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## Comparative anatomical study of the taxa from series *Verni Mathew (Crocus L.)* in Serbia

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### **Abstract:**

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The comparative leaf anatomy of three *Crocus L.* taxa from series *Verni Mathew (Crocus heuffelianus* Herb., *Crocus tommasinianus* Herbert and *Crocus kosaninii* Pulević) in Serbia is introduced. The general outlook of the cross sections of the leaves was defined with microphotographs. More precisely, leaf shape, leaf surface, mesophyll parenchyma and vascular bundle features were examined. The biggest differences were found at the level of vascular bundles (xylem area, phloem area, sclerenchyma area) and leaf blade features (section height, section length, arm length, white stripe width, lacuna area). In general, listed parameters had the lowest values in *C. tommasinianus* population, while the highest values could be found in population of *C. heuffelianus*. Further investigations should be focused on discovering more localities followed by surveying the ecological factors of the habitats.

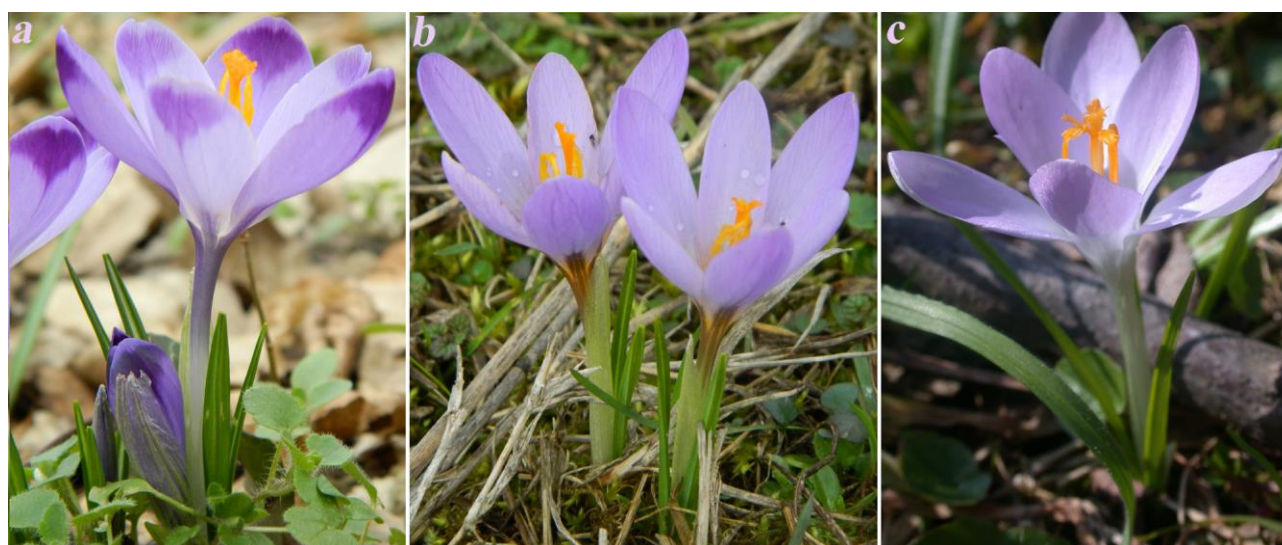
**Key words:** *Crocus*, series *Verni*, cross section, leaf

### **Apstrakt:**

**Raca, I., Ljubisavljević, I., Jušković, M., Randelović, N., Randelović, V.: Usporedna anatomska studija taksona serije *Verni Mathew (Crocus L.)* u Srbiji. *Biologica Nyssana*, 8 (1), Septembar 2017: 15-22.**

Komparativna anatomska analiza listova tri taksona roda *Crocus L.* a serije *Verni Mathew (Crocus heuffelianus* Herb., *Crocus tommasinianus* Herbert i *Crocus kosaninii* Pulević) iz Srbije prikazana je na mikrofotografijama. Konkretnije, razmatrani su oblik poprečnog preseka lista, površinske strukture listova, lisni mezofil i karakteristike provodnih snopića. Najveće razlike zabeležene su na nivou provodnih snopića (površina ksilema, floema i sklerenhima) i parametara koji se odnose na oblik preseka (visina i dužina preseka, dužina ručice, širina bele pruge, površina centralnog parenhima). Generalno, navedeni parametri imaju najniže izmerene vrednosti u populaciji vrste *C. tommasinianus*, dok su najviše izmerene vrednosti zabeležene u populaciji vrste *C. heuffelianus*. Komparativna anatomska analiza obezbeđuje dodatne informacije o karakterima značajnim za diferencijaciju vrsta. Kako god, buduća istraživanja bi trebalo da budu fokusirana na pronalaženje novih lokaliteta populacija od interesa, kao i na praćenje ekoloških uslova njihovih habitata.

**Ključne reči:** *Crocus*, serija *Verni*, poprečni presek, list



**Fig. 1.** Verni taxa in Serbia: 1a. *Crocus heuffelianus*, 1b. *C. kosaninii*, 1c. *C. tommasinianus*

## Introduction

Saffrons are known as “golden spice” and ornamentals (Candan, 2007). Moreover *Crocus* species have antitumor, antimutagenic, cytotoxic activities and inhibitory effect on nucleic acid synthesis in human malignant cells (Fatehi et al., 2003). As a matter of fact, the genus *Crocus* contains more than 200 taxa (Rukšāns, 2017) from the West Mediterranean to Central Asia with Poland as Northern limit of distribution. In the latest detailed revision of the genus (Mathew et al., 2009), the genus was divided into: section *Crocus* Mathew and section *Nudiscapus* Mathew. The Balkan Peninsula is known as one center of diversity (Mathew, 1982). In Serbia, there are 17 noticed species (Randelović et al., 1990). They belong to section *Crocus* Mathew (series *Verni* Mathew, *Scardici* Mathew, *Crocus* Mathew) and section *Nudiscapus* Mathew (series *Reticulati* Mathew, *Biflori* Mathew, *Flavi* Mathew) (Randelović et al., 1990). However, there are many contentious questions related to systematic positions of *Crocus* taxa. One of them certainly refers to taxa from series *Verni*. Series *Verni* is represented by three species in Serbia:

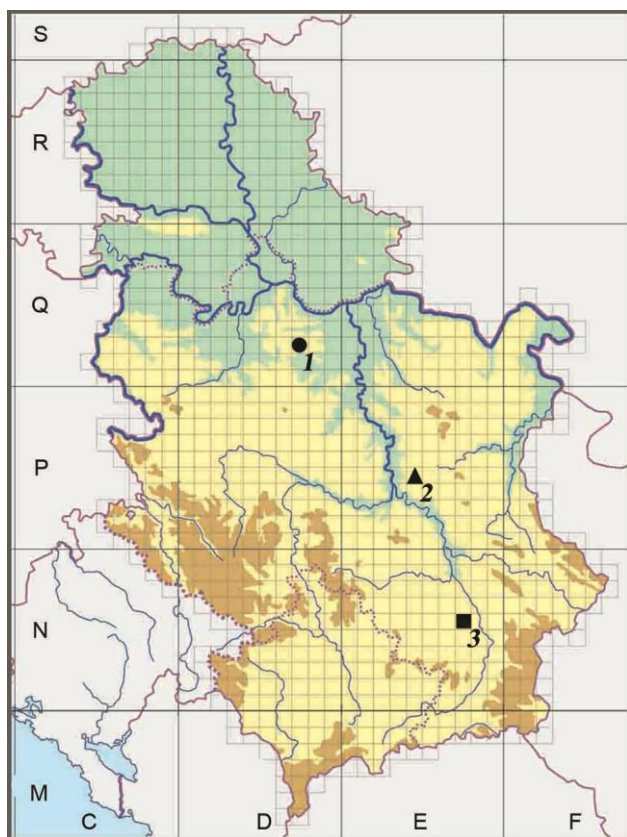
*Crocus heuffelianus* Herb. (Fig. 1a), *Crocus kosaninii* Pulević (Fig. 1b) and *Crocus tommasinianus* Herb. (Fig. 1c) (Randelović et al., 1990). Since good differential markers can be found on the level of micromorphological aspects of the leaves, there have been several investigations about leaf anatomy of *Crocus* taxa from different parts of its distribution range (Rudall & Mathew, 1990; Akan, 2007; Erol & Kuçuker, 2007; Satil, 2007; Candan, 2007, 2015; Kandemir, 2009, 2010, 2012; Coşkun, 2010; Özdemir, 2010; Yetişen, 2013). As can be seen in the literature, no detailed comparative study of series *Verni* has been available, yet. Therefore, the initial objective of this paper is to introduce the comparison of leaf anatomy in cross sections of series *Verni* taxa in Serbia and to identify their possible differences.

## Material and methods

Plant samples were collected from natural populations in the flowering time. The sampling localities (Fig. 2) with the associated information are given below (Tab. 1). Taxonomic description of the species was made according to Randelović et al.

**Table 1.** Localities with associated information

Species	<i>Crocus heuffelianus</i>	<i>Crocus kosaninii</i>	<i>Crocus tommasinianus</i>
Locality	Velika Ivanča	Strojkovce	Mađere
N lat/E long	44° 42' 33" 20° 59' 20"	42° 54' 00" 21° 55' 00"	43° 41' 29" 21° 30' 34"
Altitude	173 m	305 m	207 m



**Fig. 2.** The geographical position of the investigated populations in Serbia (1-*Crocus heuffelianus*; 2-*C. tommasinianus*; 3-*C. kosaninii*)

(1990). Specimens were deposited in Herbarium Moesiacum Niš. Five individuals per population were fixed in 50% alcohol for anatomical study, which was done in the laboratory for Plant Systematics and Ecology, Faculty of Science and Mathematics, University of Niš. Manual microtome (Gligorijević & Pejčinović, 1983) was utilized in order to make cross sections of the leaves. 30 transverse sections per population were stained with Safranin - Alcian Blue and examined by *Leica DM 1000* microscope afterwards. Following parameters were considered in leaf cross sections: section height, section length, arm length, white stripe width, lacuna area, adaxial epidermis cells height and width, palisade cells height and width, palisade tissue height, spongy cells height and width, spongy tissue height, abaxial epidermis cells height and width, number of big vascular bundles, xylem area, phloem area and sclerenchyma area. Listed anatomical features were measured with ImageJ software. Anatomy characteristics were also described by microphotographs.

## Results and discussion

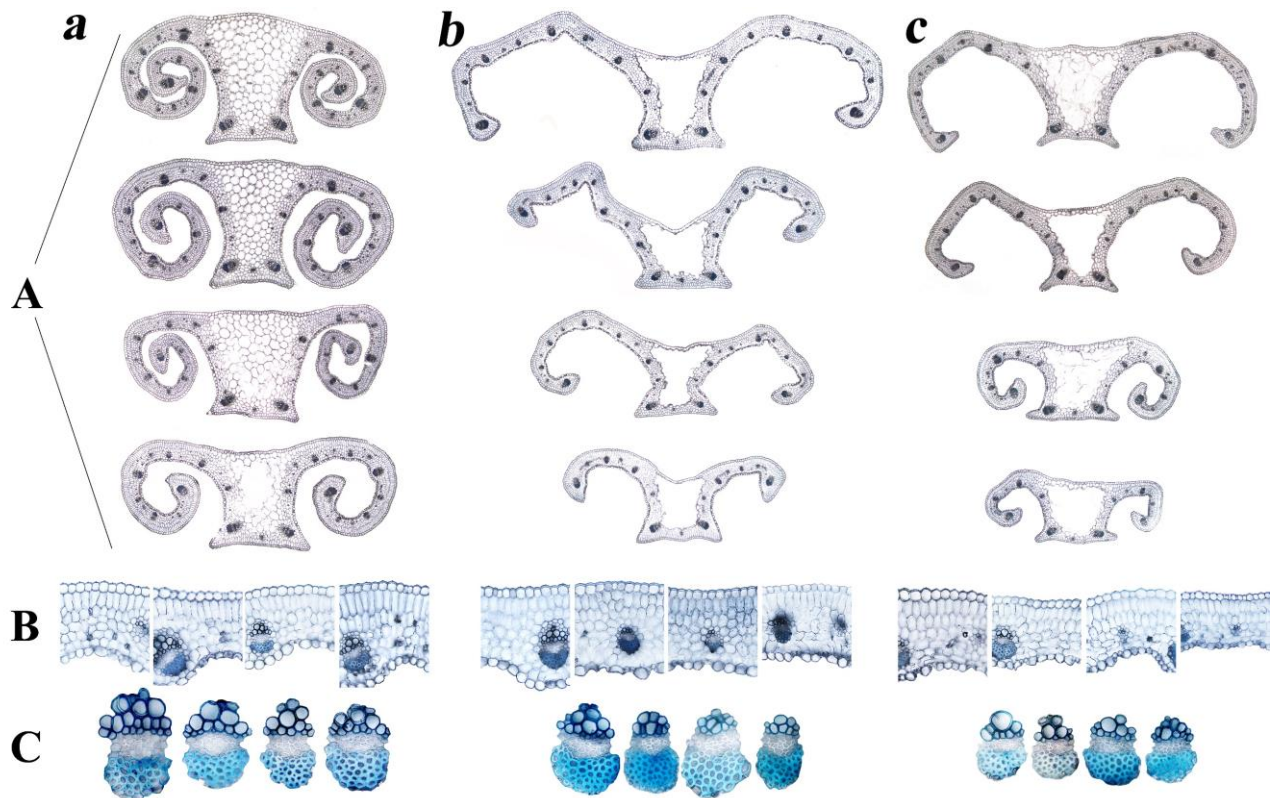
Typical microphotographs of leaf cross sections for all of three *Verni* species from Serbia are presented in **Fig. 3**. The first part of collage refers to the general outline of leaf cross sections. Lateral arm details are given in the middle. The last part of collage represents the structure of big bundles positioned at the end of the arms.

**Leaf blade:** *Crocus* leaves cross sections have a unique shape - with a square and almost rectangular central keel and two lateral arms recurved towards the keel (Rudall & Mathew, 1990). However, there are some exceptions where the keel and arms are of the same length (*C. scharojanii*, *C. carpetanus*, *C. scardicus*). The leaf type was declared as morphologically and physiologically bifacial (Erol, 2007). The keel is squared with wide base and acute corners in all *Verni* cross sections. The length of arms and their curving degree differ amongst the species (**Fig. 3A**) (**Tab. 2**). The papillae are detected on the adaxial side at the end of the arms. Also, the abaxial side of the corner of keel might have hair. The mentioned hair abundance pattern is characteristic of each of three species.

**Leaf surface:** Adaxial epidermis cells are rectangular, while abaxial cells are elliptical in shape. The cuticle is thicker on the adaxial side of the leaf. Stomata occurs on the abaxial surface in the region of arms and lateral sides of the keel. The stomata type is anomocytic (Erol, 2007).

**Mesophyll:** Large, almost round parenchyma cells are located in the central part of the keel. Because of their thin walls, they might break down, forming an air space called lacuna. Disintegration of parenchyma cell walls varies between species (**Fig. 3A**, **Tab. 2**). Since parenchyma cells of the keel lack chloroplasts, lacuna area can be noticed as typical white stripe all along the central part of the *Crocus* leaves (Erol, 2007; Yetişen, 2013). There are two types of parenchyma cells in the mesophyll of arms – palisade and spongy cells. Palisade is oriented to the adaxial surface and it is made of two layers of polygonal cells. Spongy parenchyma is three – four layered and consists elliptical - irregular shaped cells sometimes with intercellular space within (**Fig. 4**). Two layers of palisade cells are noticed at the base of the keel as well.

**Vascular bundles:** Collateral vascular bundles are located between the palisade and spongy cells in one row. There are four big vascular bundles (two of them are placed at the ends of the lateral arms, while two others are positioned in the base of the central keel) (**Fig. 3A**). The number of small and the smallest vascular bundles depends on the leaf dimensions. Additionally, they are located all along



**Fig. 3.** Anatomic characters of leaves of *Crocus* ser. *Verni* species from Serbia: A-Leaf cross sections (5x), B-Arm details (20x) and C-Big bundles (40x) (a-*C. heuffelianus*; b-*C. kosaninii*; c-*C. tommasinianus*)

the arms and lateral and basal side of the keel. The xylem is oriented towards the adaxial side with the phloem beneath. Sclerenchyma “caps” below the phloem are well developed (Fig. 5).

The anatomy features measurements expressed through the average values and standard deviation are given in the table below (Tab. 3).

Apparently, the highest values of leaf blade features (section height and length, arm length and lacuna area) are noticed in *C. heuffelianus* population. In the other two species values of those parameters are much lower. The white stripe is narrow in *C. kosaninii* cross sections (Fig. 6). Both adaxial and abaxial epidermis cells were found to be

**Table 2.** Qualitative anatomic characters of leaves of *Crocus* ser. *Verni* species from Serbia

	<i>Crocus heuffelianus</i>	<i>Crocus kosaninii</i>	<i>Crocus tommasinianus</i>
<b>Leaf shape</b>			
Arms	<b>the longest with the highest curving degree</b>	shorter and slightly recurved	
Keel	<b>rectangulared, with ± acute corners</b>	<b>squared, with ± acute corners</b>	<b>squared, with conspicuously acute corners</b>
<b>Epidermis</b>			
Cell shape	adaxial rectangular, abaxial elliptical		
Papillae	finger-like, at the end of the arms and in the corner of the keel		
<b>Mesophyll parenchyma</b>			
Lacuna area	<b>large round cells</b>	disintegration of parenchyma cell walls is more frequent	
Palisade	2 layers of polygonal cells		
Spongy	3 - 4 layers of elliptical – irregular shaped cells		
<b>Big Vascular Bundles</b>			
	2 of them at the ends of the lateral arms, 2 others at the base of the central keel		
	<b>sometimes one more pair in the middle of the arms</b>		

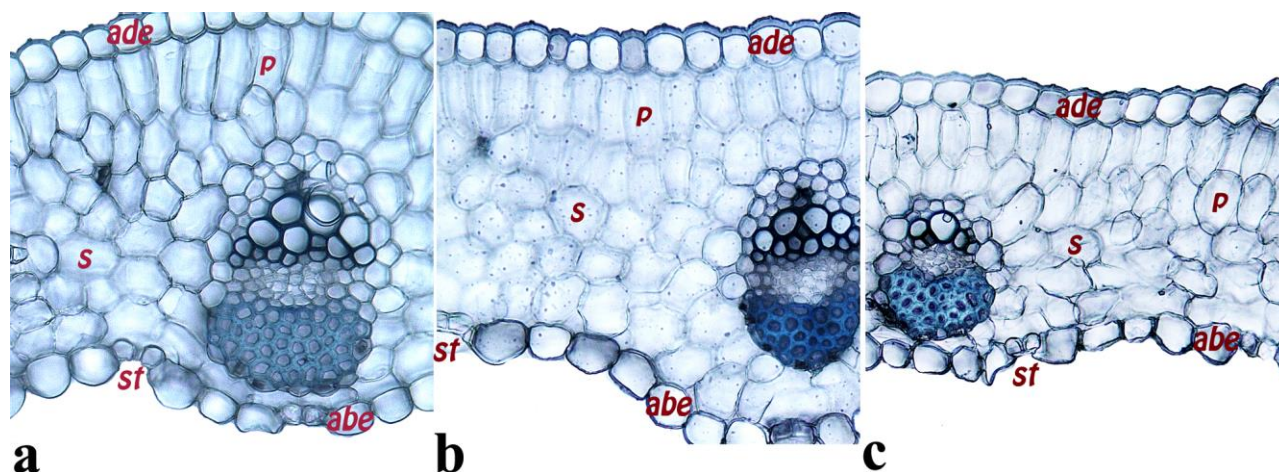
**Table 3.** Quantitative anatomic characters of leaves of *Crocus* ser. *Verni* species from Serbia

Species	<i>Crocus heuffelianus</i>	<i>Crocus kosaninii</i>	<i>Crocus tommasianus</i>
Character	Mean ± StDev Min - Max	Mean ± StDev Min - Max	Mean ± StDev Min - Max
<b>Section height</b> (µm)	<b>881 ± 173</b> 570 - 1210	<b>654 ± 124</b> 494 - 1017	<b>630 ± 125</b> 382 - 916
<b>Section length</b> (µm)	<b>7003 ± 1095</b> 3611 - 9035	<b>4527 ± 946</b> 2810 - 6298	<b>4152 ± 959</b> 2391 - 5598
<b>Arm length</b> (µm)	<b>3139 ± 492</b> 1684 - 4263	<b>1908 ± 429</b> 1109 - 2725	<b>1701 ± 462</b> 892 - 2467
<b>White stripe width</b> (µm)	<b>752 ± 122</b> 500 - 996	<b>590 ± 93</b> 434 - 792	<b>740 ± 111</b> 584 - 955
<b>Lacuna area</b> (µm <sup>2</sup> )	<b>371375 ± 105510</b> 193667 - 648276	<b>209992 ± 69767</b> 111999 - 335380	<b>248603 ± 74234</b> 141373 - 395804
Adaxial epidermis cell height (µm)	<b>20 ± 3</b> 16 - 28	<b>22 ± 2</b> 19 - 27	<b>16 ± 2</b> 11 - 20
Adaxial epidermis cell width (µm)	<b>18 ± 3</b> 15 - 30	<b>19 ± 2</b> 16 - 25	<b>17 ± 2</b> 13 - 20
Palisade cell height (µm)	<b>37 ± 6</b> 27 - 55	<b>34 ± 4</b> 27 - 42	<b>34 ± 4</b> 28 - 43
Palisade cell width (µm)	<b>15 ± 2</b> 11 - 21	<b>14 ± 1</b> 12 - 17	<b>16 ± 2</b> 13 - 20
Palisade tissue height (µm)	<b>59 ± 13</b> 35 - 88	<b>59 ± 9</b> 45 - 82	<b>54 ± 7</b> 40 - 66
Spongy cell height (µm)	<b>22 ± 3</b> 16 - 29	<b>22 ± 2</b> 17 - 27	<b>16 ± 2</b> 12 - 20
Spongy cell width (µm)	<b>19 ± 3</b> 15 - 26	<b>20 ± 2</b> 18 - 23	<b>24 ± 3</b> 17 - 30
Spongy tissue height (µm)	<b>68 ± 11</b> 38 - 91	<b>69 ± 16</b> 38 - 107	<b>47 ± 9</b> 27 - 66
Abaxial epidermis cell height (µm)	<b>19 ± 2</b> 14 - 24	<b>22 ± 2</b> 15 - 25	<b>14 ± 2</b> 11 - 18
Abaxial epidermis cell width (µm)	<b>18 ± 2</b> 14 - 21	<b>22 ± 2</b> 17 - 27	<b>16 ± 3</b> 12 - 23
Number of big bundles	<b>4 ± 0</b> 4 - 4	<b>4 ± 0</b> 4 - 4	<b>4 ± 0</b> 4 - 4
<b>Sclerenchyma cap area</b> (µm <sup>2</sup> )	<b>2614 ± 636</b> 1323 - 3710	<b>2219 ± 375</b> 1596 - 2929	<b>1459 ± 409</b> 895 - 2303
<b>Phloem area</b> (µm <sup>2</sup> )	<b>1230 ± 245</b> 669 - 1645	<b>720 ± 337</b> 338 - 1965	<b>609 ± 87</b> 442 - 768
<b>Xylem area</b> (µm <sup>2</sup> )	<b>2477 ± 586</b> 1657 - 3946	<b>1234 ± 491</b> 621 - 2323	<b>1059 ± 249</b> 496 - 1509

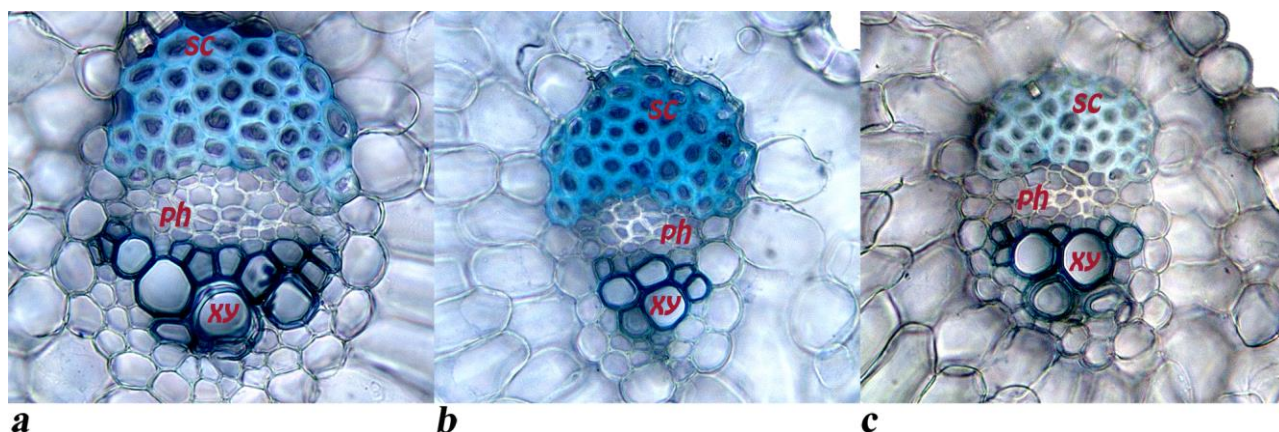
the largest in *C. kosaninii* population. Palisade tissue characters are of a similar value. The thinnest spongy layer was observed in the investigated *C. tommasianus* population. The most developed vascular tissue features (xylem, phloem and sclerenchyma cap area) were found in *C. heuffelianus* cross sections. *Crocus tommasianus* vascular bundles are the smallest, while *C. kosaninii* big bundles are middle-sized (Fig. 6).

The objective of this study was to introduce and compare leaf anatomy of the three *Crocus*

species from series *Verni* present in Serbia. Leaf blade, epidermis, mesophyll and vascular bundle features were analyzed. As might be expected (Rudall & Mathew, 1990), those species tend to correspond closely in their leaf anatomy. However, major differences can be found on the level of big vascular bundles and their examined parameters (xylem area, phloem area and sclerenchyma area). Moreover, leaf blade features (section height and length, arm length, white stripe width and lacuna area) are good distinguishing characters as well. The



**Fig. 4.** Arm details on leaves cross sections of *Crocus* ser. *Verni* species from Serbia (20x): a-*C. heuffelianus*; b-*C. kosaninii*; c-*C. tommasinianus* (ade-adaxial epidermis, p-palisade parenchyma, s-spongy parenchyma, abe-abaxial epidermis, st-stomata)



**Fig. 5.** Big bundles on leaves cross sections of *Crocus* ser. *Verni* species from Serbia (40x): a-*C. heuffelianus*; b-*C. kosaninii*; c-*C. tommasinianus*; (sc-sclerenchyma cap; ph-phloem; xy-xylem)

length of arms and their curving degree differ amongst the species (Erol, 2007). *Crocus heuffelianus* cross sections have the longest arms with conspicuously high curving degree. This population is also characterized by the most prominent vascular bundles. The features referred to leaf blade are of a higher value as well (section height and length, arm length, white stripe width, lacuna area). While *C. tommasinianus* can be distinguished from the other two species by the smallest vascular bundles, thinnest spongy layer and wide white stripe, *C. kosaninii* has middle - sized vascular bundles and the biggest epidermal cells (both adaxial and abaxial). Also, disintegration of parenchyma cell walls in lacuna area is more frequent in *C. tommasinianus* and *C. kosaninii* populations in comparison to *C. heuffelianus*.

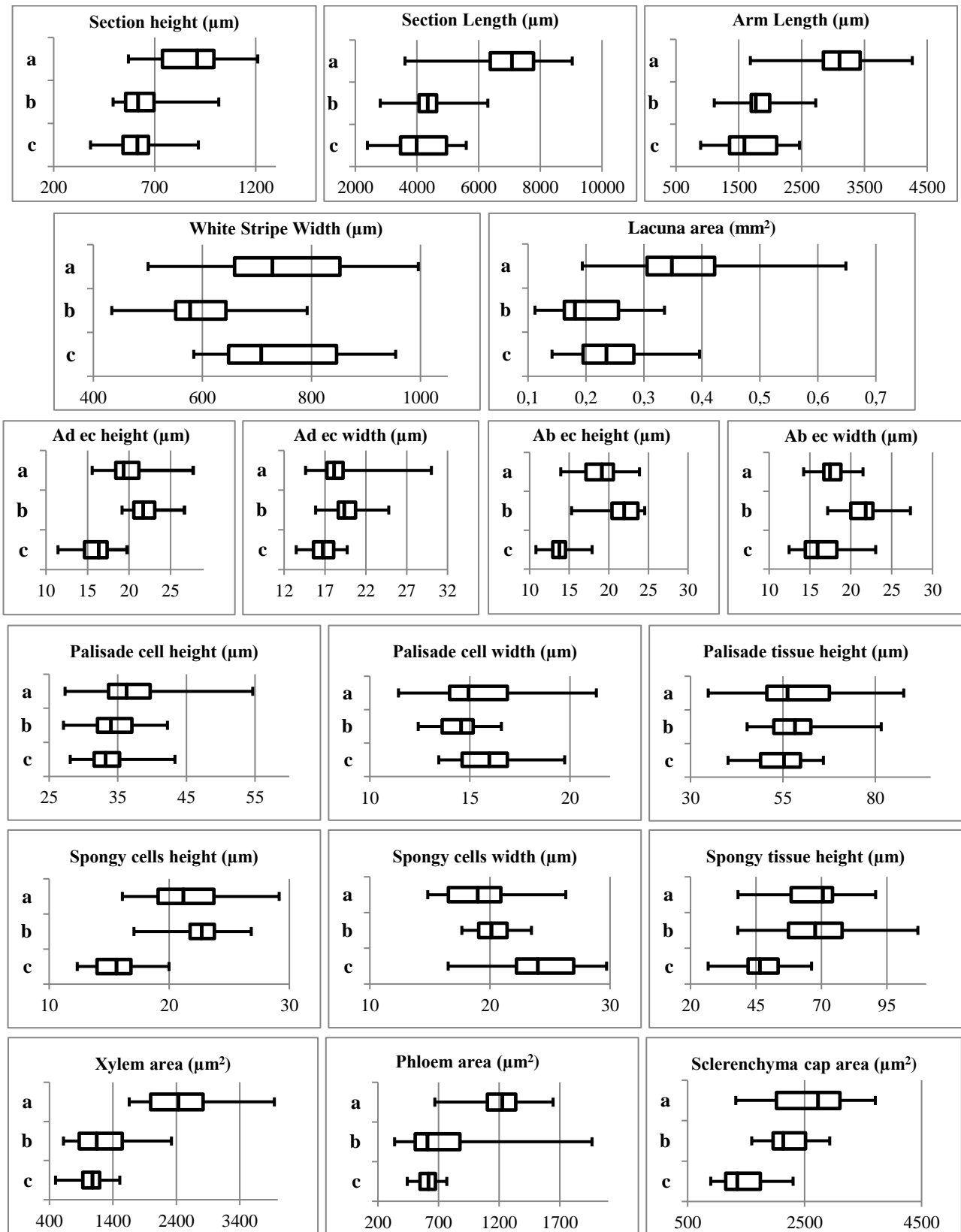
Further researches should be carried out including more populations, other closely related species and surveying also the ecological conditions

in each locality. This would allow us to evaluate which impact habitat factors have on the anatomy of leaves as well as the potential of leaf anatomy features as diagnostic characters.

## Conclusion

As could be seen from the literature, no detailed comparative study of series *Verni* had been done before. Therefore, the major aim of this paper was to introduce a leaf anatomy comparison of series *Verni* taxa in Serbia and to highlight the differences between them. This study is a basis for further researches within series *Verni*.

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**Fig 6.** Box plots of quantitative anatomic characters of leaves of *Crocus* ser. *Verni* species from Serbia: a-*C. heuffelianus*; b-*C. kosaninii*; c-*C. tommasinianus* (Ad ec-Adaxial epidermal cell; Ab ec-Abaxial epidermal cell)

## References

- Akan, H., Eker, I., Satil, F. 2007: The morphological and anatomical properties of endemic *Crocus leichtlinii* D. Dewar Bowles (Iridaceae) in Turkey. *Pakistan Journal of Botany*, 39 (3): 711-718.
- Akyol, Y., Durmuskahya, C., Kocabaş, O., Pekönür, S., Özdemir, C. 2014: The morphological and anatomical investigation of two endemic *Crocus* L. (Iridaceae) species of Turkey. *Pakistan Journal of Botany*, 46 (3): 833-839.
- Akyol, Y., Yetişen, K., Özdemir, C., Bozdağ, B., Kocabaş, O. 2012: The Morphological and Anatomical Studies on *Crocus biflorus* Miller subsp. *tauri* (Maw) Mathew (Iridaceae) in Turkey. *Journal of the Institute of Science and Technology*, 2 (ek: a): 15-20.
- Candan, F. 2015: Comparative Morphological and Leaf Anatomical Investigations of *Crocus flavus* Weston from Turkey. *International Journal of Agriculture, Forestry and Fisheries*, 3 (3): 99-104.
- Candan, F. 2015: Morphological and Leaf Anatomical Investigations on 2 Yellow Flowered Endemic Taxa of *Crocus* L. (*Crocus ancyrensis*, *Crocus sieheanus*) from Turkey. *International Journal of Agriculture, Forestry and Fisheries*, 3 (3): 93-98.
- Erol, O., Küçükler, O. 2007: Leaf Anatomy of Some Endemic *Crocus* L. (Iridaceae) Taxa from Western Anatolia. *International Journal of Botany*, 3 (3): 290-295.
- Gligorijević, S., Pejčinović, D. 1983: Contribution to the Methodology of Anatomical Sections Preparation. *Acta Biologiae et Medicinae Experimentalis*, Priština, 8: 43-45.
- Kandemir, N. 2009: Morphology, anatomy and ecology of critically endangered endemic *Crocus pestalozzae* Boiss. (Iridaceae) in North-West Turkey. *Bangladesh Journal of Botany*, 38 (2): 127-132.
- Kandemir, N. 2010: A morphological and anatomical investigation about two rare and endemic *Crocus* taxa (Iridaceae) from Southern Anatolia. *EurAsian Journal of BioSciences*, 4: 54-62.
- Kandemir, N. 2011: Comparative leaf anatomy of some endemic *Crocus* L. taxa from Turkey. *Bangladesh Journal of Botany*, 40 (2): 155-162.
- Kandemir, N., Çelik, A., Yayla, F. 2012: Comparative anatomic and ecologic investigation on some endemic *Crocus* taxa (Iridaceae) in Turkey. *Pakistan Journal of Botany*, 44 (3): 1065-1074.
- Mathew, B. 1982: The Crocus. A Revision of the Genus *Crocus* (Iridaceae). Batsford Ltd, London.
- Mathew, B., Petersen, G., Seberg, O. 2009: A reassessment of *Crocus* based on molecular analysis. *The Plantsman*, 8 (1): 50-57.
- Özdemir, C., Akyol, Y., Yetişen, K., Bozdağ, B., Kocabaş, O. 2013: Morphological and Anatomical Study on *Crocus chrysanthus* (Herbert) Herbert (Iridaceae). *Journal of the Institute of Science and Technology*, 3 (1): 25-30.
- Özdemir, C., Akyol, Y., Alçitepe, E. 2014: Morphological and anatomical studies on two endemic *Crocus* species of Turkey area. *Pakistan Journal of Botany*, 36 (1): 103-113.
- Randelović, N., Hill, D. A., Randelović, V. 1990: *The genus Crocus L. in Serbia*. The Serbian Academy of sciences and arts, Belgrade.
- Rudall, P. 1990: Leaf Anatomy in *Crocus* (Iridaceae). *Kew Bulletin*, 45 (3): 535-544.
- Rukšāns, J. 2017: *The World of Crocuses*. Latvian Academy of Sciences, Riga.
- Satil, F. & Selami, S. 2007: An anatomical and ecological study of some *Crocus* L. taxa (Iridaceae) from the west part of Turkey. *Acta Botanica Croatica*, 66 (1): 25-33.
- Stjepanović-Veseličić, L. 1976. Rod *Crocus* L. In: Josifović, M. (ed.), *Flora SR Srbije VIII*: 2-12, Srpska Akademija Nauka i Umetnosti, Beograd.
- Yetişen, K., Şen, U., Yıldırım, T., Özdemir, C. 2013: Morphological and anatomical study on endemic *Crocus olivieri* Gay subsp. *istanbulensis* Mathew subspecies (Iridaceae). *Anadolu University Journal of Science and Technology-C Life Sciences and Biotechnology*, 3 (1): 31-37.