

# A comparative anatomical study of the genus *Puschkinia* Adams in Turkey

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**Abstract** – In the study, *Puschkinia scilloides* Adams, *P. bilgineri* Yıldırım, and *P. peshmenii* Rix et B. Mathew were compared anatomically. Although the stem vascular bundles were arranged in two rows in *P. peshmenii*, they can be observed in three rows in *P. scilloides* and *P. bilgineri*. In addition, some differences were found, such as the metaxylem number in the root, number of vascular bundles in the scape, presence of raphide crystals, and number of protruding epidermis cells at the tip of the leaf. These differences have been found to be taxonomically useful for distinguishing these species. Moreover, similarities and differences between *Puschkinia* Adams and the taxonomically closely related *Scilla* L. section *Chionodoxa* Boiss. and *Scilla bifolia* L. taxa were also discussed.

**Keywords:** anatomy, *Puschkinia*, *Scilla*, taxonomy, Turkey

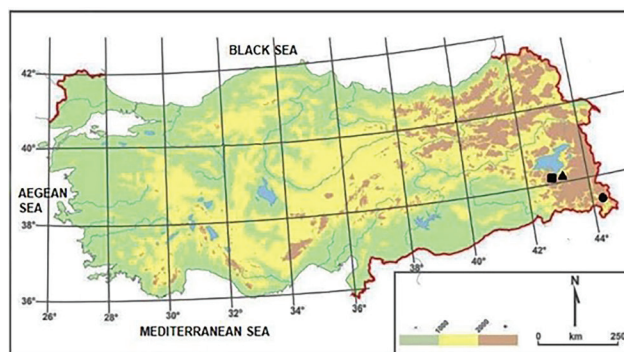
## Introduction

The genus *Puschkinia* is represented by three species in Turkey (*P. scilloides* Adams, *P. peshmenii* Rix et B. Mathew, and *P. bilgineri* Yıldırım). The species of this genus show high morphological variations, especially *P. scilloides* (Edmondson 1984, Yıldırım 2012, 2013, 2014). These species are distributed naturally in Turkey. *Puschkinia scilloides* is distributed in north Caucasus, Lebanon, northern Iran, Iraq and Syria (Rix and Mathew 2007, Yıldırım 2013). As for the other species, *P. bilgineri* is endemic to Turkey, and *P. peshmenii* is distributed in Turkey and Iran. In the present study, *P. scilloides*, *P. bilgineri* and *P. peshmenii* are compared anatomically and it is aimed at contributing to the taxonomic separation of the species. In addition, the anatomical properties of the *Puschkinia* taxa and the taxonomically closely related *Scilla* L. section *Chionodoxa* Boiss. and *Scilla bifolia* L. taxa are also discussed.

## Materials and methods

### Study area

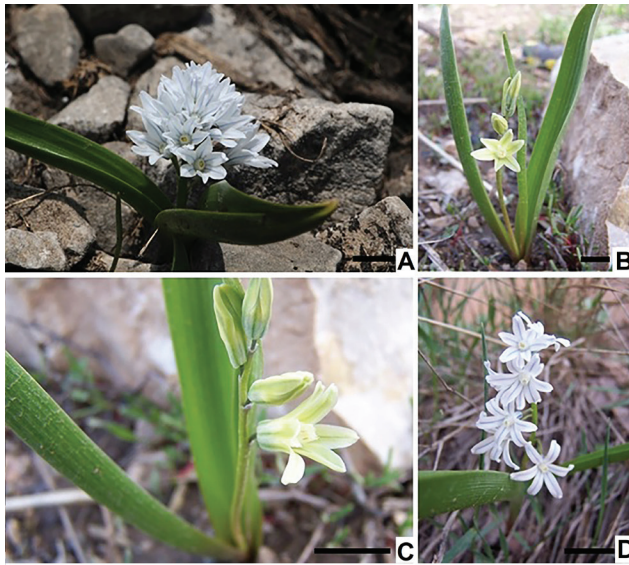
The species were collected from natural habitats in eastern and southeastern Anatolia (Fig. 1). The study area is situated in the Armenio-Iranian subregion of the Irano-Turanian phytogeographic region (Rivas-Martínez et al. 2004), and mostly has a continental semiarid climate with hot and



**Fig. 1.** Location of sampling sites of the *Puschkinia* species in Turkey: *P. scilloides* Adams (▲), *P. bilgineri* Yıldırım (■), *P. peshmenii* Rix et B. Mathew (●).

dry summers, and cold and snowy winters. *Puschkinia bilgineri* (Fig. 2A) was gathered from Van Province, Kavuşşahap mountain chain, at Karabet Gateway, alpine mountain meadows and mountain slopes, from 2885–3000 m a.s.l. (type locality). *Puschkinia peshmenii* (Fig. 2B, C) was collected from Hakkari Province, near the Yüksekova district, in the gorge leading into the valley from the west, among dwarf shrubs, from 1850–1900 m a.s.l. (type locality). *Puschkinia scilloides* (Fig. 2D) was gathered from Van Province,

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**Fig. 2.** Habitus of the *Puschkinia* species: *P. bilgineri* (A), *P. peshmenii* (B, C), *P. scilloides* (D). Scale bar = 1 cm.

Gevaş district, Artos Mountain, opening slopes and plateau, from 2400–2550 m a.s.l.

**Sampling**

For the anatomical studies, the plant specimens were fixed in 70% ethanol. Ten specimens of each species were studied. The paraffin method (Algan 1981) was used for preparing cross-sections of the root, scape, and leaves of the species. Transverse sections of 15–20 µm were made with a sliding microtome and stained with safranin-fast green. Measurements of the root and stem, and the leaf cell sizes of the species were taken using an ocular-micrometer and photographed with a motorized Leica DM 3000 microscope. The minimum, maximum, mean, and standard deviation were determined and are shown in Tab. 1. Fifteen cross-sections from each plant were made for each measurement (Tab. 1).

**Results**

**Root**

The rhizodermis, which consisted of elongated cells, was single-layered on the outer surface of the root in all of the species. There was no cuticle layer on the hypodermal cells. The cortex consisted of 6–8, 6–9, and 6–10 layered parenchymatous cells with intercellular spaces in *P. scilloides*, *P. bilgineri*, and *P. peshmenii*, respectively (Fig. 3A, C, F). The endodermis cell wall of all of the species was thick on all four sides. On the inside of the endodermis and adjacent to it was the cylinder of parenchyma cells called the pericycle in all of the studied species. The pericycle was 1 cell wide and consisted of thin-walled cells. *Puschkinia scilloides* had 1–2 metaxylem-vessels in the center of the root while *P. bilgineri* and *P. peshmenii* had just one. There were four xylem strands on the midpoint of the vascular cylinder in all of the spe-

**Tab. 1.** Measurements of anatomical properties of the studied *Puschkinia* species (n = 10).

	<i>P. bilgineri</i>						<i>P. peshmenii</i>						<i>P. scilloides</i>						
	Width			Length			Width			Length			Width			Length			
	Min-Max (µm)	Mean±SD		Min-Max (µm)	Mean±SD		Min-Max (µm)	Mean±SD		Min-Max (µm)	Mean±SD		Min-Max (µm)	Mean±SD		Min-Max (µm)	Mean±SD		
<b>Root</b>																			
Epidermis	10.0–23.0	15.0±1.4		8.0–27.0	14.5±2.1		10.0–25.0	16.0±2.7		7.5–17.5	15.0±3.4		17.0–25.0	20.0±3.4		7.50–17.0	11.0±1.2		
Cortex (diameter)	22.0–55.0	29.0±2.8					20.0–37.0	28.0±5.1					17.0–38.0	23.0±4.1					
Metaxylem (diameter)	30.0–37.0	34.0±1.3					30.0–42.0	27.0±9.0					20.0–37.0	31.0±2.3					
<b>Stem</b>																			
Epidermis	12.0–33.0	25.0±5.1		12.0–34.0	22.0±6.7		10.0–19.0	16.0±3.7		17.0–37.0	30.0±5.2		15.0–27.0	20.0±3.1		20.0–32.0	25.0±5.4		
Cortex (diameter)	16.0–43.0	37.0±7.5					25.0–55.0	46.0±3.1					20.0–52.0	34.0±3.6					
Trachea (diameter)	12.0–26.0	16.1±4.3					13.0–35.0	20.0±4.2					10.0–20.0	14.0±1.7					
Pith (diameter)	25.0–96.0	57.1±22					52.0–87.0	66.0±13					30.0–90.0	60.0±11					
<b>Leaf</b>																			
Adaxial epidermis	12.0–27.0	19.0±3.6		15.0–25.0	20.0±2.7		17.0–30.0	19.0±3.8		20.0–33.0	27.0±4.1		25.0–42.0	34.0±5.2		17.0–30.0	23.0±1.9		
Palisade cell	12.0–30.0	23.0±5.0		25.0–75.0	50.0±12		22.0–50.0	30.0±4.1		45.0–75.0	55.0±9.2		21.0–37.0	28.0±2.1		38.0–59.0	47.0±7.1		
Spongy cell	10.0–25.0	16.0±4.7		32.0–28.0	19.0±3.7		12.0–28.0	15.0±1.7		12.0–35.0	23.0±6.2		20.0–43.0	31.0±3.1					
Abaxial epidermis	10.0–22.0	17.0±2.9		13.0–21.0	18.0±2.0		20.0–37.0	28.0±3.9		23.0–30.0	27.0±3.5		25.0–32.0	28.0±3.7		22.0–30.0	24.0±1.7		

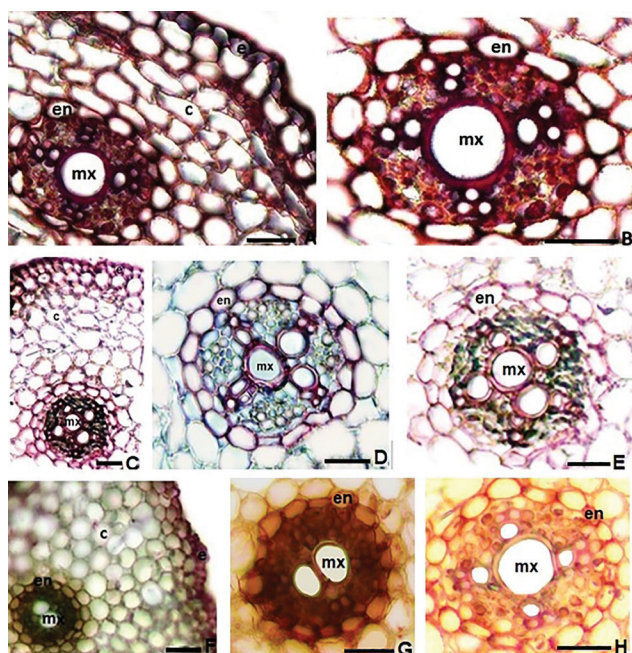


Fig. 3. Root cross-sections of the *Puschkinia* species: *P. bilgineri* (A, B), *P. peshmenii* (C, D, E), *P. scilloides* (F, G, H). c – cortex, e – epidermis, en – endodermis, mx – metaxylem. Scale bar = 40  $\mu$ m.

cies root cross-sections (Fig. 3B, D, E, G, H). Needle-shaped raphide crystals were present in the root cortex of *P. scilloides* and *P. bilgineri*.

### Scape

A very thick cuticle layer covered the scape epidermis in all of the studied species. The cortex was composed of 6–9, 5–8, and 4–7 layered thin-walled, circular parenchyma cells with intercellular spaces in *P. scilloides*, *P. bilgineri* and *P. peshmenii*, respectively, under the epidermis (Fig. 4A, C, E). In *P. scilloides*, 23–29 collateral vascular bundles with thick-walled cells were arranged in 3 rings, while in *P. bilgineri* there were 16–20 vascular bundles arranged in 3 rings, and in *P. peshmenii*, there were 14–17 vascular bundles arranged in two rings. In *P. peshmenii*, a 1–3 cell lined collenchyma layer was present, whereas a 3–5 cell lined layer was present in the other two species. There were pith cells in the center of the scape in all of the species (Fig. 4).

### Leaf

Leaf anatomy was quite similar in all of the studied species. The epidermis comprised a single layer of cells covering both the abaxial and adaxial surfaces of the leaf, where there was a thick cuticle layer in all of the species. Outward epidermis cell protrusions were present at the ends of the leaves with, respectively 4–7, 3–5, and 3–6 in *P. scilloides*, *P. bilgineri* and *P. peshmenii* (Fig. 5G, J). Two regions of the mesophyll were distinguishable, and the adaxial and abaxial palisade parenchyma consisted of a rectangle, and oval-shaped cells were arranged in 2–3 rows in all of the species. The middle region consisted of loosely arranged spongy pa-

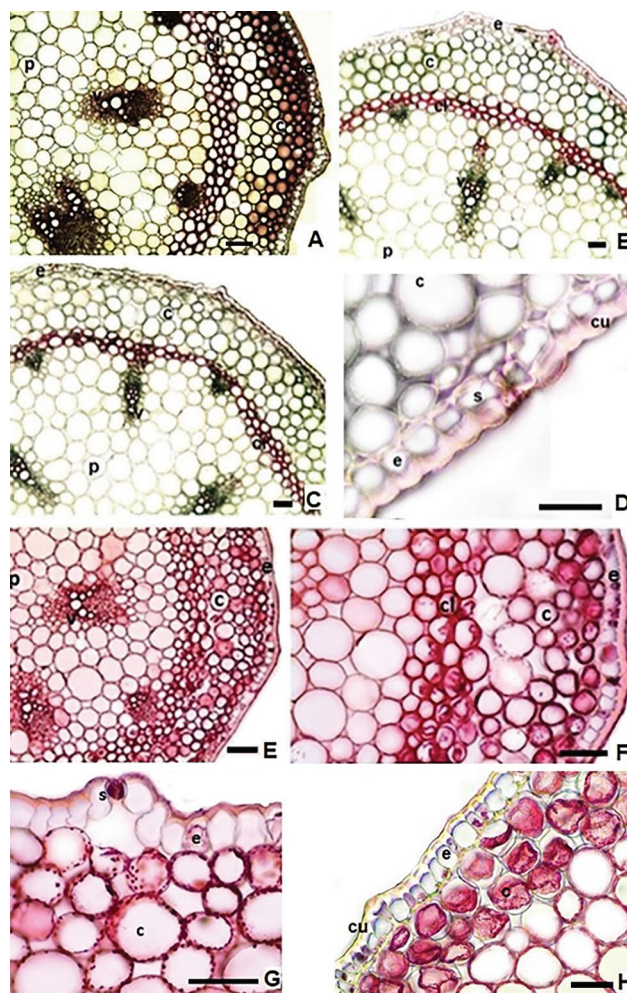


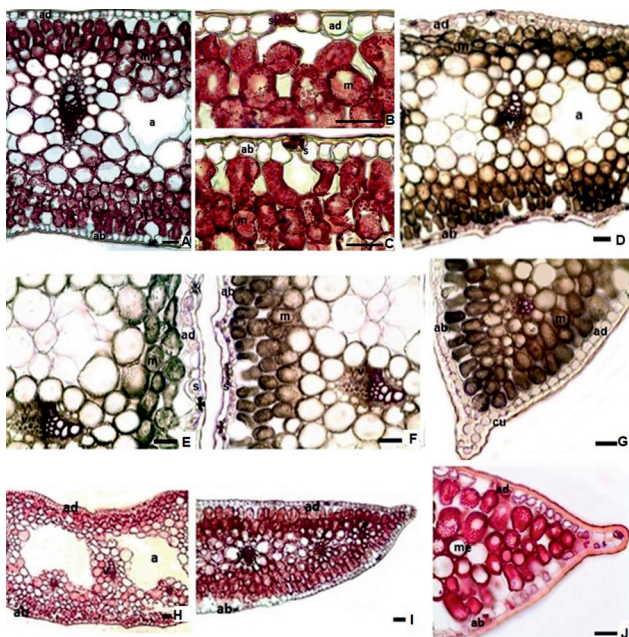
Fig. 4. Scape cross-sections of the *Puschkinia* species: *P. bilgineri* (A), *P. peshmenii* (B, C, D), *P. scilloides* (E, F, G, H). c – cortex, cl – collenchyma, cu – cuticle, e – epidermis, p – pith, s – stoma, v – vascular bundle. Scale bar = 40  $\mu$ m.

renchyma cells with abundant air spaces between them in all of the taxa. The raphides present in the mesophyll of *P. peshmenii* were different from those in the other species.

The vascular bundles were arranged regularly in the center of the mesophyll in all of the species (Fig. 5). When the anatomical measurements were taken, the cell widths and lengths were generally close to each other. The measured values are detailed in the Tab. 1.

### Discussion

When the anatomical features of the roots were compared, it was understood that *P. scilloides* differed from the other species by its 1–2 metaxylem. When the scape properties were considered, the vascular bundles were arranged in two rings in *P. peshmenii*, while the other species had three rings and the number of vascular bundles in the species differed. The scape epidermis cells protruded outward in *P. peshmenii*, while they were almost non-existent in the other *Puschkinia* species. Scape cortex and pith cell measurements of *P. peshmenii* are bigger than the other two species. Leaf



**Fig. 5.** Leaves cross-sections of *Puschkinia* species: *P. bilgineri* (A, B, C), *P. peshmenii* (D, E, F, G), *P. scilloides* (H, I, J). a – aerenchyma, ab – abaxial epidermis, ad – adaxial epidermis, cu – cuticle, m – mesophyll, s – stoma, v – vascular bundle. Scale bar = 40  $\mu$ m.

spongy cells of *P. scilloides* are larger than in the other two species (Tab. 1). The location and type of crystals in a plant are taxonomically important (Metcalf and Chalk 1983, Fahn 1990). In this study, needle-shaped crystals were pres-

## References

- Algan, G., 1981: Microtechnics for plant tissues. PhD Thesis. Firat University, Faculty of Science, Istanbul, Turkey (in Turkish).
- Almeida da Silva, R., Rosselló, J.A., 1990: Anatomical studies on the *Scilla verna* (Hyacinthaceae) complex. *Israel Journal of Plant Sciences* 47, 103–110.
- Edmondson, J., 1984: *Puschkinia* Adams. In: Davis, P.H. (ed.), *Flora of Turkey and the East Aegean islands* 8, 226–227. Edinburgh University Press, Edinburgh.
- Fahn, A., 1990: *Plant Anatomy*. 4<sup>th</sup> ed. Pergamon Press, Oxford, New York.
- Metcalf, C.R., Chalk, L., 1983: *Anatomy of the Dicotyledons*. Vol. 1. Oxford University Press, Oxford.
- Rivas-Martínez, S., Penas, A., Díaz, T.E., 2004: Mapa Biogeográfico de Europa. Servicio Cartográfico de la Universidad de León, España. Retrieved December 24, 2019 from <http://webs.ucm.es/info/cif/form/maps.htm>
- Rix, M., Mathew, B., 2007: 582. *Puschkinia peshmenii*. *Curtis's Botanical Magazine* 24, 54–57.
- Satıl, F., Akan, H., 2006: Anatomical investigations on some endemic and rare geophytes from the Liliaceae family. *Ekoloji* 58, 21–27 (in Turkish).
- Yıldırım, H., 2012: *Puschkinia* Adams. In: Güner, A., Aslan, S., Ekim, T., Vural, M. ve Babaç, M.T. (eds.), *Turkey Plant List (Vascular Plants)*. Nezahat Gökyiğit Botanical Garden and Flora Research Association Publication, Istanbul (in Turkish).
- Yıldırım, H., 2013: Domestic distribution and species variation limits of *Puschkinia scilloides* Adams (Asparagaceae/Liliaceae) in Turkey. *Manas Journal of Agriculture and Life Sciences* 3, 7–12 (in Turkish).
- Yıldırım, H., 2014: *Puschkinia bilgineri* (Asparagaceae subfamily Scilloideae): A new species *Puschkinia* Adams from the eastern Anatolia region. *Bağbahçe Bilim Dergisi* 1, 1–10 (in Turkish).
- Yıldırım, H., Yetişen, K., Özdemir, A., Özdemir, C., 2017: An anatomical study of *Scilla* (Scilloideae) Section *Chionodoxa* and *Scilla bifolia* in Turkey. *Plantha Daninha* 35.

ent in the root cross-sections of *P. scilloides* and *P. bilgineri*, but the presence of raphide crystals in the mesophyll layer of the leaves of *P. peshmenii* distinguished this species from the others. The differences in the number of vascular bundles in the scape, the presence of raphide crystals, and the number of protruding epidermis cells at the tips of the leaves can be used to differentiate species of the genus *Puschkinia*.

These taxa are known to be taxonomically closely related to *Scilla* section *Chionodoxa* and *Scilla bifolia* taxa in Turkey (Yıldırım et al. 2017). The number of metaxylems in the root of *Scilla* taxa was higher than that in species of *Puschkinia*. Disintegration areas in the scape pith of the *Scilla* taxa were wider than that of the genus *Puschkinia*. A single-layered epidermis formed projections that formed longitudinal ribs in all of the taxa, except *Scilla*  $\times$  *allenii* in the scape. These protrusions were present in the endemic *P. peshmenii* scape, but were almost non-existent in other *Puschkinia* species. *Hyacinthoides* Heist. ex Fabr. and *Scilla* taxa generally have aerenchyma cavities in the leaf mesophyll (Almeida da Silva and Rosselló 1990). We also observed aerenchyma cavities in the leaves of studied *Scilla* and *Puschkinia* taxa. Needle-shaped raphide crystals were present in all three species of *Puschkinia*. In the studies carried out previously, such crystals were found in the leaves and stems of *Scilla* taxa (Satıl and Akan 2006, Yıldırım et al. 2017). In the three *Puschkinia* species, 3–7 epidermis cells protruded outwardly at the tip of the leaves. Such protrusions were not observed in the studied *Scilla* taxa (Yıldırım et al. 2017). This feature can be used to distinguish between the two genera.