

Studies on *Verbascum ovalifolium* and *V. purpureum* (*Scrophulariaceae*) from the vicinity of Edirne (European Turkey)

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Abstract. The morphological, anatomical, palynological and karyological properties of two subspecies of *Verbascum ovalifolium* (subsp. *ovalifolium* and subsp. *thracicum*), as well as of *V. purpureum* were investigated. In the anatomical study, epidermal cells of the cauline leaves belonging to *V. ovalifolium* subsp. *thracicum* were pentagonally or hexagonally shaped, while the epidermal cells of the other taxa were undulating in shape. There were raphid crystals in the upper epidermis cells of the leaves belonging to *V. ovalifolium* subsp. *ovalifolium*, which do not exist in other taxa. Idioblasts were observed in the epiderma of the leaves of *V. purpureum*, but could not be seen in *V. ovalifolium*. All examined taxa had tricolpate pollen type, prolate pollen shape and reticulate exine ornamentation. The highest pollen sterility rate was found in *V. ovalifolium* subsp. *thracicum*. The diploid chromosome numbers of all three taxa were determined as $2n = 30$.

Key words: anatomy, karyology, morphology, palynology, *Verbascum*

Introduction

The genus *Verbascum* L. (*Scrophulariaceae*) comprises some 360 species across the world (Heywood 1993). In Turkey, with addition of 129 hybrids, the genus is represented by 243 species, which are divided into 13 partly artificial groups. The endemism ratio of the genus is very high, with 193 endemic species (80%) (Huber-Morath 1978; Davis & al. 1988; Ekim 2000). Subsequently, eight species and six hybrids were described (Vural & Aydoğdu 1993; Sutory 2001, 2004; Karavelioğulları & al. 2004, 2006, 2008, 2009; Özhatay 2006; Kaynak & al. 2006; Parolly & Tan 2007; Parolly & Eren 2008; Dane & Yılmaz 2009) and five new species were recorded (Dane & Yılmaz 2005; Yılmaz & Dane 2008; Karavelioğulları 2009; Bani & al. 2010; Karavelioğulları & al. 2011). Anatomical investigation has been carried out by Koktay (1974) and Lersten & Curtis (1997). Pollen morphology of the family *Scrophulariaceae* has been examined by a number of researchers, including Erdtman (1952), Moore & Webb

(1978), Inceoglu (1982), Vargehese (1986), Karim & El-Oqlah (1989), Minki & Eshbaugh (1989), Dane & Yılmaz (2002a); Karavelioğulları & al. (2005), Juan & al. (1997, 1999, 2000), Vujicic & al. (1993).

Few studies have been conducted into the chromosomes of the *Verbascum* species and, as it has been pointed out, they have very small chromosomes. Some species have been karyologically studied by Mori 1957, Arts-Damler 1960, Nilsson & Lassen 1971, Koktay 1974, Dane & Yılmaz 2002b, etc.). There is no anatomical and karyological record for the taxa subject of this study, which are Balkan endemics.

The aim was to investigate the morphological, anatomical, palynological and karyological features of *V. ovalifolium* and *V. purpureum*.

Material and methods

The specimens of *V. ovalifolium*, *V. thracicum* and *V. purpureum* were collected from a natural population

in Edirne in European Turkey. Voucher specimens were deposited in the Herbarium of Trakya University (EDTU).

Anatomical studies were carried out of samples kept in 70% ethyl alcohol. Freehand sections were taken from the leaf, calyx and corolla. Leaf portions of 5 mm² were taken and cleared with chloral hydrate, stained with safranin and Delafield's hematoxyline, and the slides were sealed with glycerine-gelatin (Johansen 1940). Pollen slides were prepared, according to the methods described by Wodehouse (1935) and Erdtman (1952). An Olympus photomicroscope with an apochromatic oil immersion objective (×100) and a periplan eyepiece (×10) were used for the measurements. Thus, the polar axis, equatorial diameter, exine thickness, and colpi length were measured. Generally, 100 pollen grains of the specimens were measured (Sokal & Rohlf 1960). The general morphological terminology used for pollen de-

scription was in accordance with Erdtman (1952) and pollen viability was examined by staining with lactophenol anilin blue and IKI (Mclean & Cook 1941; Johansen 1940). The chromosome preparations were made by a standard root-tip squash technique of Johansen (1940). The slides were examined under an Olympus BH-2 photomicroscope. Drawings were made with the same microscope.

Results

Examined specimens. *V. ovalifolium* subsp. *ovalifolium* – A1 (E) Edirne: Karakasım, Elçili village, 19.06.2001, coll. F. Dane, E. Düzalan, EDTU (8309)! (Figs 1, 2); *V. ovalifolium* subsp. *thracicum* – A1 (E) Edirne: Elçili – Uzunköprü 17. km., 02.07.2002, coll. G. Yılmaz, E. Düzalan, EDTU (8380)! (Figs 1, 3); *V. purpureum* – A1 (E) Edirne: Lalapaşa, around Bağlık deresi, 19.06.2002, coll. G. Yılmaz, EDTU (8340)! (Figs 1, 4).

The data received for the anatomical sections taken from the leaf, calyx and corolla of the plants are shown in Tables 1–5 and Figs 5–7. Stomata frequencies of the cauline leaves are indicated in Table 2. In the palynological study, the mature pollen grains are two-celled and have three colpate apertures, and a thick exine in all taxa. The pollen characteristics are given in Table 6. In the karyological study, all examined taxa were found to be diploid. The chromosome number was determined as $2n = 2x = 30$ for all taxa (Fig. 8).

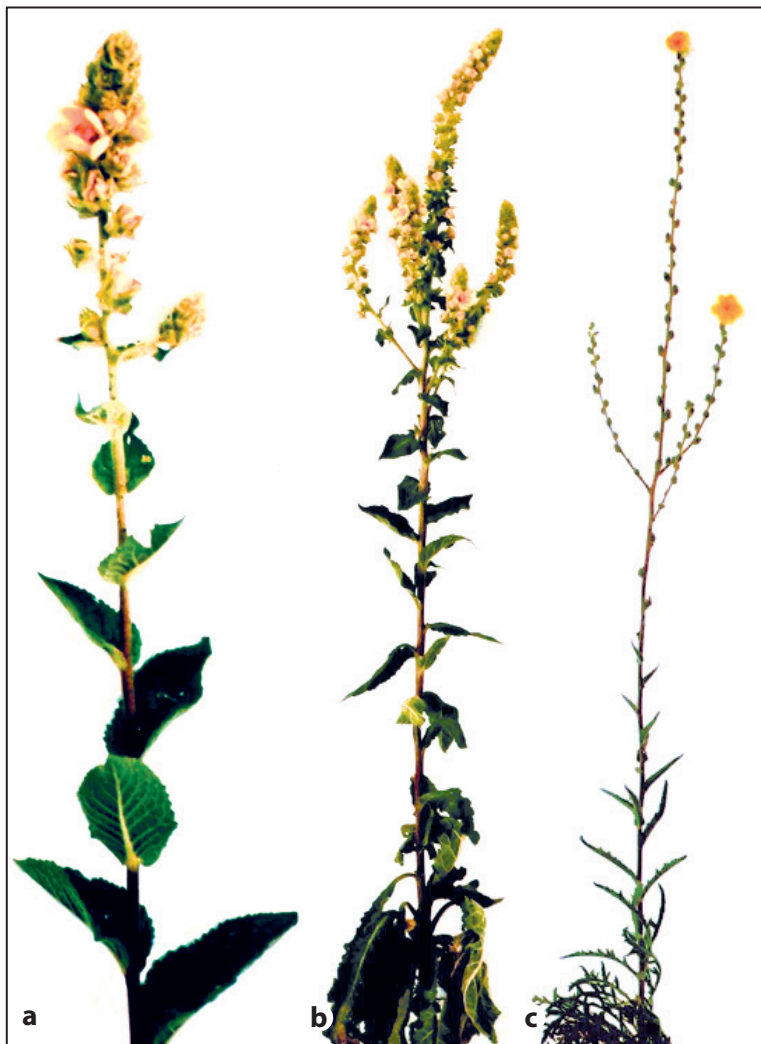


Fig. 1. General view of *Verbascum*: **a**, *V. ovalifolium* subsp. *ovalifolium*; **b**, *V. ovalifolium* subsp. *thracicum*; **c**, *V. purpureum*.

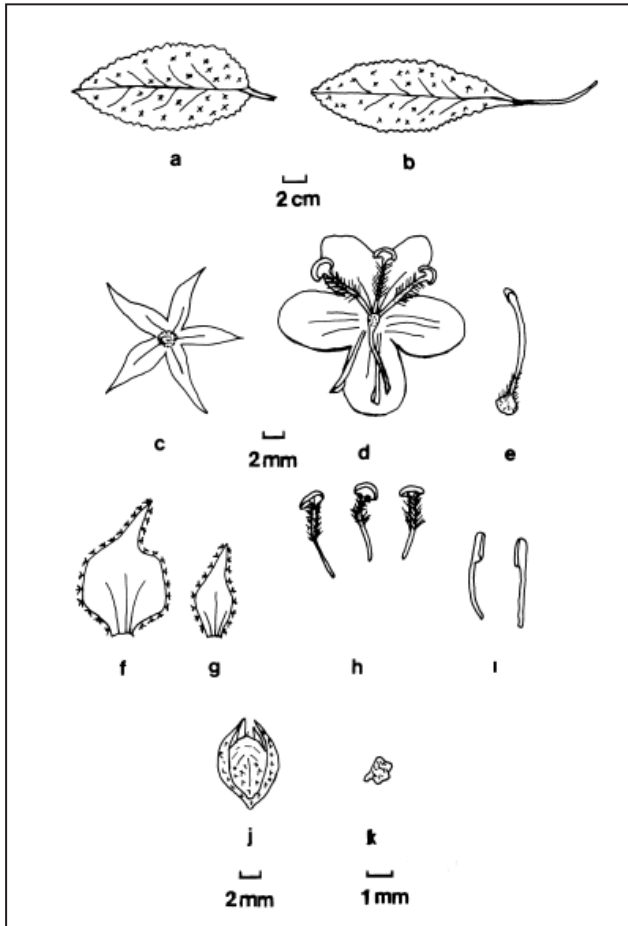


Fig. 2. Drawings of *V. ovalifolium* subsp. *ovalifolium*: a, cauline leaf; b, basal leaf (scale 2 cm); c, calyx; d, corolla with stamens; e, gynoecium; f, bract; g, bracteol; h, upper stamens; i, lower stamens; j, capsula with calyx (scale 2 mm); k, seed (scale 1 mm).

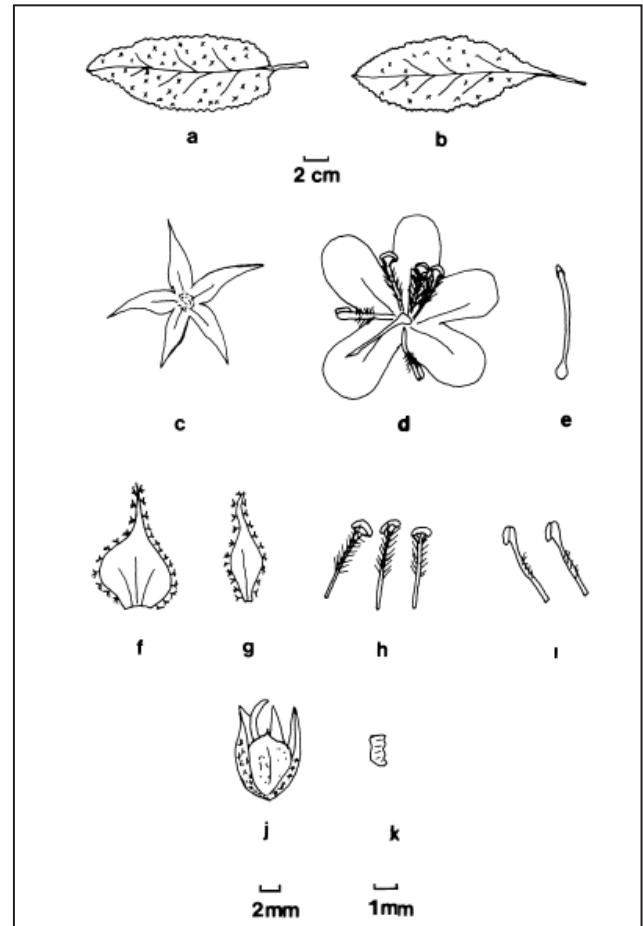


Fig. 3. Drawings of *V. ovalifolium* subsp. *thracicum*: a, cauline leaf; b, basal leaf (scale 2 cm); c, calyx; d, corolla with stamens; e, gynoecium; f, bract; g, bracteol; h, upper stamens; i, lower stamens; j, capsula with calyx (scale 2 mm); k, seed (scale 1 mm).

Table 1. Anatomical properties of the corolla.

Taxa	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (Fig. 7) (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (Fig. 7) (EDTU 8380)	<i>V. purpureum</i> (Fig. 7) (EDTU 8340)	Species investigated in Koktay's study
Adaxial face of corolla	Larger epidermal cells than on the adaxial face, anticlinal cell walls of epidermis cells extremely thin and wavy, isodiametric epidermal cells, short stalked glandular hairs	Larger epidermal cells than on the adaxial face, anticlinal cell walls of epidermis cells extremely smooth and thick, candelabriform hairs	Larger epidermal cells than on the adaxial face, anticlinal cell walls of epidermis cells extremely undulated and thin, unicellular and multicellular unbranched hairs, or bicellular branched hairs	Epidermis cells isodiametric and anticlinal walls of epidermis cells of most species thin and wavy, according to <i>V. georgicum</i> and <i>V. blattaria</i> anticlinal walls of epidermis cells of corolla of <i>V. georgicum</i> and <i>V. blattaria</i> smooth and without hairs
Abaxial face of corolla	Isodiametric epidermal cells, anticlinal cell walls of epidermis cells extremely thin and wavy, candelabriform hairs denser than on the adaxial face	Anticlinal cell walls of epidermis cells extremely smooth and thick, candelabriform hairs denser than on the adaxial face	Anticlinal cell walls of epidermis cells more undulated than on the adaxial face and in the other taxa, unicellular or bicellular unbranched hairs denser than on the adaxial face	Anticlinal walls of epidermis cells of <i>V. georgicum</i> and <i>V. blattaria</i> smooth. Epidermis cells isodiametric and their walls thinner and wavier than on the adaxial face; anticlinal walls of epidermis cells smooth in <i>V. lasianthum</i> and <i>V. sinuatum</i> ; one-celled unbranched hairs in <i>V. xanthophoeniceum</i> and multicelled unbranched hairs in <i>V. pinnatifidum</i> and <i>V. sinuatum</i> . In most studied species various types of glandular hairs on corolla; those in <i>V. blattaria</i> and <i>V. bithynicum</i> resembled each other

Table 2. Stomata frequencies of the species (%).

Taxa	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (EDTU 8380)	<i>V. purpureum</i> (EDTU 8340)
Stomata frequency on adaxial face of cauline leaf	14	15	17
Stomata frequency on abaxial face of cauline leaf	18	17	22

Table 3. Anatomical properties of the calyx.

Taxa	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (EDTU 8380)	<i>V. purpureum</i> (EDTU 8340)	Species investigated in Koktay's study
Adaxial face of calyx	Almost rectangular-shaped epidermis cells, anticlinal walls rather thick, no hairs	Almost like rectangular-shaped epidermis cells, anticlinal walls rather thick, no hair	Anticlinal walls of epidermis cells with more marked undulation, smaller epidermal cells than in <i>V. ovalifolium</i> , no hairs	No information
Abaxial face of calyx	Candelabriform hairs	Candelabriform and stellate hairs	Anticlinal walls of epidermis cells with more marked undulation, smaller epidermal cells than in <i>V. ovalifolium</i> unidirectional, laterally inclined, unicellular unbranched, or bicellular, branched hairs and long-stalked, unidirectional, laterally inclined glandular hairs	The surface sections of calyx examined by Koktay (1974) revealed dense one-celled or branched hairs, or glandular hairs with a small head

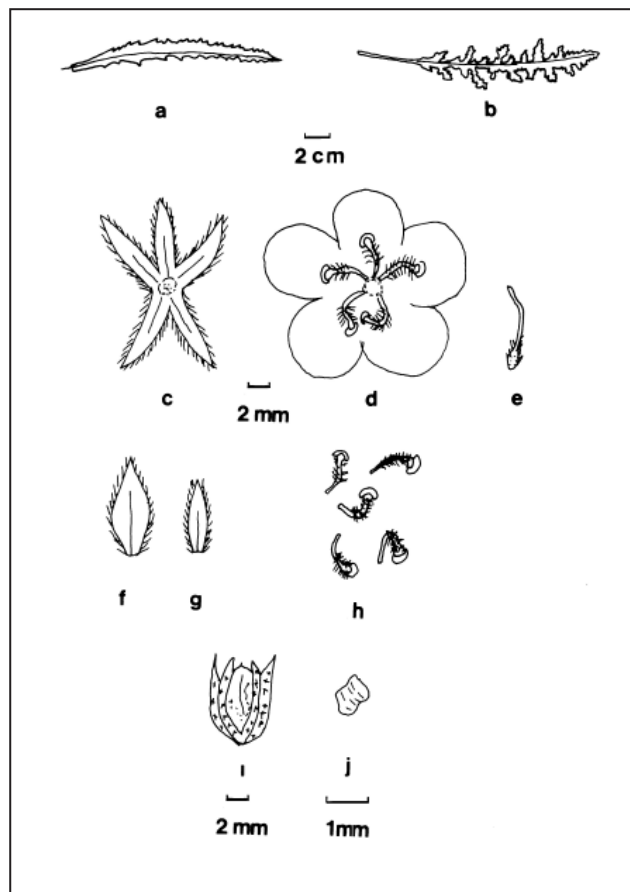


Fig. 4. Drawings of *V. purpureum*: a, cauline leaf; b, basal leaf (scale 2 cm); c, calyx; d, corolla with stamens; e, gynoecium; f, bract; g, bracteol; h, stamens; i, capsula with calyx (scale 2 mm); j, seed (scale 1mm).

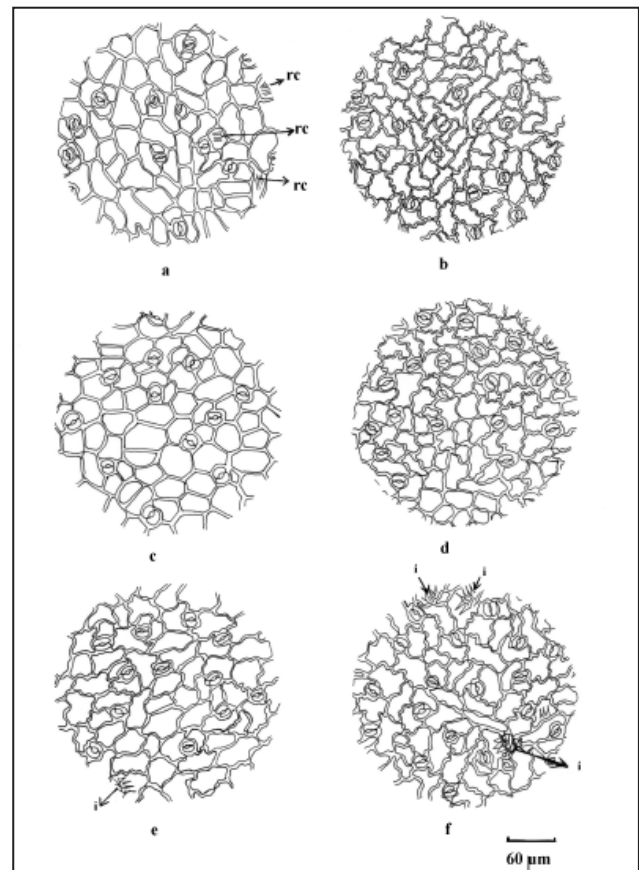


Fig. 5. *V. ovalifolium* subsp. *ovalifolium* leaf: a, upper surface section; b, lower surface section; *V. ovalifolium* subsp. *thracicum* leaf: c, upper surface section; d, lower surface section; *V. purpureum*: e, upper surface section; f, lower surface section (i: idioblast, rc: raphid crystal) (scale 60 μ m).

Table 4. Surface sections of the cauline leaves.

Taxa Features	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (EDTU 8380)	<i>V. purpureum</i> (EDTU 8340)	Species investigated in Koktay's study
Epidermal cells on the adaxial face	Anticlinal walls undulated and thin, raphid crystals in epiderma	Anticlinal walls flattened and thin	Anticlinal walls thin and more undulated than in ssp. <i>ovalifolium</i> and ssp. <i>thracicum</i> , carbohydrate derived materials	No information
Stomata type	Amaryllus type, isocytic or unisocytic, adjacent cell numbers vary between 3 and 5	Amaryllus type, isocytic or unisocytic, adjacent cell numbers vary between 3 and 5	Amaryllus type, isocytic or unisocytic, adjacent cell numbers vary between 3 and 5.	Some stomata with 4 adjacent cells, others with 3–6. Stomata in <i>V. pinnatifidum</i> considerably more flattened and more elongated than in other <i>Verbascum</i> species
Leaf type	Amphystomatic, bifacial	Amphystomatic, bifacial	Amphystomatic, bifacial	Amphystomatic, bifacial
Epidermal cells on the abaxial face	Anticlinal walls thin and more undulated than on the adaxial face,	Anticlinal walls flattened and thin,	Anticlinal walls thin and more undulated than on the adaxial face	No information

Table 5. Cross sections of the cauline leaves.

Taxa Features	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (EDTU 8380)	<i>V. purpureum</i> (EDTU 8340)	Species investigated in Koktay's study
Adaxial face of cauline leaf	Thick cuticula, epidermal cells ovoidal flat shaped, twice bigger than on the abaxial face, candelabrifiform hairs and short-stalked glandular hairs	Thick cuticula, epidermal cells ovoidal flat shaped, twice bigger than on the abaxial face, candelabrifiform hairs and short-stalked glandular hairs	Normal cuticula, epidermal cells somewhat square-shaped, unidirectionally inclined, uni- or multi-cellular unbranched hairs and long-stalked glandular hairs	Epiderma of <i>V. pinnatifidum</i> , <i>V. degenii</i> , <i>V. sinuatum</i> covered with a thick layer of cuticle, epidermis cells flat-shaped and approximately of equal size
Palisade parenchyma	2–3 layers of cylindrical-shaped cells	3–6 layered	3 layered	3–5 layered in <i>V. pinnatifidum</i> and <i>V. degenii</i> growing in sandy and salty soils. 2–3 layered in the other studied species
Spongy parenchyma	4–6 layers of spherical or nearly flat ovoidal cells	4–6 layers, contrary to the former nearly cylindrical cells	5 layers of round or ovoidal cells	9–10 layers in <i>V. pinnatifidum</i> and <i>V. degenii</i>
Abaxial face of cauline leaf	Thin cuticula, epidermis cells somewhat square-shaped, dense candelabrifiform hairs and short-stalked glandular hairs	Thin cuticula, epidermis cells somewhat square-shaped, dense candelabrifiform hairs and short-stalked glandular hairs	Normal cuticula, epidermis cells somewhat square shaped, unidirectionally inclined, dense uni- or multi-cellular unbranched hairs and long-stalked glandular hairs	Epiderma of <i>V. Pinnatifidum</i> , <i>V. degenii</i> end <i>V. sinuatum</i> covered with a thick layer of cuticle, epidermis cells flat-shaped and approximately equal in size, dense candelabrifiform hairs in <i>V. lagurus</i> , <i>V. phlomooides</i> , and <i>V. georgicum</i> . In <i>V. pinnatifidum</i> , unidirectionally and laterally inclined branching prevails. In <i>V. xanthophoeniceum</i> , occasional multicellular simple hairs, while in <i>V. blattaria</i> there were no hairs. Glandular hairs kidney-shaped and composed of a head of 2–4 cells and a more or less long stalk

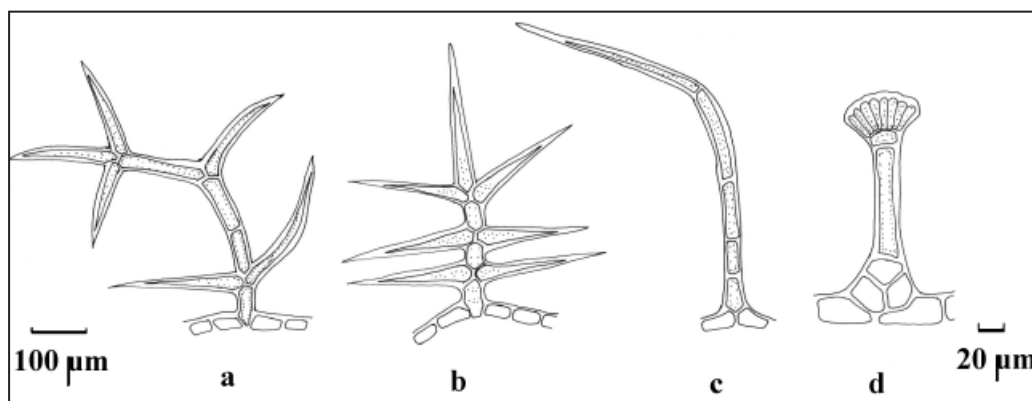


Fig. 6. Candelabrifiform hair on calyx in *V. ovalifolium* subsp. *ovalifolium* (a) and in *V. ovalifolium* subsp. *thracicum* (b); c, multicellular and unidirectional unbranched hair on calyx in *V. purpureum* (scale 100 µm); d, Long-stalked glandular hair on calyx in *V. purpureum* (scale 20 µm).

Table 6. Pollen characteristics of the taxa.

Features	Taxa	<i>V. ovalifolium</i> subsp. <i>ovalifolium</i> (EDTU 8309)	<i>V. ovalifolium</i> subsp. <i>thracicum</i> (EDTU 8380)	<i>V. purpureum</i> (EDTU 8340)
Pollen Type		Tricolpate	Tricolpate	Tricolpate
Pollen Shape (W)		Prolate, P/E = 1.57	Prolate, P/E = 1.81	Prolate, P/E = 1.71
Exine thickness (μm) (W)		1.13 \pm 0.37	1.19 \pm 0.31	1.70 \pm 0.37
Apertures		Colpi, long and wide, borders regular and distinct	Colpi, long and wide, ; borders regular and distinct	Colpi, long and wide, borders regular and distinct
Structure		Tectate ect/end = 3 / 1	Tectate ect/end = 3 / 1	Tectate ect/end = 3 / 1
Sculpture		Reticulate, angular; large shapes between colpus, narrow shape near colpus	Reticulate, angular	Reticulate, slightly angular and very narrow
Intine thickness (μm) (W)		0.62 \pm 0.18	0.61 \pm 0.21	0.80 \pm 0.30
P (μm)		25.05 \pm 2.58	29.88 \pm 1.76	31.00 \pm 2.93
E (μm)		15.9 \pm 2.72	16.5 \pm 2.08	18.07 \pm 2.4
Clg (μm)		18.97 \pm 3.31	22.44 \pm 2.83	25.2 \pm 3.02
Clt (μm)		4.05 \pm 1.44	4.41 \pm 2.13	3.18 \pm 1.47

Abbreviations: (W) – Wodehouse method; P – polar axis; E – equatorial axis; Clg – colpus length; Clt – colpus width; ect – ectexine; end – endexine.

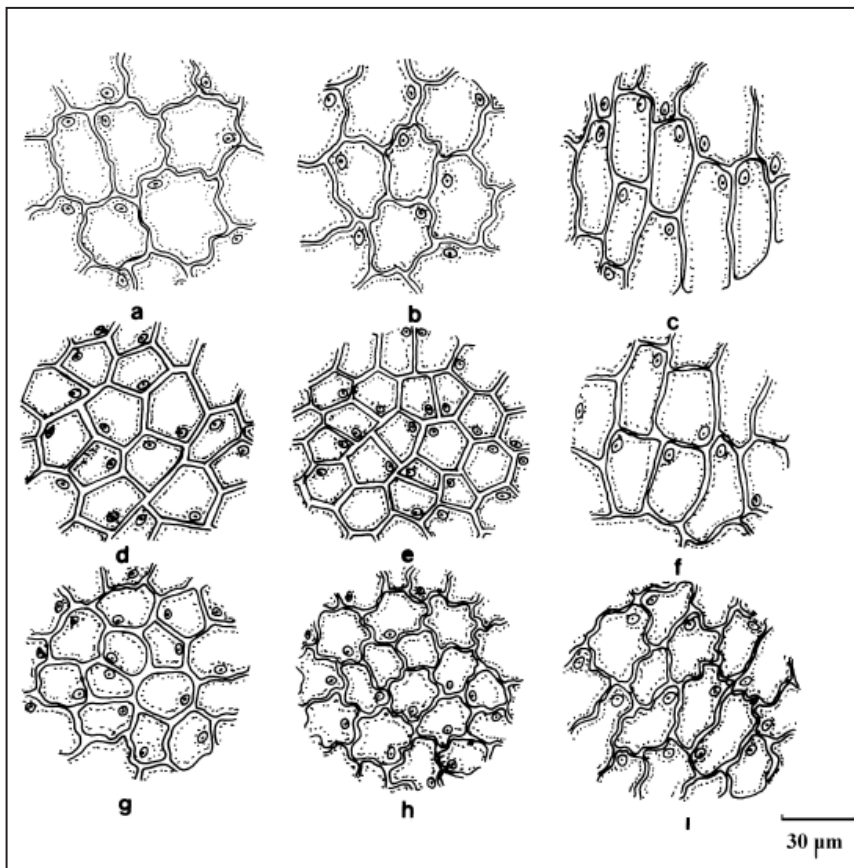


Fig. 7. *V. ovalifolium* subsp. *ovalifolium*: a, adaxial face of corolla; b, abaxial face of corolla; c, adaxial face of calyx; *V. ovalifolium* subsp. *thracicum*: d, adaxial face of corolla; e, abaxial face of corolla; f, adaxial face of calyx; *V. purpureum*: g, adaxial face of corolla; h, abaxial face of corolla; i, adaxial face of calyx (scale 30 μm).

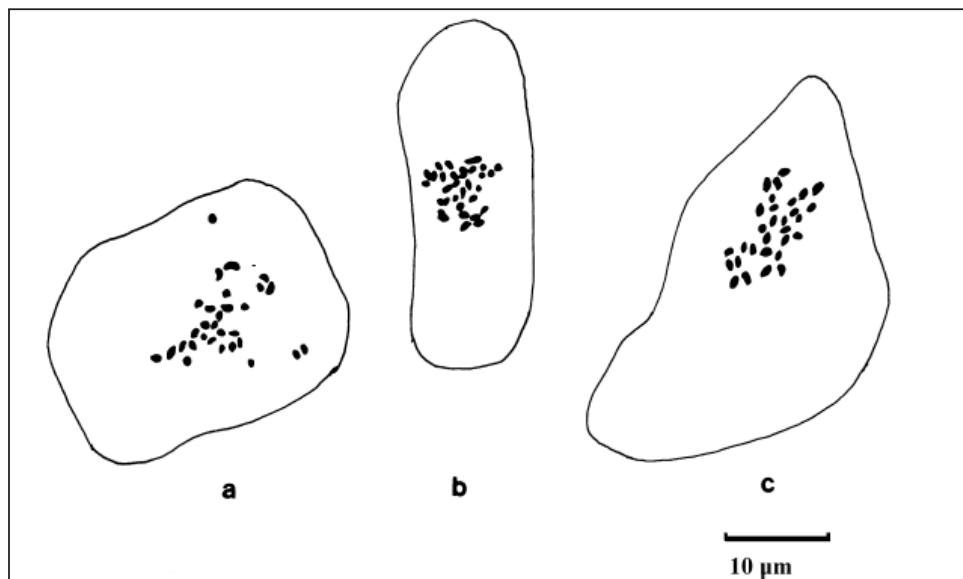


Fig. 8. Drawings of metaphase chromosomes: a, *V. ovalifolium* subsp. *ovalifolium*; b, *V. ovalifolium* subsp. *thracicum*; c, *V. purpureum* (scale 10 μ m).

Discussion

Morphology. Some morphological differences of the examined taxa are pointed out at the end of this study. When compared to data of Huber-Morath (1978), these are as follows: the length of the bract in both subspecies of *V. ovalifolium* is longer – 1-1.5 mm, the calyx lobe of *V. purpureum* is 2 mm longer, and candelabriform hairs are also present on the upper surface of the corolla in subsp. *thracicum*. The deviations in some properties of the studied species are presumably due to ecological factors.

Anatomy. The anticlinal walls of epidermis cells of the leaf, calyx and corolla of subsp. *thracicum* were less undulated, as compared to the other taxa. There were raphid crystals in the upper epidermis cells of the leaf of subsp. *ovalifolium*, which do not exist in other taxa. Koktay (1974) provided information on the general anatomical characteristics of 11 *Verbascum* species. These species also grow in European Turkey, and we have compared our results with hers in Tables 1, 3, 4, 5.

Palynology. The pollen grains of the taxa generally did not differ morphologically from each other, but when their size was investigated from the viewpoint of poles, the largest belonged to *V. purpureum*, followed by *V. ovalifolium* subsp. *thracicum* and subsp. *ovalifolium*. Diameters of the lumens of the reticulate exine were the largest in subsp. *ovalifolium*, followed by subsp. *thracicum* and *V. purpureum* (Table 6). There were differences in the aperture shapes among the species of *Scrophulariaceae*. Pollen sterility rates were 2.04%

in subsp. *ovalifolium*, 18.34% in subsp. *thracicum* and 1.61% in *V. purpureum*.

Karyology. The chromosomes are very small and its number was determined as $2n = 2x = 30$ for all taxa.

In conclusion, *V. ovalifolium* and *V. purpureum* have been investigated in terms of morphology, anatomy, palynology and karyology. The differences between these species are obvious and are presented in Tables 1-6 and Figs 1-7.

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