

A New Distribution Area of *Verbascum stenostachyum* Hub.-Mor. (Scrophulariaceae) From Bursa (Turkey) and Contributions on its Micromorphological, Anatomical and Palynological Properties

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ABSTRACT

This study aims to obtain detailed information about the morphological, micromorphological, anatomical and palynological characteristics of the *Verbascum stenostachyum* Hub.-Mor., which is reported for the first time to the flora of Bursa. Micromorphological, palynological and anatomical properties were examined with SEM (Scanning Electron Microscope) and LM (Light Microscope). According to micromorphological examinations, *Verbascum stenostachyum* has dense stellate and rare glandular hairs. In anatomical examinations, single-row epidermis cells in the stem, leaf cross-sections, and anomocytic-type stomata are at the same level as the epidermis cells. Idioblast cells of different sizes containing druse crystals were found under the epidermis cells. The pollen shape was determined to be prolate-spheroidal, and the pollen to be tricolpate. The pollen ornamentation was determined as reticulate. The seeds have different shapes, such as prismatic, oblong, and ovate and are dark brown-black. Surface ornamentation was determined as reticulate.

Keywords: *Verbascum stenostachyum* Hub.-Mor., Micromorphology, Anatomy, Palynology, Türkiye

INTRODUCTION

The genus *Verbascum* L. (Scrophulariaceae) is represented by 360 species worldwide. There are 241 species in Türkiye, 165 of which are endemic to the flora (Güner 2012, Ekim 2014). Anatolia is one of the diversity centres of the genus *Verbascum* (Kaynak *et al.* 2006; Sotoodeh 2015). Endemic species in Türkiye are mostly distributed in the Eastern, Southern and Central Anatolia regions. The genus *Verbascum* is divided into two sections according to their seed morphologies: *Aulacospermae* Murb. and *Bothrospremae* Murb. All *Verbascum* species found in Türkiye belong to *Bothrospremae* (Sotoodeh 2015). Chemical structures have been studied in *Verbascum* (Hartleb *et al.* 1997; Kalpoutzakis *et al.* 1999; Abou Gazar *et al.* 2003; Magiatis *et al.* 2000). The flowers and leaves are widely used in Europe. Additionally, the flowers of some *Verbascum* species are collected and dried to prepare the Flores Verbasci drug. It has been reported that the saponins contained in this drug cause hemolysis in human blood (Çakır and Bağcı 2006; Baytop 1994; Zeybek *et al.* 1994). Florium Verbasci drug is obtained from dried *Verbascum* leaves (Baytop 1994). Florium Verbasci is rich in saponin, mucilage, resin and bitter substances. Drugs obtained from *Verbascum* are used as chest softeners, expectorants and diuretics (Baytop 1999; Çakır and Bağcı 2006; Zeybek 1985).

The aims of this study are: (a) to present the new localities of *Verbascum stenostachyum* Hub.-Mor. detected in Bursa (Turkey); (b) to investigate its micromorphological, anatomical, and palynological features to provide a useful source for future studies on the identification of other *Verbascum* species.

MATERIALS AND METHODS

The study materials were collected from Orhaneli and Harmancık (Bursa / Türkiye). The samples used in the anatomical study were placed in 70% alcohol and labelled. The remaining samples are kept in Bursa Uludağ

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University Herbarium (BULU). Deposited specimens from the Bursa Uludağ University Herbarium (BULU) were studied for morphological observations. Stem, stem leaf, basal leaf, sepal, petal, bract, bracteole, pedicel and capsule measurements were made on the collected samples. The data obtained were compared with the characters in Flora of Turkey. For anatomical observations, the stems and leaves of the plant were studied. After cross-sections were taken by hand, they were made into a fixed preparation with glycerin-gelatin. To examine the anatomical features by electron microscopy, samples were coated with gold-palladium and examined in the electron microscope in the microscope room of Bursa Uludağ University. For the palynological observations, preparations were made using the Wodehouse method (1935). A LEICA DM4000M camera microscope was used for imaging and photographing.

RESULTS AND DISCUSSION

Morphological characteristics

Verbascum stenostachyum Hub.-Mor. in *Bauhinia* 1(1):75 (1955) (Figure 1).

Type: [Turkey B3] Kütahya: Eskişehir- Kütahya, Kalkschutt 20 km nordöstlich Kütahya, 900 m, 13 vi 1954, Huber-Morath 12301 (holo. Hb. Hub.-Mor.!) (Figure 2).

Biennial, 60-97 cm tall, stellate, woolly tomentose, glandular-hairy. The stem has a straight or slightly branched, rounded and striped structure. Basal leaves lanceolate to linear-lanceolate, 10.3-30 x 2-3.9 cm, flat acute-acuminate; The stem leaves are 3.6-11.9 x 0.6-2 cm and have shapes ranging from linear-lanceolate to lanceolate. The inflorescence is unbranched or branched, with 3-6 flowers, cylindrical or simple panicle-shaped. Bracts are 3-5 mm long, ovate-lanceolate, and acuminate. Flower stalks are 0.5-9 mm long. Bracteoles are 2 mm long. Calyx 3x1 mm, lanceolate, acute. The corolla is 5-7 x 3-5 mm in diameter, yellow in colour, and its outer surface is stellate-tomentose hairy. Stamens are 5; all anthers are reniform, filaments are woolly hairy up to the anther, and hairs are yellowish white at the base and bright violet at the top. Capsule 5 x 2-3 mm, oblong-cylindrical in shape, densely stellate-tomentos. Seed 1 x 0.5-1 mm, dark brown-black, prismatic-oblong, oblong, ovate, surface ornamentation is reticulate.

Known Distribution: C. Anatolia. B2 Kütahya: Murat Da. between Kütahya and Gediz, 1500 m, M&D. Zohary 553! B3 Kütahya: 21 km from Eskişehir to Kütahya, 900 m, Hub.-Mor.12302! (Figure 3).

Examined specimens: Bursa: Orhaneli – Harmancık, 678 m, Ö. Yılmaz 28/17 and A. Yılmaz (BULU)! Harmancık, Harmancık – Tavşanlı, 675 m, Ö. Yılmaz 33/17 and A. Yılmaz (BULU)!

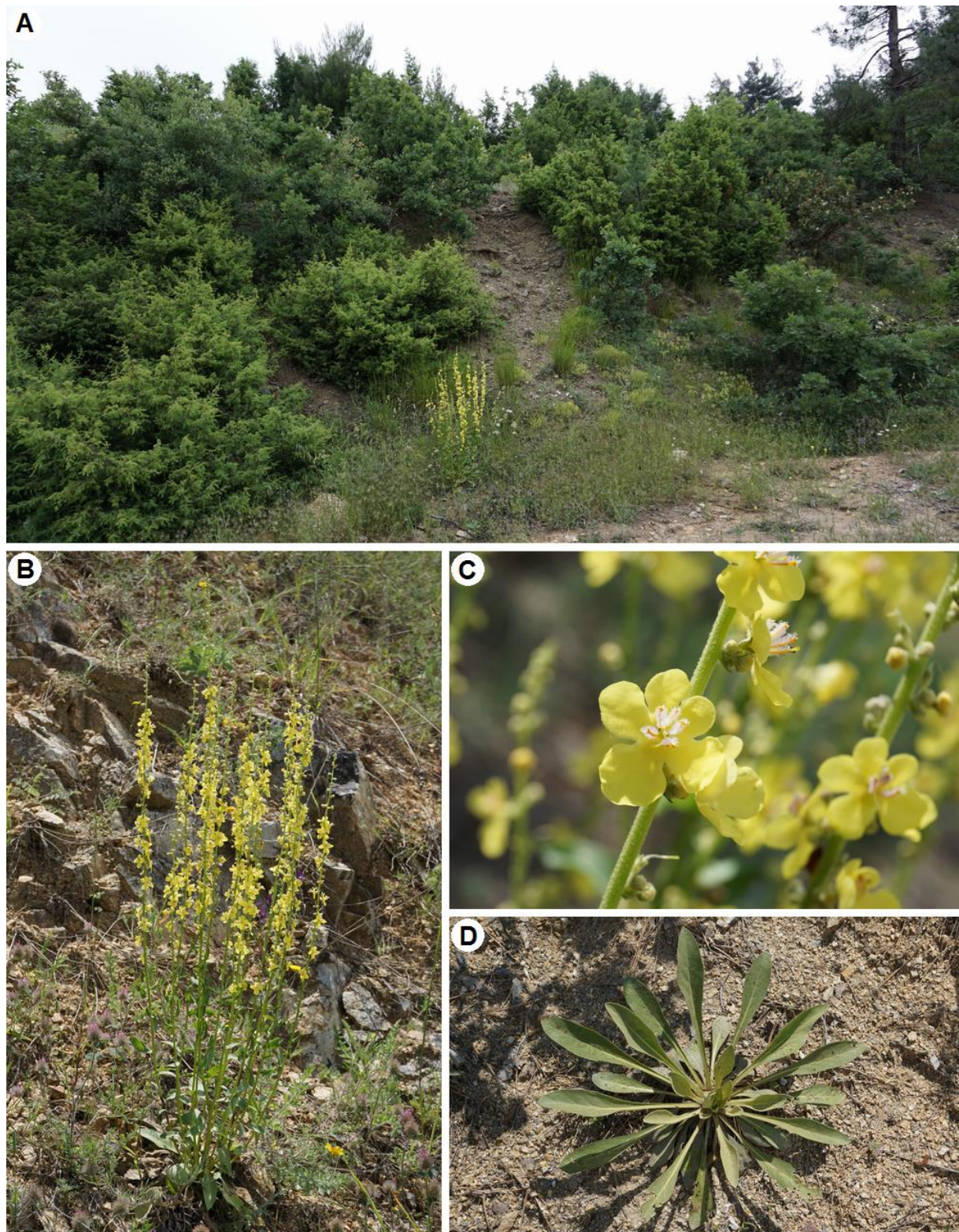


Figure 1. A- General view of *Verbascum stenostachyum* habitat, B- Close-up view *V. stenostachyum*, C- Close-up flower, D- Close-up base leaf

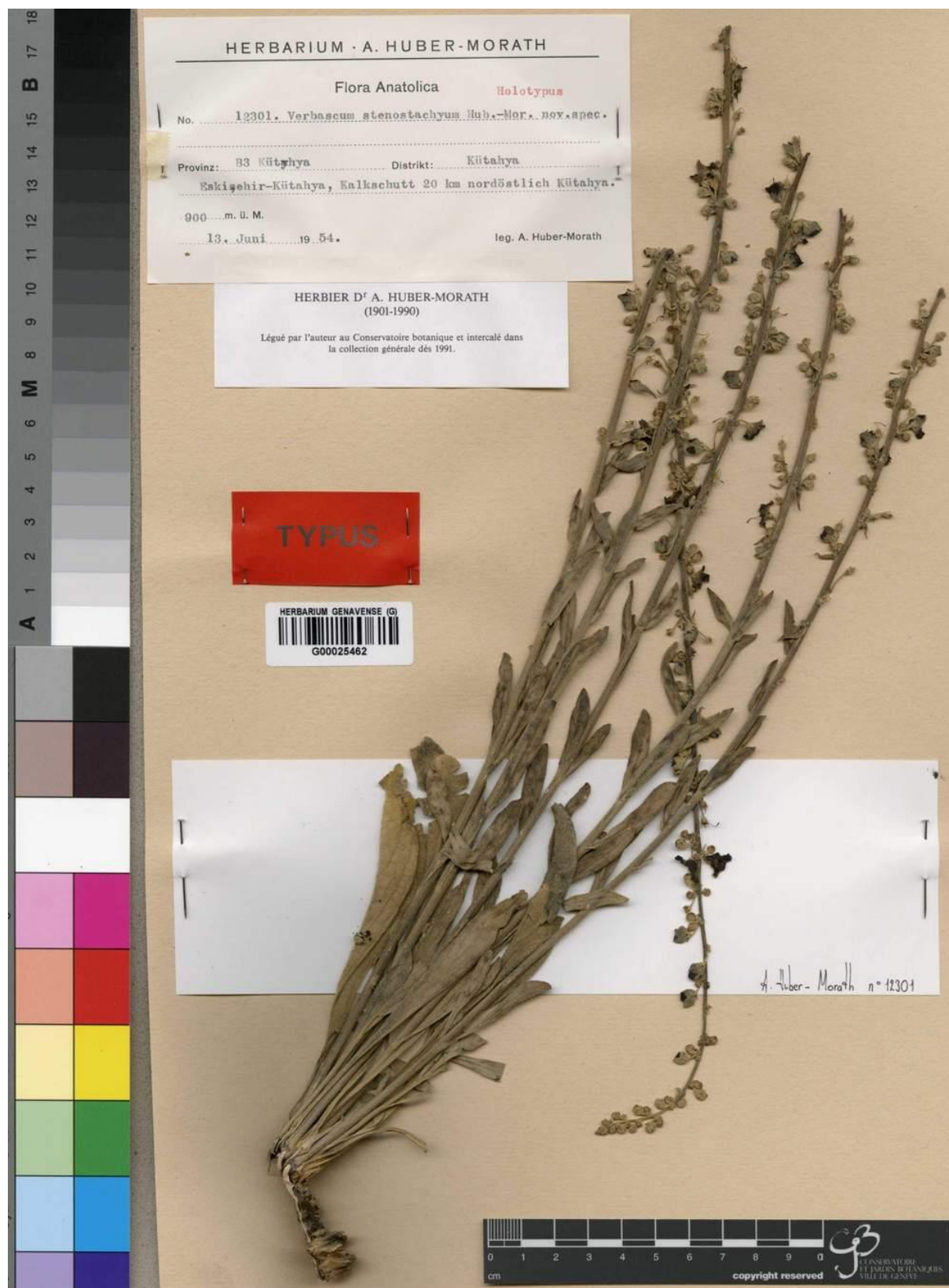


Figure 2. Holotype of *Verbascum stenostachyum* (Catalogue des herbiers de Genève (CHG).Conservatoire & Jardin botaniques de la Ville de Genève. 28-02-2024<<http://www.ville-ge.ch/musinfo/bd/cjb/chg>>.

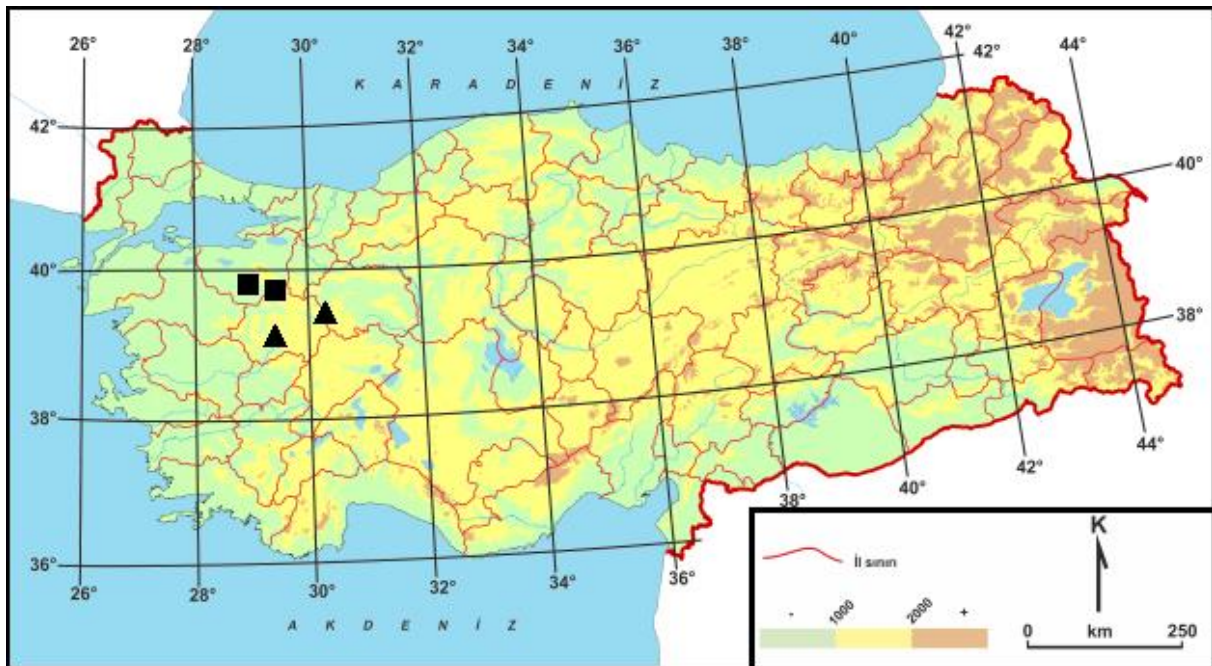


Figure 3. Distribution map of *Verbascum stenostachyum*, known localities (black triangle) and new localities (black square)

Micromorphological characteristics

The stem only contains stellate hairs (Figure 4). Its stem structure has a striped and wavy cuticle layer. There are stomas at the same level as the epidermis. Also, the leaf has stellate hairs on the upper and lower surfaces. The stoma was found on both surfaces. There are dense stellate hairs on the outer surfaces of the sepals and petals, just like the leaves. The pollen ornamentation is reticulate. The seeds have different shapes, such as prismatic, oblong, and ovate, and are generally dark brown and black. Seed surfaces are reticulate (Figure 5).

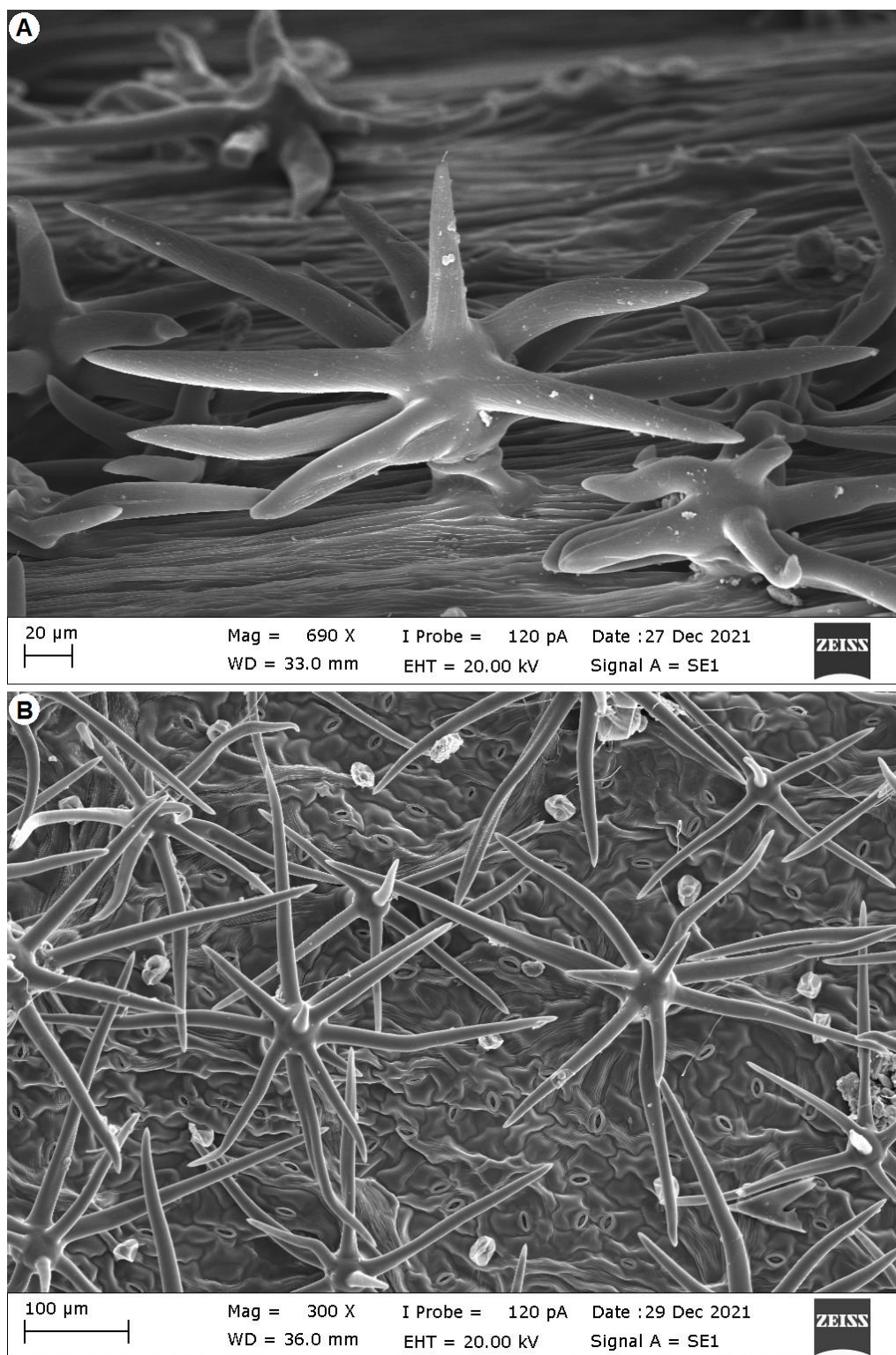


Figure 4. Hair Structure of *Verbascum stenostachyum* (SEM) A- Stem surface, B- leaf surface

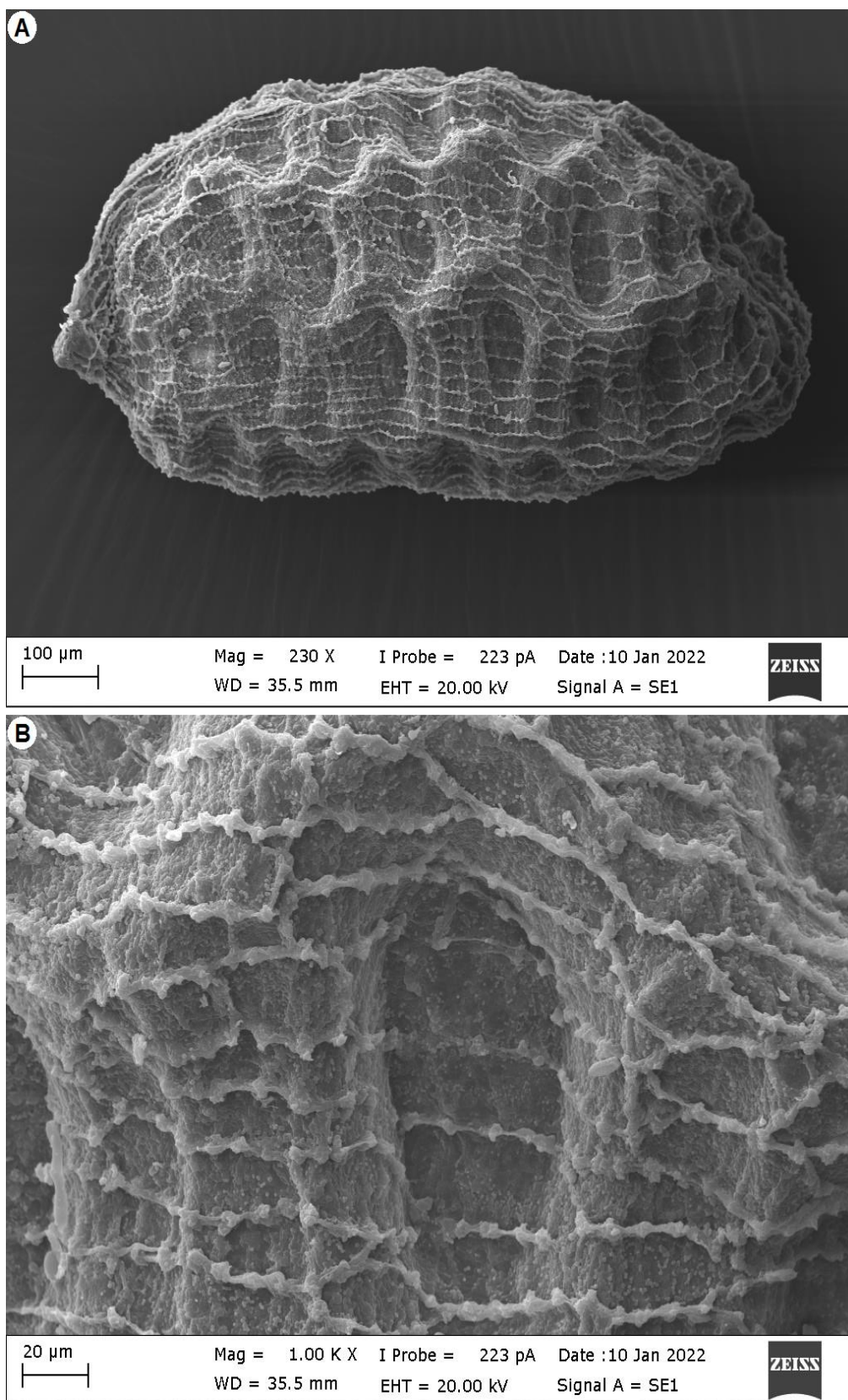


Figure 5. Seed and seed ornamentation of *Verbacum stenostachyum* (SEM)

Anatomical characteristics

When the cross-sections of the stem are examined, there is a cuticle layer at the outermost part. The epidermis cells, which consist of a single row, are rectangular-oval in shape. There are two different types of hairs: stellate and glandular hairs. There are stomas at the same level as the epidermis cells. Under the single-row epidermis, many idioblast cells have diameters of 13.20-27.57 x 30.30-70.91 μm . Druse crystals were observed in some idioblast cells. There are 4-10 rows of cortex parenchyma under the epidermis. The phloem has 2-12 x 3-6 rows of sclerenchyma cells. Phloem cells are in 3-7 rows and are mostly flattened. The cambium is observed thinly (Figure 6).

There is a cuticle layer in the outermost part of the leaf cross-section. Epidermis cells consist of a single layer and mostly oval and rectangular cells. There are two different hairs, stellate and glandular hair, on both the abaxial and adaxial surfaces of the section. As in the stem cross-section, regularly arranged idioblast cells, mostly oval-shaped, with diameters of 41.47-84.25 x 38.26-86.67 μm , were found on both sides of the leaf cross-section. It was observed that the idioblast cells in the leaf were larger than those in the stem. Some idioblast cells contain druse crystals. The leaf structure is isolateral because the palisade parenchyma is present on both leaf surfaces. Amphistomatic leaves have anomocytic-type stomata and are mesomorphic. Vascular bundles are of open collateral type. There are phloem and xylem cells underneath the sclerenchyma cells. The side veins on both sides of the middle vein are anatomically the same as the middle one (Figure 7).

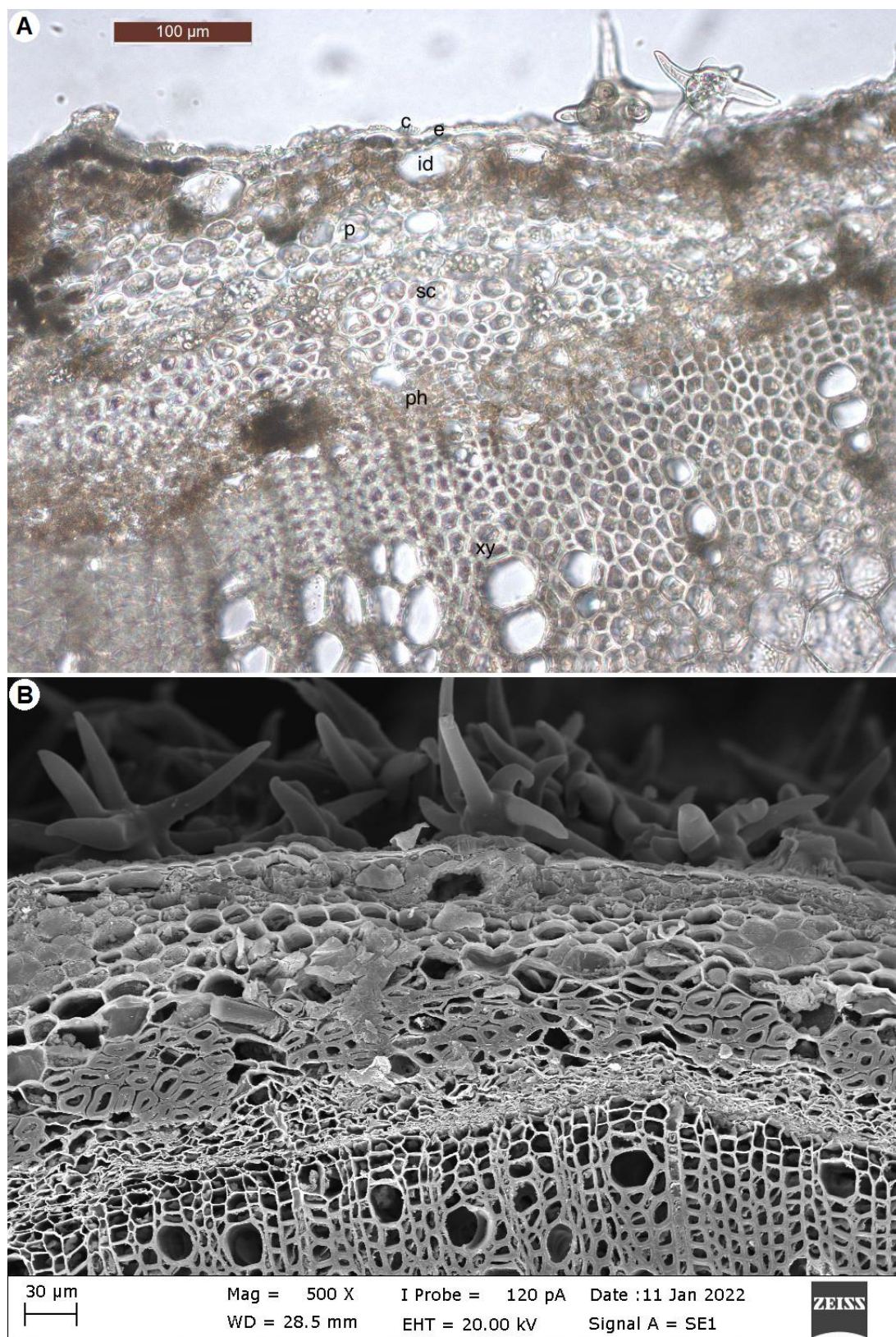


Figure 6. Cross-section of *Verbascum stenostachyum* stem A- LM, B- SEM; c-cuticula, e- epidermis, id- idioblast, p parenchyma, sc- sclerenchyma, ph- phloem, xy- xylem.

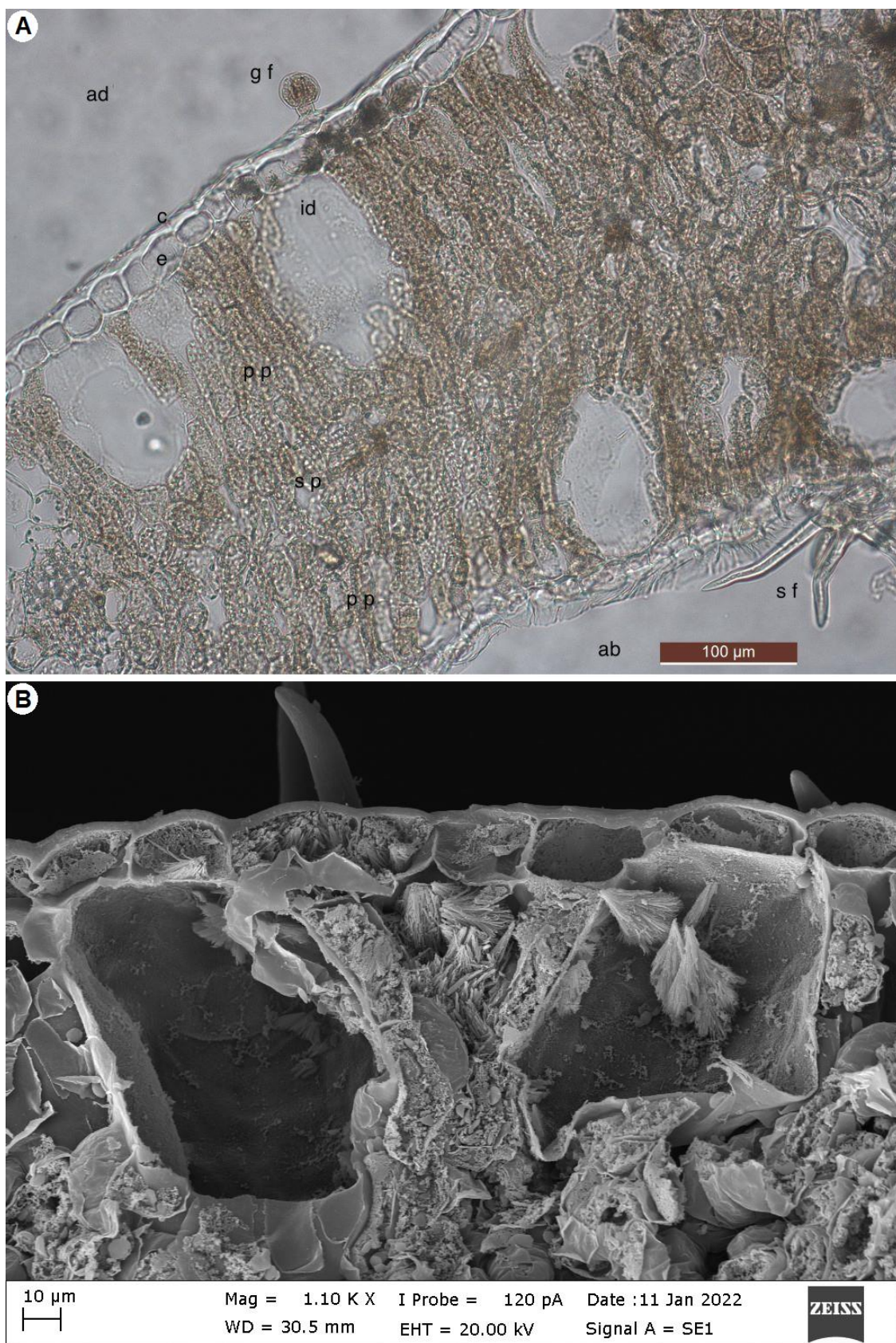


Figure 7. Cross-section of *Verbascum stenostachyum* leaf. A- LM, B- SEM. ad- adaxial, ab- abaxial, gf- glandular hair, c- cuticula, e- epidermis, id- idioblast, pp- palisade parenchyma, sp- sponge parenchyma, sf- stellate hair

Palynological characteristics

The polar axis (P) of the *V. stenostachyum* was determined as $23.725 \pm 1.270 \mu\text{m}$, the equatorial axis (E) was $22.418 \pm 0.734 \mu\text{m}$, the P/E ratio was 1.058 μm , and the pollens shape was prolate-spheroid and the pollens were tricolpate. Colpus length (Clg) $20.941 \pm 1.370 \mu\text{m}$, colpus width (Clt) $6.015 \pm 0.478 \mu\text{m}$, Clg/Clt ratio 3.481 μm , Exine thickness (E) $0.878 \pm 0.175 \mu\text{m}$, intine thickness (I) $0.512 \pm 0.102 \mu\text{m}$. Pollen ornamentation was observed as reticulate (Figure 8).

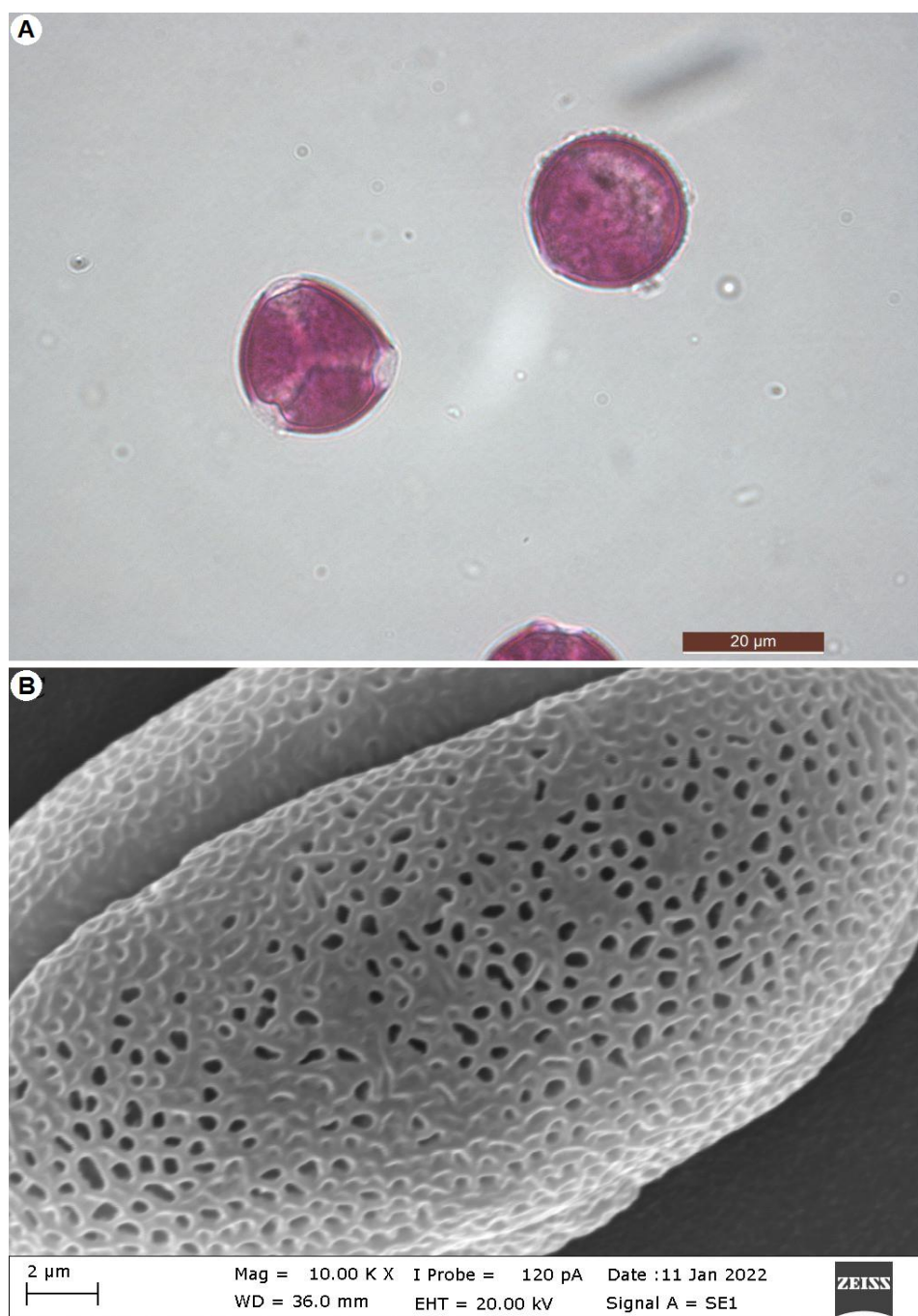


Figure 8. Pollen grains of *Verbascum stenostachyum* A- LM, B- SEM

According to the Flora of Turkey (Huber-Morath 1978), the endemic species *V. stenostachyum* is included in group J and closely similar to *V. cheiranthifolium*. Some differences were determined when the morphological data obtained was compared with the data in the Flora of Turkey. Additionally, the values of some morphological characters not specified in the Flora of Turkey. However, there are many morphological, anatomical and palynological studies for Turkish *Verbascum* species (Alan and Gökman 2015; Cabi *et al.* 2011; Çakır and Bağcı 2006; Demirezen *et al.* 2021)

Stem length 60-97 cm, basal leaf 10.3-30 x 2-3.9 cm, sepal 3 x 1 mm, petal 5-7 x 3-5 cm, number of bundles 3-6, pedicel length 6-9 mm and capsule size is determined as 5 x 2-3 mm. It was determined for the first time that the stem leaf size was 3.6-11.9 x 0.6-2 cm, the bract size was 3-5 mm, its shape was lanceolate, and the bracteole size was 2 mm, its shape was lanceolate (Table 1). Unlike the previous studies, many idioblast cells with diameters of 13.20-27.57 x 30.30-70.91 µm were found in the stem.

Table 1. Comparison of some morphological features of *Verbascum stenostachyum*.

	Hub.-Mor. 1955 (Davis)		In this study
	Length	60-90 cm	60-97 cm
Stem	Hair Structure	Flattened yellowish	Stellate-tomentos,
		Stellate-tomentos, eglandular	Stellate-tomentos, glandular
	Shape	Terete	Terete
	Branching	Unbranched or slightly branched	Unbranched or simply branched
Stem Leaf	Size	-	3,6-11,9 x 0,6-2 cm
	Shape	Lanceolate to linear lanceolate	Linear lanceolate, lanceolate
Basal Leaf	Size	5-15 x 1-3,5 cm	10,3-30 x 2-3,9 cm
	Shape	Complete, acuminate or acute	Lanceolate, acute-acuminate
Sepal	Size	2-3 mm	3 x 1 mm
	Shape	Lanceolate to linear, acute	Lanceolate
	Hair Condition	Stellate-tomentos	Stellate-tomentos
Petal	Size	20 mm	5-7 x 3-5 cm
	Color	Yellow	Yellow
	Hair Condition	Outer surface stellate-tomentos	Outer surface stellate-tomentos
Bract	Size	-	3-5 mm
	Shape	-	Lanceolate
Bracteole (1 pair)	Size	-	2 mm
	Shape	-	Lanceolate
Cluster	Number	2-9	3-6
Pedicel	The longest	8 mm	6-9 mm
	The shortest	-	0,5-2 mm
Anther Shape		Reniform	Reniform
Filament Hair Color		Hairy up to the anther, base whitish yellow, upper part bright violet	Hairy up to the anther, base yellowish white, upper part bright violet
Capsule	Size	6-8 x 2 mm	5 x 2-3 mm
	Shape	Cylindrical	Prismatic-oblong, oblong, ovate,
	Hair Condition	Dense tomentose	Dense stellate-tomentose
Seed	Shape	-	Prismatic oblong, ovate
	Color	-	Dark brown-black
	Surface shape	-	Retikulat
	Size	-	0.7-1 x 0.5-1 mm

Lersten and Curtis (1997) have studied the leaf idioblast anatomy of *Verbascum* and *Scrophularia* species. According to the results, the presence of idioblast cells was reported in 13 of the 39 *Verbascum* species investigated. Although there are many small and large idioblast cells in the petals of *V. thapsus* L., no idioblast cells were found in the leaf. Özdemir and Altan (2007) reported the presence of large-scale idioblast cells surrounded by parenchymatic cells in the lower part of the epidermis or the mesophyll tissue of *V. oreophilum*

K.Koch var. *oreophilum*. They reported that idioblast cells close to the abaxial epidermis were smaller and more numerous than those close to the abaxial epidermis. Kheiri *et al.* (2009) determined in *V. oreophilum*, *V. szovitsianum* Boiss., *V. agrimonifolium* (K.Koch) Hub.-Mor. and *V. macrocarpum* Boiss. single-celled idioblasts. They reported that the idioblasts found in *V. oreophilum* and *V. agrimonifolium* species were round-elliptical in shape. In contrast, the idioblasts found in *V. szovitsianum* and *V. macrocarpum* species were elliptical. They also reported that idioblasts and mesophyll types helped determine the species. Al-Hadeethy *et al.* (2014) examined the leaves of 20 *Verbascum* species. All species were determined to have amphistomatic leaves and stoma type as anomocytic. They divided the mesophyll layer into two, namely regular and irregular mesophyll, according to the shape of the idioblasts. Idioblast cells were found on both surfaces of the leaf in all species. The shapes of idioblast cells are determined as rectangular-polygonal or rectangular-oval. In our study, the idioblasts identified on the leaves were determined to be oval-elliptical in shape and of similar size on both surfaces. These data are similar to other studies.

Eyüpoğlu (2022) studied the pollen morphologies of some *Verbascum* species in group J. According to the results, the P/E ratio of *V. stenostachyum* was 0.95 mm, pollen shape was determined as oblate spheroid, and pollen was tricolporate. Polar axis (P) 23.93 ± 1.00 mm, equatorial axis (E) 25.15 ± 0.94 mm, colpus length (Clg) 20.74 ± 1.29 mm, colpus width (Clt) 3.76 ± 0.59 mm, Clg/Clt ratio 5.51 mm, exine thickness (E) 0.98 mm, intine thickness (I) 0.55 mm. Exine ornamentation was determined as microreticulate. Çakır and Bağcı (2006) determined that the pollen types of *V. melitenense* Hub.-Mor. and *V. euphraticum* Benth. were tricolpate, their pollen shapes were oblate, and their ornamentation was reticulate.

Karavelioğulları *et al.* (2014) reported that pollen shapes of *V. duzgunbabadagensis*, *V. luciliae* and *V. rupicola* were determined as prolate-spheroidal, subprolate, and their ornamentations were determined as reticulate and reticulate-perforate. Öztürk *et al.* (2018) reported that *V. cariense* pollen shape was determined as prolate-spheroidal, *V. nudatum* pollen shape was determined as prolate, *V. bithynicum* pollen shape was determined as subprolate, *V. lasianthum* pollen shape was determined as prolate-spheroidal, *V. pycnostachium* pollen shape was determined as oblate-spheroidal, *V. georgicum* pollen shape was determined as oblate-spheroidal. The ornamentation of all species was reported as reticulate. We determined the pollen type of *V. stenostachyum* as tricolpate, prolate-spheroidal shape, and ornamentation reticulate. Compared to the studies conducted, pollen shape, type and ornamentation are similar to other species. However, when compared to the study conducted by Eyüpoğlu (2022), differences were observed in P/E ratio, pollen shape, colpus width, Clg/Clt ratio and exine thickness.

In the seed micromorphology of *V. stenostachyum*, seed sizes were determined to be between 0.7-1 x 0.5-1 mm, dark brown-black, prismatic-oblong, oblong, ovate, and the seed surface was reticulate. Juan *et al.* (1997) studied the seed morphology of 11 *Verbascum* taxa. It has been reported that the seed sizes are between 0.5-1.8 x 0.4-1.2 mm, dark brown or black in colour, prismatic, and in different shapes from rectangular to triangular. Cabi *et al.* (2011) studied the seed morphologies of 30 *Verbascum* taxa in group A. Seed size 0.3-1.5 x 0.1-0.7 mm, dark brown-black colour, prismatic, elliptical-rectangular, triangular-prismatic, triangular shaped, seed surface irregular polygonal cell, regular elongated polygonal cell, wide regular polygonal cell, small rectangular cell. Seed sizes, shapes and colours of *V. stenostachyum* appear to be compatible with other studies.

CONCLUSIONS

In this study, a new locality for *Verbascum stenostachyum* was reported. In addition to the morphological-micromorphological characters used to identify the species, new characters such as stem leaf size, bract size and shape, bracteole size and shape, seed size, shape, colour and surface shape were determined. In the stem and leaf anatomy, two different hairs, stellate and glandular hairs were observed. Many oval-shaped idioblast cells were detected under the single-row epidermis cells. Druse crystals were observed in some idioblast cells.

This study is thought to help determine the morphological and biological characteristics of the endemic *V. stenostachyum* species.

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