BIOLOGICA NYSSANA
8 (1) • September 2017: 07-13
DOI: 10.5281/zenodo.962907

Original Article

Received: 02 July 2017
Revised: 18 July 2017
Accepted: 06 September 2017

A new Serbian endemic species of the genus *Crocus* (Iridaceae)

Dörte Harpke[1], Helmut Kerndorff[2], Irena Raca[3], Erich Pasche[4]

[1] *Leibniz Institute of Plant Genetics and Crop Research (IPK), Gatersleben, Germany*

[2] *Casa da Eira, São Romão, São Bras de Alportel, Portugal*

[3]*University of Niš, Faculty of Science and Mathematics, Department of Biology and Ecology, Višegradska 33, Niš, Serbia*

[4] *Feldstraße, Velbert, Germany*

** E-mail: raca.irena@gmail.com*

***Abstract*:**

Harpke, D., Kerndorff, H., Raca, I., Pasche, E.: A new Serbian endemic species of the genus *Crocus* (Iridaceae). *Biologica Nyssana, 8 (1), September 2017: 07-13.*

Recent research within the genus *Crocus* (Iridaceae) let us doubt that *Crocus adamii* Gay from Serbia represents the same taxon as *C. adamii* s. str. of the locus classicus in the Caucasian Mountains. The latter belongs to a group of crocuses, which is distributed from the Anatolian Diagonal, a mountain belt in inner Anatolia, to Iran and the Caucasian Mountains. To infer (*i*) if the Serbian *C. adamii* represents a new species and (*ii*) its taxonomical and phylogenetic affiliation within the genus we combined morphological and molecular investigations. The results show the presence of a morphologically and molecularly differentiated lineages, which both share a close relationship e.g. to *C. alexandrii*, *C. chrysanthus*, and *C. weldenii* but not to *C. adamii* s. str., which indicates a new species. As a result, we here describe *C. randjeloviciorum* to honor the Serbian botanists Novica and Vladimir Ranđelović.

Key words: *Crocus*, new species, Serbia

***Apstrakt*:**

Harpke, D., Kerndorff, H., Raca, I., Pasche, E.: Nova endemična vrsta roda *Crocus* (Iridaceae) u Srbiji. *Biologica Nyssana, 8 (1), Septembar 2017: 00-00.*

Skorašnja istraživanja u okviru roda *Crocus* (Iridaceae) dovela su u sumnju teoriju da *Crocus adamii* Gay iz Srbije predstavlja takson istovetan *C. adamii* s. str. čiji je locus classicus na Kavkazu. Drugi navedeni takson pripada grupi šafrana, distribuiranih od planinskog pojasa u centralnom delu Anatolije, do Irana i Kavkaskih planina. Da bi se zaključilo o tome (*i*) da li takson *C. adamii* iz Srbije predstavlja novu vrstu, te (*ii*) sudilo o njegovom taksonomskom i filogenetskom položaju unutar roda, kombinovana su morfološka i molekularna istraživanja. Rezultati ukazuju na postojanje morfološki i molekularno diferenciranih linija, srodnim *C. alexandrii*, *C. chrysanthus* i *C. weldenii*, ali ne i *C. adamii* s. str., što sugeriše da je *C. adamii* iz Srbije nova vrsta za nauku. Kao rezultat, u ovom radu biće opisana vrsta *C. randjeloviciorum*, čiji je naziv dodeljen u čast srpskih botaničara Novice i Vladimira Ranđelovića.

Ključne reči: *Crocus*, nova vrsta, Srbija

Introduction

Crocus L. (Iridaceae) comprises currently about 200 species. Within the last few years, over 50 new species were described (Erol et al., 2012; Ranđelović et al., 2012; Kerndorff et al., 2013; Schneider, 2014; Harpke et al., 2014, 2015; Erol et al., 2015; Ruksans, 2015; Miljković et al., 2016). Taxonomically the genus is currently in a complicated situation as many of the newly described taxa are unplaced in the system. Moreover, recent phylogenetic analyses proved several units within the genus *Crocus* to be para- or polyphyletic. As a consequence of ongoing revisions of taxonomical groups, with the aim to define monophyletic units, several other taxa are unplaced, too. One of those groups of currently unplaced taxa comprises *C. adamii* Gay and its allies (Kerndorff et al., 2013). It is a very homogenous group and occupies the sister group position to a large clade comprising series *Aleppici* B. Mathew, *Flavi* B. Mathew, *Speciosi* B. Mathew, *Reticulati* B. Mathew (Harpke et al., 2016). The *C. adamii* group comprises about 20 taxa (Kerndorff et al., 2013). The taxa of this group are characterized e.g. by toothless basal tunic rings (with the exception of *C. aerius* Herb. which has no rings), silvery bract and bracteole (in some taxa they turn brown with aging), glabrous throat, trifurcate style and vernal flowers. The species of the *C. adamii* group are inhabitants of the Anatolian Diagonal, a mountain belt dividing inner Anatolia, and occur north-, east-, and south-east of it.

Considering that despite extensive field studies *C. adamii* and its allies were never found west of the Anatolian Diagonal, the presence of *C. adamii* in Serbia became questionable. The Serbian plant was mentioned by Randjelović et al. (1990), located in Serbia in the regions of Timok, Niš, and south Morava. From the photograph shown in this work it has obviously some affinity to *C. adamii* but also to some forms of *C. nubigena* (Harpke et al., 2016). Furthermore, its chromosome number (2n) is 18, members of the C. *adamii* group from the mentioned eastern areas have mostly 2n = 20.

Material and methods

Morphological analyses

Leaf-parameters were measured on fresh material (45 specimens for number, 10 specimens for white stripe, diameter, colour of cataphylls, bract and bracteoles, and ribs underneath). Corm and flower parameters were measured on 36 randomly collected and dried specimens from the type-locality HKEP 1328.

For the investigation of leave anatomy, samples were collected during flowering time (06.03.2016) and preserved in 50% alcohol. Anatomical studies were done in the laboratory of Plant Systematics and Ecology, Faculty of Science and Mathematics, University of Niš. Thirty transverse leaf cross sections were made by manual microtome (Gligorijević & Pejčinović, 1983) and stained with Safranin - Alcian Blue. The slides with coverslips were then photographed using a *Leica DM 1000* microscope. Nineteen anatomical features were obtained of 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, sclerenchyma area, phloem area, xylem area and number of vascular bundles. Measurements of the listed parameters were done in ImageJ.

Molecular and phylogenetic analyses

Molecular analyses were carried out using 3 individuals of one *C.* cf. *adamii* populations. Extraction of genomic DNA and amplification of the nuclear rDNA ITS were conducted according to Harpke et al. (2014). Both strands of the PCR products were directly sequenced with Applied Biosystems' BigDye Terminator technology on an ABI 3730xl automatic DNA sequencer using either the primers from PCR amplifications.

Forward and reverse sequences were manually checked, edited where necessary, and combined in consensus. The newly obtained sequence was submitted to the EMBL nucleotide database and is accessible through accession numbers MF766260. Sequences were aligned manually. If sequences were found to be identical within the same species or population they were included only once in the analyses. The nuclear data were subjected to phylogenetic analyses using Bayesian phylogenetic inference (BI) with MrBayes 3.2 (Ronquist et al., 2012). For BI 2 times 4 chains were run for 2 million generations under the appropriate models of sequence evolution (nuclear data set: GTR+Γ+I), sampling a tree every 1000 generations. Converging log-likelihoods, potential scale reduction factors for each parameter and inspection of tabulated model parameters in MrBayes suggested that stationary had been reached in all analyses. The first 25% of trees of each run were discarded as burn-in. Two independent runs of BI analysis were performed to confirm that separate analyses converged on the same

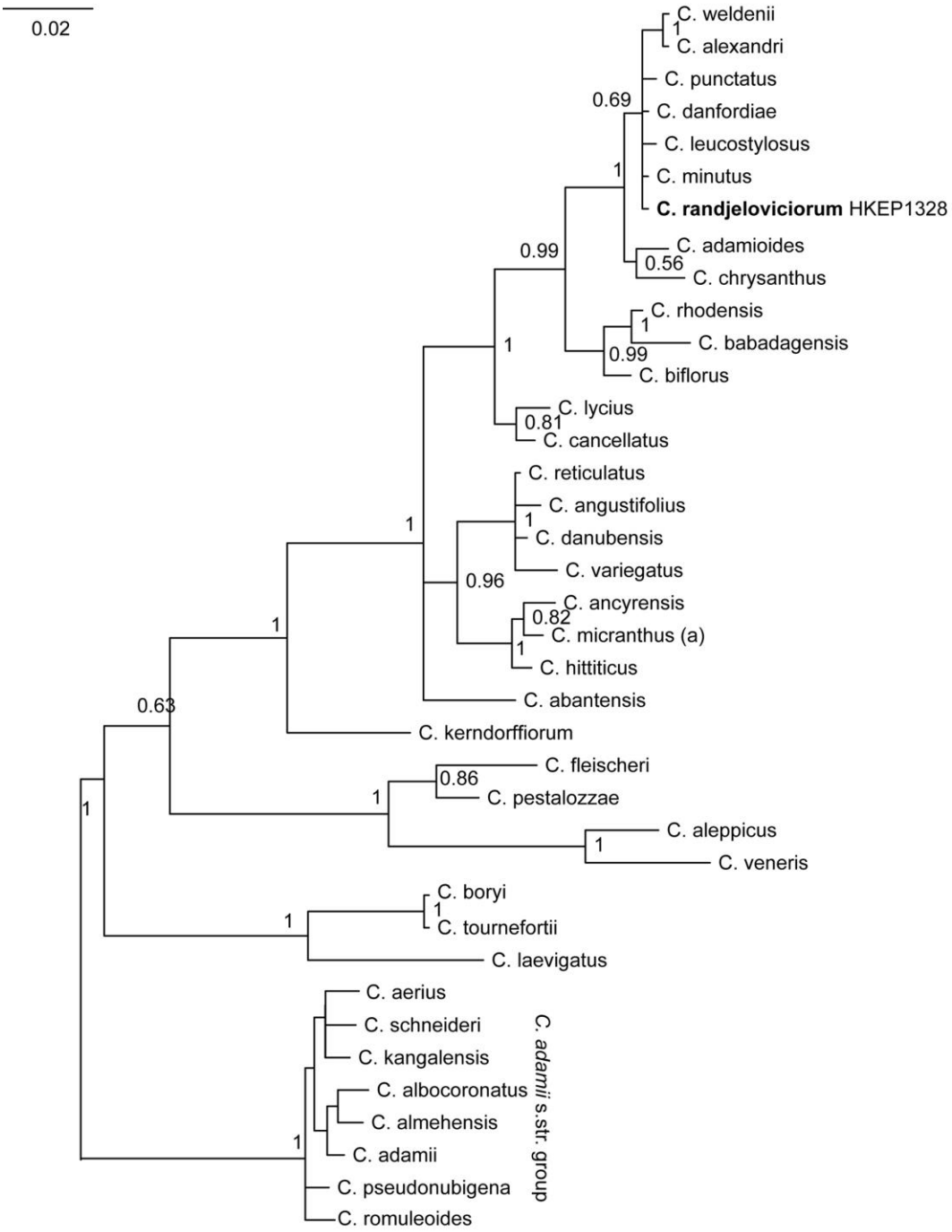

Fig. 1. Phylogenetic tree obtained by Bayesian phylogenetic inference of the nuclear rDNA ITS regions. Numbers along branches give posterior probabilities. Sequences obtained by cloning are indicated by a small letter. *Crocus randjeloviciorum* is indicated in bold.

result. In each of the 2 analyses the same topology and similar posterior probabilities (pp) of nodal support resulted.

Results and discussion

Affiliation within the genus. *Crocus* cf. *adamii* from Serbia groups within a polytomic clade comprising *C. puncatus* and its relatives with strong support (pp 1.0; **Fig. 1**). Its ITS sequence differs in at least three positions from its closest relatives *C. alexandri* (Serbia), *C. weldenii* (NE-Italy) and five from *C. adamioides* (NW-Turkey). The seeds (**Fig. 2D**) have more similarity with the latter species than to those of the relatives of the *C. adamii*-group (K e r n d o r f f et al. 2016). Further morphological characters like corm tunics (**Fig. 2A, Fig. 3**) also clearly show that it does not belong to the *C. adamii*-group.

Description of new species

The Serbian crocus considered as *C. adamii* is phylogenetically and morphological quite distinct from *C. adamii* s. str.. It represents a new species.

***Crocus randjeloviciorum* Kernd., Pasche, Harpke & Raca sp. nova**

Type: East Serbia, Tupižnica Mt., 950m a.s.l., HKEP 1328 (holotype 23042 GAT!)

Diagnosis. *Crocus randjeloviciorum* differs from other species by intermediate outer corm tunic between coriaceous and membranous (e.g. in *C. biflorus* it is coriaceous, in *C. tauri* membranous), the 2 ribs in the grooves of the leaves (none in *C. biflorus*, 2 in *C. tauri*), no dark spot at the basis of the outer segments, unusually long lobes of the anthers (1.7-3 mm) and very broad connectives. *Crocus randjeloviciorum* has also a very rare property in the genus. The styles are compared to the stamens in more or less the same proportion. This means approximately in 1/3 of the specimens they are shorter, in 1/3 they are equal and in 1/3 they are longer. In the majority of species there is either a tendency to have longer or to have shorter styles compared to the stamens.

Description. Corm sub-globose about 9-11 mm in diameter. Outer and inner tunics coriaceous to membranous, the inner ones softer; tunic splits mainly into segments of 2-5 mm rarely into >5 mm, sub-splits absent. Neck around 5 mm long consistent of medium-based "triangles", caps membranous, medium-sized (Kerndorff et al. 2015). Rings narrow to normal-sized coriaceous to membranous mostly smooth-edged, very rarely with tiny teeth <0.5 mm. Basal tunics small plates of 5-7 mm in diameter. Cataphylls silvery, skinny, sometimes slightly brownish towards tips. Leaves 2 – **3.1** – 4, green, glabrous, 1.5-2.5 mm in diameter with two ribs underneath of both sides of the keel. Leaves reach or slightly overtop the flowers at anthesis. White stripe <1/3 to 1/3 of leaf diameter. Flowers 1(-2). The outer segments are between 21 and 30 mm but usually **25** mm long, between 6 and 14 mm mostly **9.5** mm wide.

The inner segments are between 18 and 28 mm but usually **23** mm long and between 6 and 14 mm in average **10.2** mm wide. Segment proportion of length/width of the outer segments is **2.6** (n = 36). The insides of all of segments are light to deep violet blue rarely whitish without markings, the inner ones also at outside with an indistinct brownish-greyish zone or spot near the basis, overlaid by the yellow of the throat which shines through. The outside of the outer segments has either the inside flower colour or can be white or yellowish (buff-coloured) dominated by different numbers of intense brownish-violet stripes, vertically orientated to the tips, occasionally accompanied by many thin ones also upwards orientated but more to the edges than to the tips of the segments, sometimes the whole outside is suffused deep violet. There is no dark blotch or area at the bases of the segments, the violet stripes merge into the perianth tube which is otherwise colourless (white) especially near the ground. Prophyll absent. Bract and bracteole present, subequal, mostly silvery-white and skinny but sometimes with brownish tips. Filaments deep yellow, hairy, 4-**5.2**-6.5 mm long; anthers yellow 7-**11.5**-15.2 mm long, having remarkably long lobes of about 1.7-**2**-3 mm (n = 15), connective very broad and conspicuous, colourless. Throat deep yellow, glabrous. Pollen yellow. The orange to orange-red red styles are divided into three branches sometimes papillous in the upper part. The branches are 3.6-**5.6**-8.5 mm long mostly expanded and fringed towards at the apex. The styles are 29% shorter, 37% equal and 34% longer compared to the stamen. Capsule small, about 1 cm long, broadly ellipsoid and peaky at top. Seeds light brown with an orange tint, main body ellipsoid about 1.6 -2 mm long and 1.3-1.4 mm wide (**Fig. 2D**), Caruncle and raphe distinct. Chromosome number 2n=18.

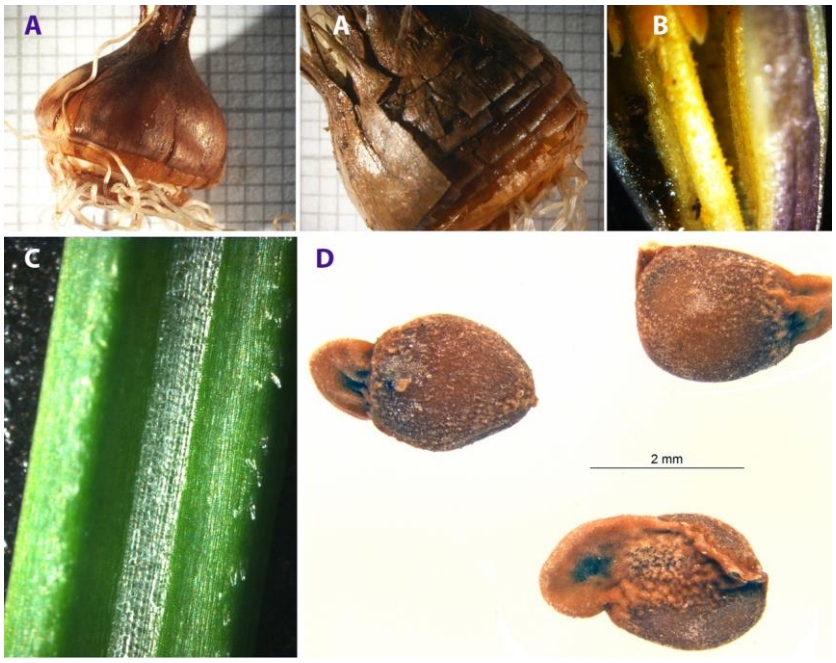

Fig. 2. Photographs of corm tunics (A), hairy filaments (B, 9x), leaves with papillae (C, 9x) and seeds (D).

Fig. 3. Photos of plants from the type population of *Crocus randjeloviciorum*.

Distribution and habitat: Serbia, in hornbeam-lilac scrub (*Syringo-Carpinetum*).

Etymology: Prof. Dr. Novica Ranđelović (1937–) and his son Prof. Dr. Vladimir Ranđelović (1965–) are among the most prominent Serbian botanists. They extensively studied the flora of the Balkan Peninsula. The results of their investigations were published in numerous papers. They both are *Crocus* enthusiasts and described several new *Crocus* species and they published a monograph about Serbian crocuses (Ranđelović et al., 1990) and authored and co-authored several publications about the genus (Ranđelović et al., 2007, 2011, Harpke et al., 2014, 2015, Miljković et al., 2016).

Phenology: Flowering period from February to April.

Taxonomic relationships: *Crocus randjeloviciorum* belongs to a group of crocuses comprising species e.g. from NE–Italy (*C. weldenii*), the Balkan Peninsula (*C. alexandri*) and West Turkey (*C. adamioides*).

Leaf cross sections

Leaf blade. Leaf cross sections of crocuses are of a unique shape consisting of a central part called the "keel" and two lateral "arms". The keel is squared, almost rectangular while the arms are re-curved towards the keel (Rudall & Mathew, 1990). Keel corners, length and curving degree of arms vary between different species. The investigated *Crocus cf. adamii* is characterized by four well defined ribs (**Fig. 4A**) on the abaxial side of the keel and arms. Velenovsky (1907) typified *Crocus* leaf type as bifacial.

Leaf surface. Finger – like papillae can be found on the adaxial side at the end of the arms (**Fig. 4A**). Adaxial epidermis cells are square or rectangular, in contrast to the abaxial cells which are more oval. Anomocytic stomata occurs in the abaxial epidermis (Erol et al., 2007).

Mesophyll. The central part of the keel consists of round parenchyma cells, these cells are mostly broken down forming air space called the lacuna. It can

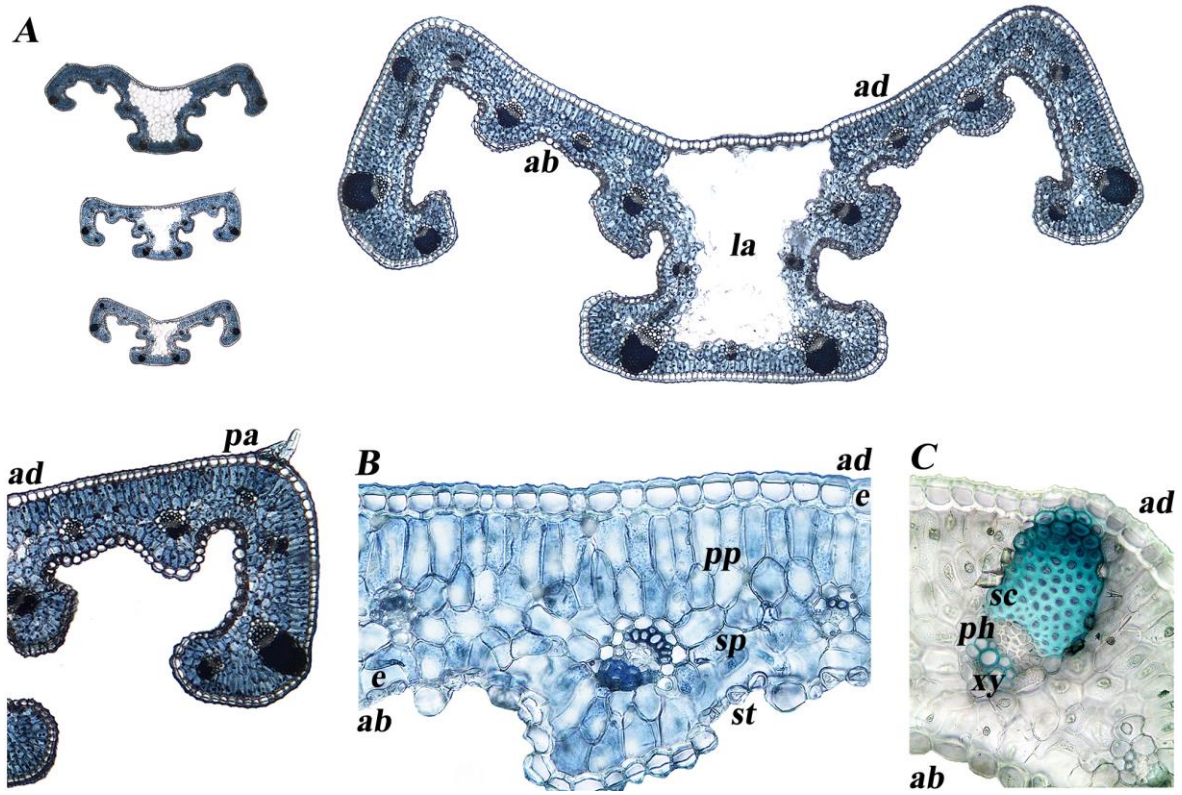

Fig. 4. Anatomic characters of leaves of *Crocus randjeloviciorum*: A-Leaf cross sections (5x), B-Arm detail (20x), C-Big bundle (40x) (ad: adaxial side, ab: abaxial side, la: lacuna area, r: rib, pp: papillae, e: epidermis, pp: palisade paremchyma, sp: spongy parenchyma, st: stomata, sc: sclerenchyma cap, ph: phloem, xy: xylem)

Table 1. *Crocus randjeloviciorum* leaf anatomy characters measurements

	Height(μm)		***Width* (*Length*)**(μm)		***Area***(μm^2)	
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**
Section	563±75	446-692	3154±311	2644-3668		
Arm			1393±170	1164-1726		
White stripe			434±91	323-599		
Lacuna					133157±37620	70665-234620
Adaxial e. cell	16±2	14-19	16±1	13-18		
Palisade cell	46±6	34-61	17±1	13-19		
Palisade tissue	68±11	51-90				
Spongy cell	18±2	14-22	26±3	21-33		
Spongy tissue	50±8	37-68				
Abaxial e. cell	16±2	12-20	18±2	13-22		
Sclerenchyma					5478±945	3022-7019
Phloem					549±123	351-954
Xylem					673±128	448-903
	Mean±SD	**Min-Max**				
V. bundles no.	18±3	11-21				

be noticed as a central white stripe all along the leaf (Erol et al., 2007; Yetişen et al., 2013) (**Fig. 4A**). The mesophyll of the arm consists of palisade (oriented to the adaxial side) and a spongy tissue below. The palisade parenchyma contains polygonal cells organized in two layers. Two or three layers of the oval or irregular shaped cells are present in the spongy parenchyma (**Fig. 4B**).

Vascular bundles. Leaf cross sections have 11-21 collateral vascular bundles positioned in one row all along the mesophyll. Four of these bundles are larger. Two of them are located in distal parts of the arms and another two in the keel base near corners. The xylem is oriented to the adaxial and phloem to the abaxial side of the leaf (bundles in the end of the arms have opposite orientation of vascular tissues). The

sclerenchyma tissue is well developed and in a form of a "cap" (**Fig. 4C**).

Measurements for leaf anatomy are given in **Tab. 1**.

Acknowledgements. The work was funded by the Ministry of Education, Science and Technological Development of Republic of Serbia (project no. 173030).

References

Erol, O., Can, L., Şık, L. 2012: *Crocus demirizianus sp. nov*. from northwestern Turkey. *Nordic Journal of Botany*, 30: 665-667.

Erol, O., Harpke, D., Yıldırım, H. 2015: A new *Crocus* L. (Iridaceae) species from SE Turkey, based on morphological and molecular data. *Phytotaxa*, 239: 223-232.

Erol, O., Küçüker, 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 sreparation. *Acta biologiae et medicinae experimentalis*, 8: 43-45.

Harpke, D., Kerndorff, H., Pasche, E., Peruzzi, L. 2016: Neotypification of the name *Crocus biflorus* Mill. (Iridaceae) and its consequences in the taxonomy of the genus. *Phytotaxa*, 260: 131-143.

Harpke, D., Meng, S., Kerndorff, H., Rutten, T., Blattner, F.R 2013: Phylogeny of *Crocus* (Iridaceae) based on one chloroplast and two nuclear loci: ancient hybridization and chromosome number evolution. *Molecular Phylogenetic and Evolution*, 66: 617-627.

Harpke, D., Peruzzi, L., Kerndorff, H., Karamplianis, T., Constantinidis, T., Ranđelović, V., Ranđelović, N., Jušković, M., Pasche, E., Blattner, F.R. 2014: Phylogeny, geographic distribution, and new taxonomic circumscription of the *Crocus reticulatus* species group (Iridaceae). *Turkish Journal of Botany*, 38: 1182-1198.

Kerndorff, H., Pasche, E. Harpke, D. 2015: The Genus *Crocus* (Liliflorae, Iridaceae): Life-cycle, Morphology, Phenotypic Characteristics, and Taxonomical Relevant Parameters. *Stapfia* 103: 27–65.

Kerndorff, H., Pasche, E., Blattner, F.R., Harpke, D. 2013: *Crocus biflorus* Miller (Liliiflorae, Iridaceae) in Anatolia-Part IV. *Stapfia*, 99: 159-186.

Mathew, B. 1982: *The Crocus. A revision of the genus Crocus (Iridaceae)*. B.T. Batsford, London.

Miljković, M., Ranđelović, V., Harpke, D. 2016: A new species of *Crocus* (Iridaceae) from southern Albania (SW Balkan Peninsula). *Phytotaxa*, 265: 39-49.

Peruzzi, L., Carta, A. 2011: *Crocus ilvensis sp. nov*. (section *Crocus*, Iridaceae), endemic to Elba Island (Tuscan Archipelago, Italy). *Nordic Journal of Botany*, 29: 6-13.

Ranđelović, N., Ranđelović, V., Hill, D.A. 1990: *The Genus Crocus L. in Serbia.* Srpska akademija nauka i umetnosti , Beograd.

Ranđelović. N., Ranđelović, V., Hristovski, N. 2012: *Crocus jablanicensis* (Iridaceae), a new species from the Republic of Macedonia, Balkan Peninsula. *Annales Botanici Fennici*, 49: 99-10.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012: MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, 61: 539-542.

Rudall, P., Mathew, B. 1990: Leaf Anatomy in Crocus (Iridaceae). *Kew Bulletin*, 45 (3): 535-544.

Rukšāns, J. 2015: Some new *Crocus* taxa (Iridaceae) from Western Turkey and East Aegean islands. *International Rock Gardener*, 64: 1-38.

Schneider, I. 2014: *Crocus brachyfilus*(Iridaceae), a new species from southern Turkey. *Willdenowia*, 44: 45-50.

Velenovsky, J. 1907: *Vergleichende Morphologie der Pflanzen.* Teil II Prag

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.