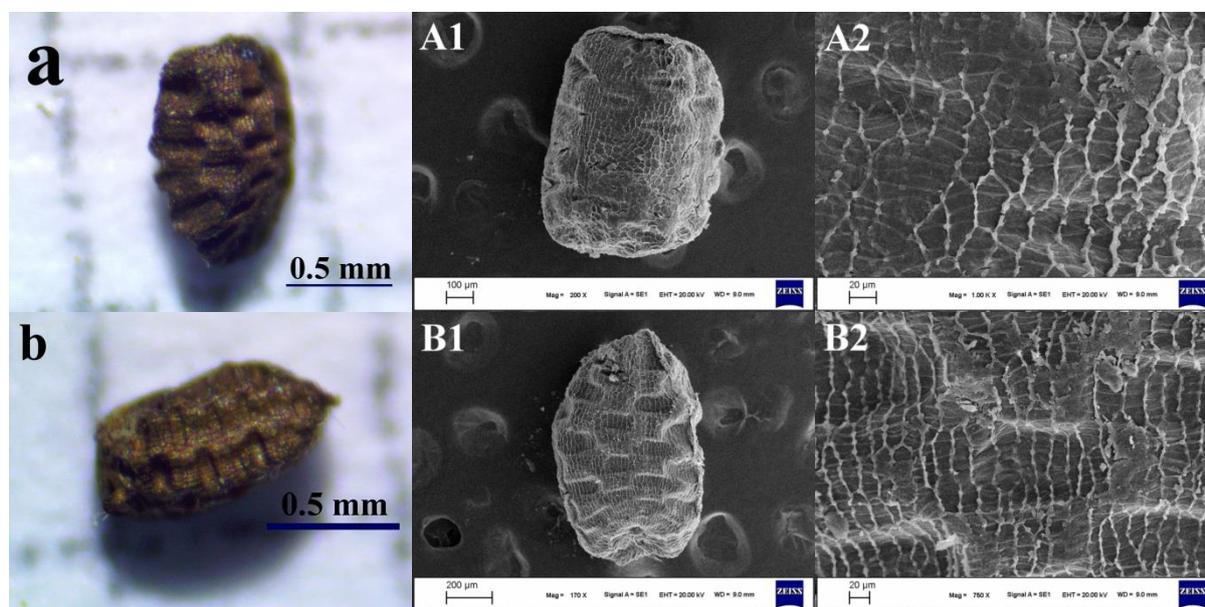


Figure 6. Seed micrographs of *Verbascum* varieties (SEM). a: *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, b: *V. sinuatum* subsp. *sinuatum* var. *sinuatum*, 1- General appearance, 2- Surface ornamentation. (A1-A2) *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, (B1-B2) *V. sinuatum* subsp. *sinuatum* var. *sinuatum*.

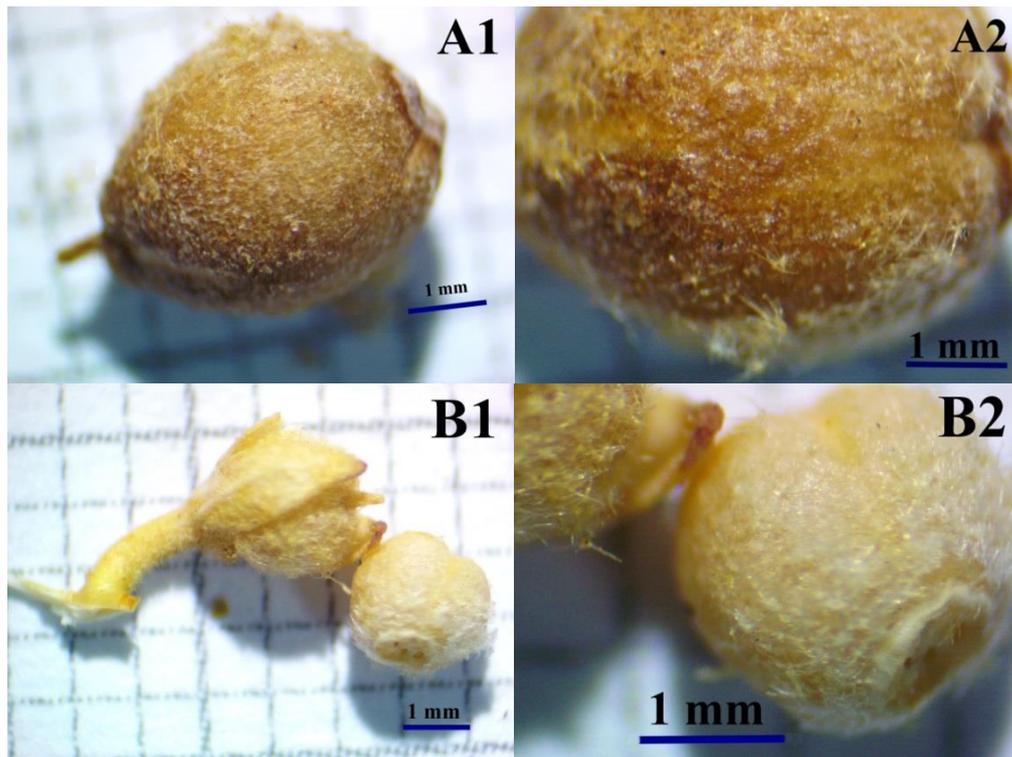


Figure 7. Capsule photographs of *Verbascum* varieties (Stereo microscopy). 1- General appearance, 2- Close view. (A1-A2) *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*, (B1-B2) *V. sinuatum* subsp. *sinuatum* var. *sinuatum*.

4. Conclusion

In this study, the anatomy, pollen and seed analyzes of *Verbascum sinuatum* subsp. *sinuatum* var. *adenosepalum* and *V. sinuatum* subsp. *sinuatum* var. *sinuatum* varieties distributed in the Southeastern Anatolia Region were examined for the first time, ensuring that they are comparable to other *Verbascum* members examined.

A very large xylem area at the root and a thick cuticle layer on the stem were observed in both varieties. Moreover, prior research [12-15, 19-22] have found similar characteristics. The cambium in the vascular bundle of the root was reported to be indeterminate in several studies [13], yet this study indicated that the root exhibited similar features for the varieties. Cross-sectional studies of the leaf's epidermal cells revealed densely glandular, eglandular, and branching multicellular hairs. Comparable outcomes have been documented for several *Verbascum* species under investigation [13]. Identification within the genus *Verbascum* may be aided by the presence of idioblasts in the leaf's mesophyll tissue [29]. Idioblasts were found in the leaf mesophyll in this study, and other studies have also reported this trait [20].

Anatomical research in vascular plants are primarily conducted on the stem since it is less subject to environmental variables [30]. This information is

supported by the stem anatomy of these types, which displays unique characteristics.

The two *Verbascum* varieties examined in this study are similar to some of the other *Verbascum* members examined based on analyses of their pollen, seeds, and capsules.

The exine of two *Verbascum* types has a tectate structure and reticulate ornamentation, and the pollen is isopolar, radially symmetric, prolate-spheroidal, prolate, and tricolporate (there is tricolpate). The pollen grain was identified as tricolpate by Aktas et al. [13] in their investigation of the endemic *Verbascum* species. Aktas [12] observed in his study on *Verbascum* species that the pollen grain was tricolpate. Öztürk et al. [7] found that *Verbascum bithynicum* (H group) tricolporate and tricolpate aperture types in their pollen analysis. According to Al-Hadeethy et al. [10], *Verbascum* species (20 species) had tricolporate aperture types. In their investigation, Tricolporate aperture type is the reported aperture type of *Verbascum* [9]. Two varieties under investigation have prolate-spheroidal and prolate shaped pollen. However, the prolate pollen morphology in the endemic *Verbascum* species under investigation was noted by Aktas et al. [13]. According to Aktas [12], the species he studied exhibits subprolate pollen shapes. According to Öztürk et al. [7], the species under investigation had prolate- and oblate-spheroidal pollen morphologies. According to Asmat et al. [9], *V. thapsus* pollen grains were oblate-spheroidal. Furthermore, Al-

Hadeethy et al. [10] noted that *Verbascum* (20 species) included both oblate and prolate-spheroidal pollen. According to Al-Hadeethy et al. [10], there was minimal taxonomic significance for this trait because the taxa under study had similarities in exine thickness. The current study's findings are consistent with earlier research on a few *Verbascum* species [7, 9-10, 13]. All of the taxa in our research, however, displayed a consistent reticulate exine sculpturing pattern.

When fully grown, the seeds were brown. There were differences in the seed shapes of the varieties under study. The size of the seed usually ranged between 0.57 to 1.11 mm in length and 0.31 to 0.68 mm in wide. The experimental variation design in size was limited among the species, despite Attar et al. [28] showing that the size of the seed displayed variability among the various populations of the same species, even among seeds in the same capsule. The current study's measurements agreed with those of the common species found in Cabi et al.'s [17] investigations. However, the measurements in this study were more than those of the species that were frequently studied in the studies by Attar et al. [28] and Kheiri et al. [16]. The longest seeds in this study were found in *V. sinuatum* subsp. *sinuatum* var. *sinuatum*, while the shortest seeds were found in *V. sinuatum* subsp. *sinuatum* var. *adenosepalum*. Within the genus *Verbascum*, there is enough variation in seed form to distinguish between species and subtaxa within the species.

The importance of a few traits in the *Verbascum* variety delimitation has been investigated in this study. The systematics of the genus can benefit from the use of certain pollen and seed traits, which are typically connected to pollen and seed micromorphological and anatomical patterns. These ancestral characters can also support the infrageneric categorization. This study examines the systematic significance of certain morphological, anatomical, and pollen characteristics of the genus *Verbascum*. More comprehensive research involving all genus taxa will be beneficial in elucidating the systematic issues facing the genus *Verbascum*.

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Author contributions MK and FMK conducted the field work. MK conducted anatomical analysis. MK and FMK wrote and gave comments to the manuscript. Authors read and approved the final version of the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals Not applicable.

Informed consent Not applicable.

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