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Morphological, Anatomical and Palynological Properties of Some Turkish *Veronica* L. Species (Scrophulariaceae)

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Abstract: In this study, pollen and seed morphology, leaf and stem anatomy of four *Veronica* L. species (Scrophulariaceae) one of which is endemic (*V. multifida*) belonging to four Sections [*V. persica* (Sect. *Pocilla*), *V. beccabunga* (Sect. *Beccabunga*), *V. officinalis* and *V. multifida* (Sect. *Veronica*)] grown in Turkey have been studied on light and scanning electron microscopy. Different and similar features of these species were described.

Key words: Scrophulariaceae, *Veronica*, pollen morphology, seed morphology, leaf anatomy, stem anatomy

INTRODUCTION

Veronica sp. L. is a quite large genus comprising approximately 200 species and polymorphic genus of the family Scrophulariaceae occurring mostly in temperate zones of both hemispheres. Its representatives from Europe, Asia and North America the herbaceous or subshrub habit is predominant (Heywood, 1978). The taxonomic history of *Veronica* clearly shows the difficulties involved in defining natural group within it, in fact, regarding delimitation of the genus and infrageneric classification (Martinez-Ortega *et al.*, 2000).

In Turkey, *Veronica* represented with over 86 species belonging to four sections (*Veronicastrum*, *Beccabunga*, *Pocilla* and *Veronica*) (Fischer, 1978).

Some biochemical studies on some Turkish *Veronica* L. species have been made by Ozipek *et al.* (1999), Sarker *et al.* (2000), Ozipek *et al.* (2002), Saracoglu *et al.* (2002), Harput *et al.* (2002a, b). The palynology and seed morphology of some *Veronica* species has already been investigated; by authors, such as Hong and Nilsson (1983), Hong (1984), Fernández *et al.* (1997), Martinez-Ortega *et al.* (2000), Martinez-Ortega and Rico (2001a,b). Other studies on the pollen of *Veronica* have been carried out, but only as part of general pollen catalogues, sometimes of particular regions (Godoy and Diez, 1987) or palynological studies of whole family (Scrophulariaceae) or of different genera from this family (Vergase, 1968; Elisens, 1986).

In this study pollen morphology, seed morphology, leaf and stem anatomy of four *Veronica* species one of which is endemic (*V. multifida*) belonging to four sections [*V. persica* (Sect. *Pocilla*),

V. beccabunga (Sect. *Beccabunga*), *V. officinalis* and *V. multifida* (Sect. *Veronica*)] have been studied by using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The different and similar features of these species have been described.

MATERIALS AND METHODS

Veronica samples were collected from different localities in Turkey. These localities are listed in Table 1. Plant samples were identified according to Fischer (1978). Specimens were preserved in the herbarium of the Faculty of Art and Science at Zonguldak Karaelmas University. Samples were fixed in 70% alcohol for anatomical studies. For light microscopy observations, samples were prepared by paraffin and hand-sectioned. For SEM study, pollen and seed samples were placed aluminum stubs, coated with gold by sputter coater and investigated with JEOL JSM 5600 Scanning Electron Microscope. The terminology used to describe seed coat surface sculpturing is mainly that proposed by Stearn (2004). The palynological terminology mainly follows Punt *et al.* (1994). For description anatomical features, the terminology of Metcalfe and Chalk (1950) was used.

Table 1: Localities of the examined *Veronica* L. taxa

Taxa	Localities and date
<i>V. persica</i> Poirlet	A5, Samsun-Kurupelit, 10 m, 21.05. 2004, Aslı Hasanoğlu
<i>V. beccabunga</i> L.	A9, Artvin Arhavi District, Kapisre river, 5 m, 19.5.2004, Aslı Hasanoğlu
<i>V. officinalis</i> L.	A9, Artvin Arhavi District, Kapisre river, 5 m, 19.5.2004, Aslı Hasanoğlu
<i>V. multifida</i> L.	A8, Erzurum-Tortum Lake, 2000 m, 19. 05. 2004, Aslı Hasanoğlu

RESULTS

Morphological characters

Sect. *Pocilla dumort*

V. persica Poiret: Annual, stems are 10-50 cm, petiole 1-8 mm; lamina 10-20×9-18 mm, bright green, ultimately blackish when dry, hairs robust; margin crenate-serrate, not revolute; pedicels 15-22 mm, 1-2 × bracts, with longer spreading hairs; calyx lobes lanceolate to elliptic, 5-6×2-2.5 mm in fruit, scarcely overlapping laterally, remaining herbaceous; corolla blue, 8-14 mm diameter; capsule compressed 4-6×8-10 mm, 2 lobed, glabrous veins prominent. Seeds are 14-18, 1.6-2.2×1-1.5 mm, irregularly rugose-reticulate (Fig. 1).

Sect. *Beccabunga (hill) domort*

V. beccabunga L.: Perennial with creeping rhizome, blackening when dry. Stem always completely glabrous, decumbent to ascending, 10-20 cm, repent at the base, usually slightly branched, thick, solid, often red, pruinose. Leaves shortly petiolate, fleshy, dark green, shining, completely glabrous; petiole 2 mm; lamina broadly oblong, 10-15×6-8 mm, base rounded-truncate, apex obtuse-rounded, margins subentire. Racemes 2-6-8, 5-10-15 flowered, always completely glabrous. Fruiting pedicels 2-7, 0.8-1.5 × bracts, suberect. Calyx is 2-2.5 mm in flower. Corolla is deep blue with centre, 5-6 mm diameter. Style is 1.5-3 mm. Capsule is slightly transversely broadly elliptic, inflated, 2.5-3×4-4.5 mm, equalling calyx, apex slightly emarginate. Seeds are 40-70, broadly-elliptic, plano-convex, 0.5-0.6×0.4-0.5 mm (Fig. 2).

Sect. *Veronica (sect. Chamaedrys griseb.)*

V. officinalis L.: Perennial stems 10-50 cm, branched, repent, hirsute. Leaves shortly petiolate (petiol 2-4 mm), lamina oblong to broad elliptic, 15-45×8-25 mm, serrate, densely emarginated. Racemes 1-4, narrow, erect, spike-like, dense, 15-35- flowered, densely glandular-pubescent, peduncle 20-35 mm. Pedicels 0.5-2 mm, ½ x length of bracts. Calyx is 2-3.5 mm, lobes 4, linear to oblong. Corolla is lilac-blue, 6-8 mm diameter. Style 2.5-4 mm. Capsule is triangular 3-5-4-5 mm, exceeding calyx, glandular-pubescent, truncate with slight or obtuse-angled emargination, base cuneate. Seeds 10-20, elliptic to suborbicular, 0.8-1.2×0.7-0.9 mm, flat and smooth (Fig. 3).

V. multifida L.: Perennial 7-15 cm, suffruticose usually branched, stems suberect, ascending, decumbent or shortly repent, glabrous; leaf sessile, pinnatisect or bipinnatisect (Fig. 4). Leaves 5-15 mm (lobes 0.5-1.5×rachis) and glabrous; leaf rachis never broader than narrowly linear to linear-lanceolate lobes; lowest leaves

sometimes ovate, serrate, leaves of shoot apex often entire, narrowly linear; 3-7 pairs of leaves present below lowest racemes. Corolla is blue, 7-12 mm diameter. Style is 2.5-5 mm. Capsule is obcordate, 2.5-4×4.6 mm, subequalling calyx puberulent or glabrous. Seeds are 2-10 ovate to suborbicular, 1-2.5×1-1.5 mm, convex, rugose.

Anatomical characters

V. persica: A transverse section taken from the middle of the stem was observed (Fig. 5). Cuticle layer 2-2.5 µm thick (Fig. 6) and trichomes are evident on the cuticle. Epidermis cells consist of a single layer and rectangular or orbicular. Under the epidermis is a monolayer plate collenchyma. Stem cortex (140-175 µm thick) consists of 4-6 layers of usually oval cells and occupies 30% of the stem. The last layer of the cortex occurs starch sheath. One layered sclerenchymatic sheath is under the starch sheath. Cambium is not distinguishable. Xylem tissue, including solitary vessels or clustered vessels and occupies of 18% of stem radius. Pith cells are large and spherical and occupy 35% of the stem.

A transverse section of the lamina and both epidermises was studied (Fig. 7). Mesophyll consists of 2 - 3 layers of isodiametric or prismatic palisade cells and 2 layers of isodiametric spongy parenchyma cells. Vascular bundle is solitary, arc-shaped. Upper epidermal cells are larger than the lower ones. Cuticle layer is 3-3.5 µm thick. Leaf is bifacial and has anomocytic stomata cells. Stomata occur on the both surfaces, level with neighbouring cells.

Veronica beccabunga: A transverse section taken from the middle of the stem was observed (Fig. 8). Cuticle layer 2-3 µm thick (Fig. 9). Epidermis consists of a single layer of rectangular or orbicular cells. Under the epidermis is two layers of plate collenchyma. Stem cortex (560-720 µm) consists of usually spherical aerenchyma cells and makes up 75% of the stem. Cambium is not distinguishable. Xylem tissue, including solitary or clustered vessels, is not extensive and occupies 25% of the stem cortex. Pith cells are spherical and occupied 25% of the stem radius (Fig. 10).

A transverse section of the lamina and both epidermises was studied (Fig. 11). Upper epidermal cells are larger than lower ones. Mesophyll consists of 3 or 4 layers of elongated palisade cells and 3 or 4 layers of isodiametric spongy parenchymatic cells with large intercellular cavities. Vascular bundles are surrounded by parenchymatous bundle sheath. Both epidermises have cuticle 2-2.5 µm. Leaf is bifacial and anomocytic stomata cells. Stomata occur on both epidermal surfaces, upper than neighboring cells (Fig. 11).

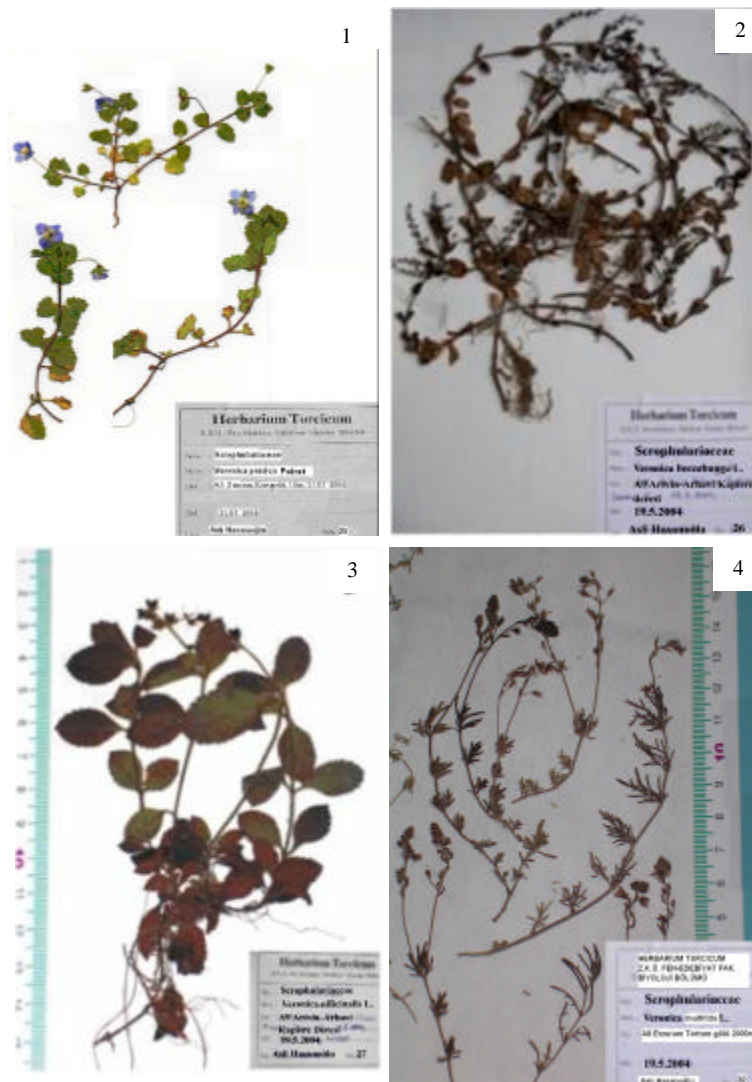


Fig. 1-4: General view of the investigated *Veronica* L. species. 1. *V. persica* Poiret, 2. *V. beccabunga* L., 3. *V. officinalis* L., 4. *V. multifida* L.

***V. officinalis*:** A transverse section taken from the middle of the stem was observed (Fig. 12). Cuticle layer 2-3.5 μm thick (Fig. 13) and trichomes are evident on the cuticle. Epidermis cells consist of a single layer and rectangular or orbicular. Under the epidermis is a monolayer plate collenchyma. Stem cortex (180-190 μm thick) consists of 6-8 layers of usually oval cells and occupies 58% of the stem. The last two layers of the cortex occur starch sheath. One layered sclerenchymatic sheath is under the starch sheath. Cambium is not distinguishable (Fig. 14). Xylem tissue, including solitary vessels or clustered vessels and occupies of 21% of stem radius. Pith cells are large and spherical and occupy 20% of the stem.

A transverse section of the lamina and both epidermises was studied (Fig. 15). Mesophyll is not well differentiated consisting of 3-5 layers of isodiametric parenchymatic cells. Vascular bundle is solitary, arch-shaped and surrounded by elliptical or spherical parenchymatous cells. Upper epidermal cells are larger than the lower ones. There are no trichomes on the both of the epidermis. Cuticle layer is 4-5 μm thick. Leaf is unifacial and has anomocytic stomata cells. Stomata occur on the both surfaces, level with neighbouring cells.

***V. multifida*:** A transverse section taken from the middle of the part of the stem was observed (Fig. 16-17). Cuticle layer 1.8-2.3 μm thick. Under the epidermis is a monolayer

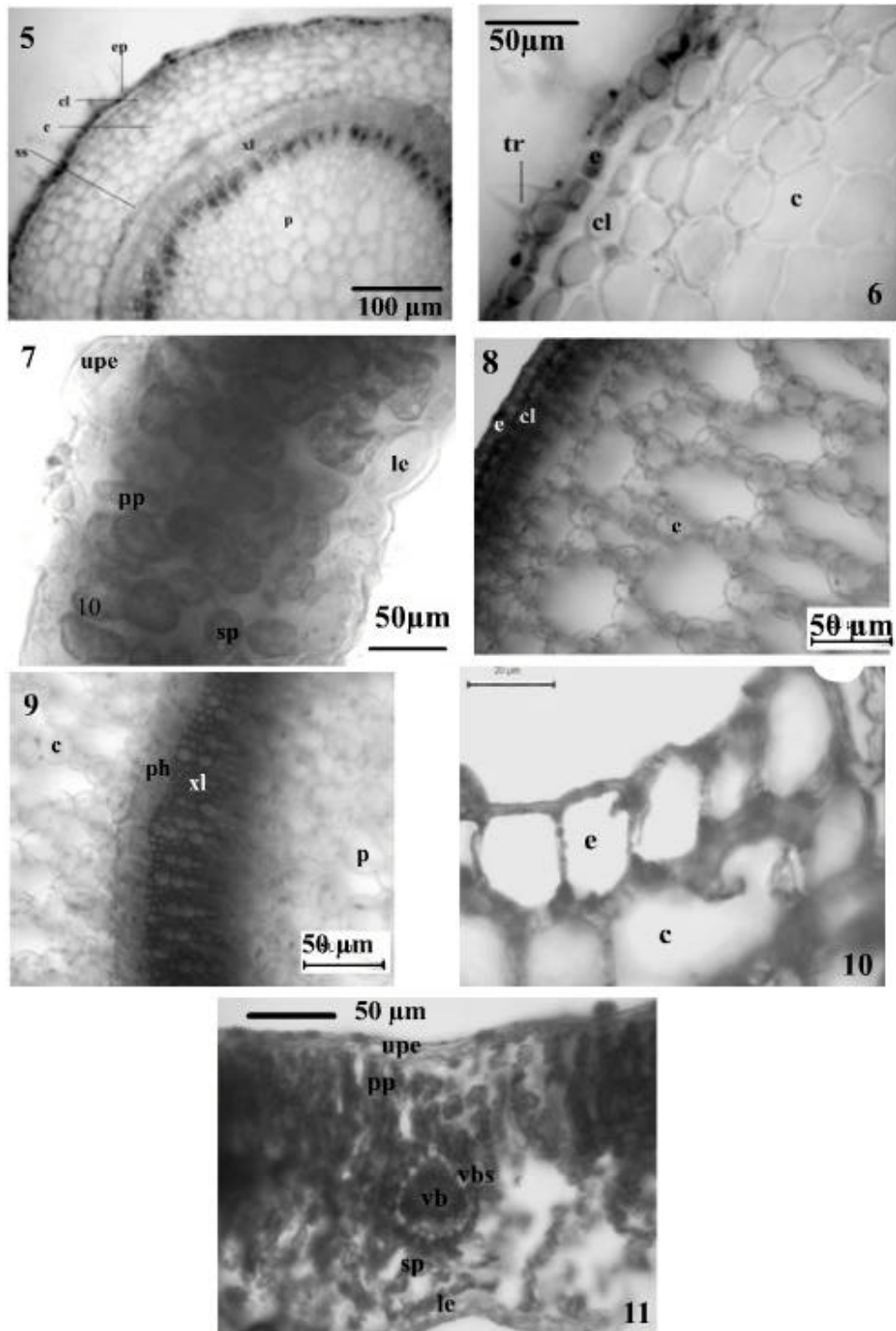


Fig. 5-11: *V. persica* and *V. beccabunga*. Fig. 5, 6. *V. persica*, transverse section of stem. Fig. 7. *V. persica*, transverse section of leaf. Fig. 8-10. *V. beccabunga*, transverse section of stem. Fig. 11. *V. beccabunga*, transverse section of leaf. e-epidermis; c-cortex; xl-xylem; ph-phloem, p-pith; cl-collenchyma; le-lower epidermis; upe-upper epidermis; sp-spongy parenchyma; pp-palisade parenchyma; tr-trichome; vb-vascular bundle; vbs-vascular bundle sheath

Fig. 12-19: *V. officinalis* and *V. multifida*. Fig. 12, 13, 14. *V. officinalis*, transverse section of stem. Fig. 15. *V. officinalis*, transverse section of leaf. Fig. 16, 17, 18. *V. multifida*, transverse section of stem. Fig. 19. *V. multifida*, transverse section of leaf. e-epidermis; c-cortex; ss-starch sheath; s-sclerenchymatic sheath; xl-xylem; ph-phloem, p-pith; cl-collenchyma; le-lower epidermis; upe-upper epidermis; sp-spongy parenchyma; pp-palisade parenchyma; tr-trichome; vb-vascular bundle; vbs-vascular bundle sheath

Fig. 20-27: *V. persica* and *V. beccabunga*. Fig. 20, 21. *V. persica*, pollen structure. Fig. 22-23. *V. persica*, seed structure. Fig. 24, 25. *V. beccabunga*, pollen structure. Fig. 26, 27. *V. beccabunga*, seed structure

Fig. 28-34: *V. officinalis* and *V. multifida*. Fig. 27, 28. *V. officinalis*, pollen structure. Fig. 29, 30. *V. officinalis*, seed structure. Fig. 31, 32. *V. multifida*, pollen structure. Fig. 33, 34. *V. multifida*, seed structure

plate collenchyma. Stem cortex (165-180 µm thick) consists of 6-7 layers of usually oval or orbicular cells and occupies 35% of the stem. The last layer of cortex differentiated as starch sheath. Bundles are of the different sizes and occupy 35% of the stem. Phloem includes sclerenchymatic tissue consisting of solitary and bundled fibers (Fig. 18). Pith cells are large and cylindrical.

The transverse section of the lamina and both epidermises was studied (Fig. 19). Vascular bundles are arc-shaped and surrounded by parenchymatic bundle sheath. Mesophyll consists of 2 or 3 layers of elongated palisade cells. Both epidermises are covered by cuticle 4.5-6 µm thick (Fig. 16). Leaf is unifacial and has anomocytic stomata.

Palynological characters: Pollen grains isopolar, tricolpate, perprolate (elongated) in *V. persica*, prolate in *V. officinalis*, subprolate in *V. multifida* and oblate-spheroidal in *V. beccabunga* (P/E ratio varying from 0.95 to 2.03) (Fig. 20, 24, 28, 31, 32), circular or elliptic outline in equatorial view and triangular outline in polar view. Regarding size, the *Veronica* pollen grains studied have mean values of 27.38 µm (P) and 19.40 µm (E). Size of the grains varies from 16.7 (P) × 13.3 (E) µm in *V. multifida* and 39.5 µm (P) and 19.5 µm (E) in *V. persica*. Colpi simple, long, terminal and relatively wide, usually with pointed ends. Apertural membrane mostly with ectexine elements which regulate-perforate in loops (*V. multifida*), or shaped like a wart (*V. persica*, *V. beccabunga* and *V. officinalis*).

There are two types of exine ornamentation, although the differences are not always very remarkable:

Type 1. Typically striate-reticulate: It is represented by *V. persica*, *V. beccabunga* and *V. officinalis*. (Fig. 21, 25 and 29).

Type 2. Striate-microreticulate: Muri with strong tendency to form a striation. This type is represented by *V. multifida* (Fig. 33).

Seed morphological characters

V. persica: Seed structure is reticulate-papillate (Fig. 22 and 23).

V. beccabunga: Reticulate coat formed by big polygonal cells. Radial walls of medium depth and irregularly thickened, striate and tangential walls reticulate to striate (Fig. 26 and 27).

V. officinalis: This seed coat pattern is colliculate to corrugate, although very rarely a tendency exist to form

a network. Irregular cells with obscure radial walls and tangential ones irregularly elevated and rugose (Fig. 29 and 30).

V. multifida: It has ribbed seed coat pattern (Fig. 34).

DISCUSSION

This is the first anatomical report on the four examined taxa of *Veronica*. Among the morphological traits, leaf characters are the most important for separation of taxa (Davis, 1978). In this study we found that the lamina shape is bipinnatisect in *V. multifida* and entire in the others. The leaves are alternate in *V. persica*, *V. beccabunga* and *V. multifida*; leaves are opposite in *V. officinalis*.

A foliar endodermis is present as one or two layered starch sheath in *V. officinalis* and *V. multifida*, but we didn't observe such feature in *V. persica* and *V. beccabunga*. Xylem is well developed in *V. beccabunga*, *V. officinalis* and *V. multifida*. Cortex is also well developed and has wide intercellular area in *V. beccabunga*.

The presence/absence and distribution of sclerenchymatic tissue in the phloem has considerable taxonomic value (Canne-Hilliker and Kampany, 1991; Makbul *et al.*, 2006). It occurred as solitary in *V. officinalis*, solitary or bundled fibers in *V. multifida*. Fibers consist of 2-6 cells in *V. multifida*. In *V. persica* and *V. beccabunga*, sclerenchymatic tissue is absent.

The presence of vascular bundle sheath in leaves of *V. beccabunga*, *V. orientalis* and *V. multifida* is an additional taxonomic trait for identifying these taxa. Vascular bundle sheath occurs small parenchyma cells in *V. beccabunga*, one layered big parenchyma cells in *V. officinalis*, two layered big parenchyma cells in *V. multifida*. Mesophyll tissue is well differentiated in *V. persica* and *V. beccabunga*, but consists of only palisade parenchyma cells in *V. multifida* and spheric cells in *V. officinalis*.

The value of pollen characters as taxonomic tools within examined species of *Veronica* studied here is restricted to a small number of cases and does not very remarkable. The differences existing among the pollen grains of the species investigated are not very important characters for taxonomy of this genus. Martinez-Ortega *et al.* (2000), said that striate-reticulate exine ornamentation is the most frequent pattern in *Veronica*. This exine pattern was observed in three species (*V. persica*, *V. beccabunga* and *V. officinalis*).

They also observed *V. officinalis*, but we found the difference in pollen size. They measured P (polar axis) as 30.4±2.06, E (Equatorial diameter) as 27.5±1.76 and P/E ratio as 1.10 (prolate-spheroidal), but we found the longer pollen grains 32.5±1.6 (P), 23.8±1.4 (E) and P/E ratio as 1.37 (prolate). They didn't investigate pollen grains of *V. persica*, *V. beccabunga* and *V. multifida*.

For *Veronica* Sect. *Veronica* and *V.* Sect. *Veronicastrum*, Martínez-Ortega *et al.* (2000) proposed the following trends in the evolution of pollen characters: Exine from rugulate-reticulate to striate-reticulate and within the latter, from one with a slight tendency to form a reticulum to one with a strong tendency to be striate. We observed the following trends in examined species: Exine from striate-reticulate to strong tendency to be striate.

According to seed coat structure, we examined four different seed coat patterns in studied species: reticulate-papillate in *V. persica*, reticulate to reticulate-corrugate in *V. beccabunga*, colliculate to corrugate in *V. officinalis*, ribbed in *V. multifida*. Martínez-Ortega and Rico (2001 a) observed reticulate seed coat ornamentation in *V. officinalis*, but we found colliculate to corrugate seed pattern in our *V. officinalis*.

These results prove *Veronica* is polymorphic genus and *V. officinalis* in Turkey shows different pollen and seed morphology from the distributed in Europe.

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