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Original scientific paper

VIOLA ORBELICA (VIOLACEAE), NEW SPECIES FOR THE FLORA OF SERBIA

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During two botanical expeditions in southeast Serbia (Mt. Dukat) one intriguing species of the genus *Viola* (*V.* sect. *Melanium*) was found. Intensive inspection and comparation with the plant material from several herbarium collections revealed that it is *V. orbelica* Pančić, which is a new species for the flora of Serbia. Morphological description, general distribution, ecological traits, chromosome number as well as threatened status of the newly discovered *Viola* plant in Serbia are presented.

Key words: *Viola orbelica*, distribution, threatened status, Serbia

INTRODUCTION

In the last five years, several articles about genus *Viola* L. in Serbia and the Balkan Peninsula were published (Niketić 2014; Stevanović *et al.* 2014; Tomović *et al.* 2013; 2014; 2016). Niketić *et al.* (2015) pointed out that the number of *Viola* species in Serbia is not final, since the intensive and long-

standing field work and inspection of the herbarium collections could result in discovering some new *Viola* species either for the whole Balkan Peninsula or for the territory of Serbia.

The prove of such assumption is discovery of a new species from *V.* sect. *Melanium* Ging. for the flora of Serbia. Namely, authors obtained first *Viola* specimens from Mt Dukat from herpetologist Lj. Tomovic in June 2014. After several personal visits of authors to the locality and collection of enough plant material, detailed comparison of these specimens with other *Viola* taxa from Serbia and surrounding Balkan countries was conducted. This included checking and inspection of the plant material from several herbarium collections (BEO, BEOU) and only after that it was proved that these intriguing *Viola* specimens belong to the species *Viola orbelica* Pančić

MATERIAL AND METHODS

Field investigation, checking and revision of herbarium material and consulting literature sources (Delipavlov 1979; Erben 1985; Micevski 1995) were essential for identification of the new species for the flora of Serbia. Distribution of the species in the Balkan Peninsula is accomplished according to the grid map with squares of 10 km × 10 km, based on the Military Grid Reference System (MGRS) projection (Lampinen 2001). Collected plant material is deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade (BEOU) and the Herbarium of the Natural History Museum in Belgrade (BEO) (Thiers 2013; http://sciweb.nybg.org/Science2/IndexHerbariorum.asp).Nomenclatureis given according to The Plant List database (http://www.theplantlist.org/) and Euro+Med (2006-) (http://ww2.bgbm.org/ EuroPlusMed/). Taxon description is given according to several botanical sources (Pančić 1883; Valentine *et al.* 1968; Delipavlov 1979; Erben 1985; Micevski 1995), with some modifications which are the results of the field observations.

Germinating seeds collected in the field were used for mitotic chromosome analyses. Root-tip meristems were pre-treated with 0.002 M 8-hydroxyquinoline for 4h at 10°C. After fixation in cold 3/1 (v/v) ethanol/ acetic acid for 48 hours, root tips were stored in 70% ethanol at 4°C. Hydrolysis was performed in 1N HCl for 11 minutes at 60°C and staining in Schiff reagent (Feulgen & Rossenbeck, 1924), followed by squash in a drop of acetic carmine. Chromosome plates were observed under a Leica DMLS light microscope (Leica Microsystems, Wetzlar, Germany) and photographs were taken with a Leica DCF 295 camera (Leica Microsystems). The chromosome number was determined from at least five individuals and from at least 10 cells per root tip.

RESULTS AND DISCUSSION

Viola orbelica Pančić, Glasn. Srpskog Učenog Društva 53: 176 (1883) [Elem. Fl. Bulg.: 16].

≡ *V. saxatilis* [subsp. *macedonica*] f. *orbelica* (Pančić) Hayek, Repert. Spec. Nov. Regni Veg. Beih. 30(1): 517 (1925) [Prodr. Fl. Penins. Balc. 1] ≡ *V. alpestris* f. *orbelica* (Pančić) Becker, Beih. Bot. Centralbl., Abt. 2 26(3): 345 (1910).

Description

Plant usually annual to biennial (rarely short-lived perennial) greyish white, glabrous to short pubescent. Stem (5)10–15(30) cm high, ascending branched from base. Lower leaves 12–30 × 6–9 mm at time of flowering, usually smaller then the other ones, with longer petioles than those from middle and upper; blade ovate to orbicular, obtuse or rounded, coarsely crenate, shortly pubescent. Stipules of lower leaves small, shortly pubescent and 1/6–1/3 as long as lower leaves, oblong to oblong-spathulate, entire or two- to three-partite. The terminal segment elongate-lanceolate, on the inner side with 0–1 and on outer side with 1–3 very small, narrow triangular to oblong-lanceolate lateral segments. Middle and upper leaves 22–45 × 5–12 mm at time of flowering wide lanceolate to oblanceolate, sometimes at the middle of the stem ovate to obovate with shorter petioles; blade ovate-lanceolate or oblanceolate, 1.5–2 times longer than stipules, gradually attenuated at the base into the petiole, obtuse or acute, crenate, densely pubescent. Petiole 0.3–0.6 as long as leaf blade, 0.7–1.1 mm wide, sparse to densely short pubescent. Stipules of middle and upper leaves 1/4–2/5 shorter than the leaves, pinnately divided, pubescent; the undivided terminal part relatively large, leaf-like; there are 1–3 lateral segments on the inner and 2–6 on the outer side of the stipules, which are tapering gradually downwards. Peduncles up to 15 cm, puberulent to pubescent only in lower part. Bracts at a distance of 1/5-1/4 of the length of the peduncle below the flower, 1.6–2.8 × 0.9–1.3 mm, narrow triangular to ovate, near the base, on either side, with 1–2 small, narrow triangular glands. Flowers small to medium size 1.3–2.0 cm, orbicular or obovate, 1–5 on the stem, solitary in the axils of the cauline leaves, not fragrant. Sepals 7–10 × 1.5–2.5 mm, elongate lanceolate, acuminate, mostly glabrous on back, long-ciliate at margin; margin with 1–3 ± conspicuous short teeth on both sides, or often entire. Appendages 1/4 as long as sepals, narrow to wide rectangular, trapezoid or semiorbicular, attenuate at base, coarsely dentate. Petals yellow, rarely bluish-violet or bicolor, at the base orange, upper petals 9–12 × 5–9 mm, obovate to orbiculate; lateral 9–13 × 4.5–7.5 mm, narrow orbiculate to obovate, lower petals (including spur) 14.5–21 × 6–11 mm,

Fig. 1. – *Viola orbelica* Pančić – habitus: a) typical plant with yellow flowers; b) plants with yellow and bluish-violet flowers (photo M. Niketić).

cordate to almost triangular, usually longitudinally folded along the midrib. Spur glabrous, 4.8–7.5 mm long, reaching 3/10 to 4/10 of the lower petal length, elliptical in cross-section (median diameter 1.2–1.7 mm), straight to slightly curved upwards, pale violet or greenish. Fruit a capsule. Seeds ellipsoid 1.7–1.8 × 0.9–1.0 mm, pale to dark brown. Flowering period: June–July; fructification from July to September (Figs 1-3).

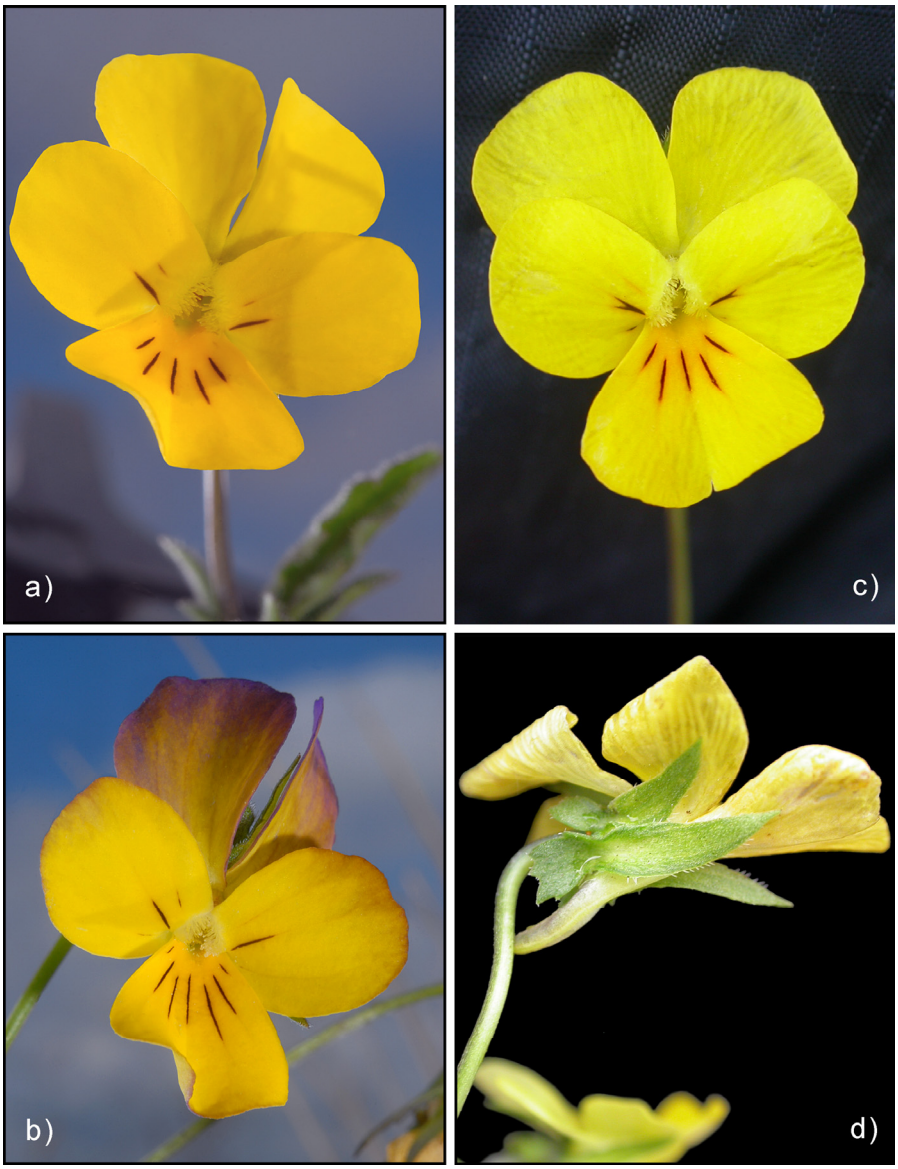

Fig. 2. – *Viola orbelica* Pančić – yellow flowers (photo: M. Niketić (a-b) and G. Tomović (c-d)).

Note: Only the yellow colour of the flowers was known so far, which we also noted on the type locality (Mts. Rila in Bulgaria) during field observations. However in Mt. Dukat (Serbia) as well as in Mt. Osogovski Planini (Macedonia) [MGRS 34T FM26] we also found some plants with bluish-violet and bicolor flowers (Fig. 1, 3). Both localities (in Serbia and Macedonia) are situated at the farthest western points of the species range (Fig. 4).

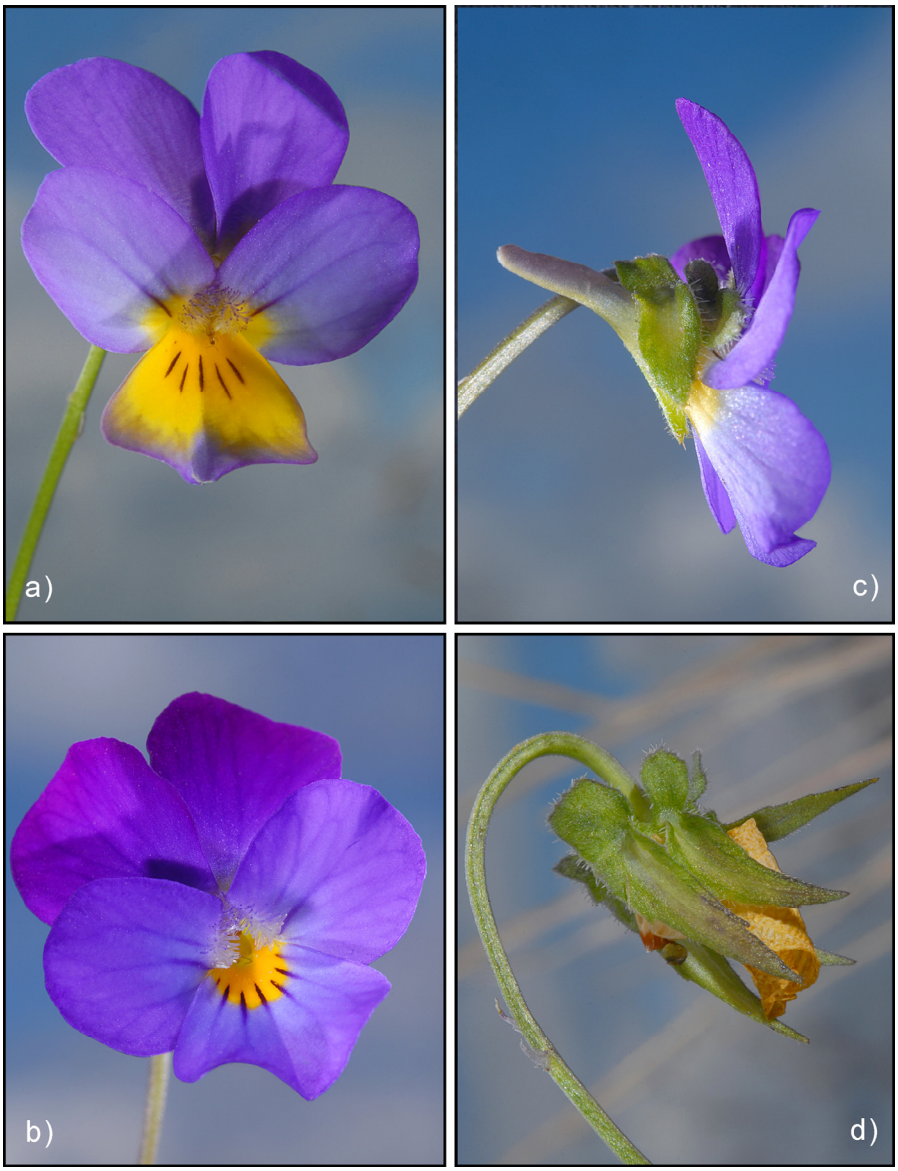

Fig. 3. – *Viola orbelica* Pančić – bicolor flower (a), bluish-violet flowers (b-c) and calyx after anthesis (d) (photo M. Niketić).

Taxonomic membership

V. orbelica Pančić belongs to *V.* sect. *Melanium* and it was firstly described as a species from Mts. Rila in Bulgaria (Pančić 1883). In later *Viola* monographic publication (Becker 1910) and Flora of the Balkan Peninsula (Hayek 1925) it was treated as form either of *V. alpestris* (DC.)

Becker or of *V. saxatilis* subsp. *macedonica* (Boiss. & Heldr.) Hayek. In the Flora Europaea it had unresolved status and been related either to *V. gracilis* Sibth. & Sm. or to *V. tricolor* subsp. *macedonica* (Boiss. & Heldr.) A. Schmidt (Valentine *et al.* 1968). However, Erben (1985) and Dimopoulos *et al.* (2013) considered that *V. gracilis* was erroneously cited for the Balkan Peninsula, since it is confined to Turkey (Anatolia) only.

Erben (1985) also considered *V. orbelica* as closely related to the taxa of *V. tricolor* group by many morphological features, such as their growth form, arrangement of the cauline leaves, shape and the degree of division of the stipules, relatively short, slender and upwardly curved spur, as well as the same chromosome number (2n = 26). On the other side, from *V. t.* subsp. *macedonica* it differs by narrower leaves, and even more by deeply pinnately incised (near to the middle veins) stipules; lateral lobes of the middle leaves stipule are 5- to 10- times longer than undivided part of the stipules (in *V. t.* subsp. *macedonica* 3- to 5-times longer); terminal segment is enlarged, linear-lanceolate, much longer than the lateral lobes.

General distribution

Delipavlov (1979) and Erben (1985) considered this species endemic to Bulgaria and confined to the mountain ranges of Rila and Pirin. Interestingly, Erben (1985) erroneously published herbarium specimens of *V. orbelica* from Mt. Centralna Stara Planina ["Balkan-Gebirge: Hütte Rai am Botev, 8.1960, Lepper & Lippold (JE)"]. Later, it was confirmed for eastern part of the Republic of Macedonia (Mt. Osogovski Planini, Mt. Golak and Mt. Plačkovica) (Micevski 1995) and only recently, it was also found in the Mts. Rhodope in NE Greece (Dimopoulos *et al.* 2013) (Fig. 4).

Distribution in Serbia

Southeast Serbia: Mt. Dukat: between Bandera peak and Beli Kamen peak [Karamanica], 42.36712 N; 22.31836 E, [MGRS 34T FM09], on silicate (quartz latite and schists) rocks, 1730 m, 22.06.2014, coll. Tomović, G., Zlatković, B., Tomović, Lj. det. M. Niketić, G. Tomović (BEOU 40775); between Bandera peak and Beli Kamen peak, [MGRS 34T FM09], on silicate (quartz latite and schists) rocks, 1610-1730 m, 29.06.2016, coll./det. M. Niketić, G. Tomović (BEO k20160604/1; BEOU 40000) (Fig. 4).

Habitat and ecology

V. orbelica inhabits alpine rocky pastures, predominantly on silicate at heights of (1200-)1500-2000(-2300) m a.s.l. in Bulgaria (Delipavlov 1979, Erben 1985). In the Republic of Macedonia it grows on subalpine rocks and

rocky grounds on silicate geological substrate, at the altitude from 1000 to 2200 m a.s.l. (Micevski 1995).

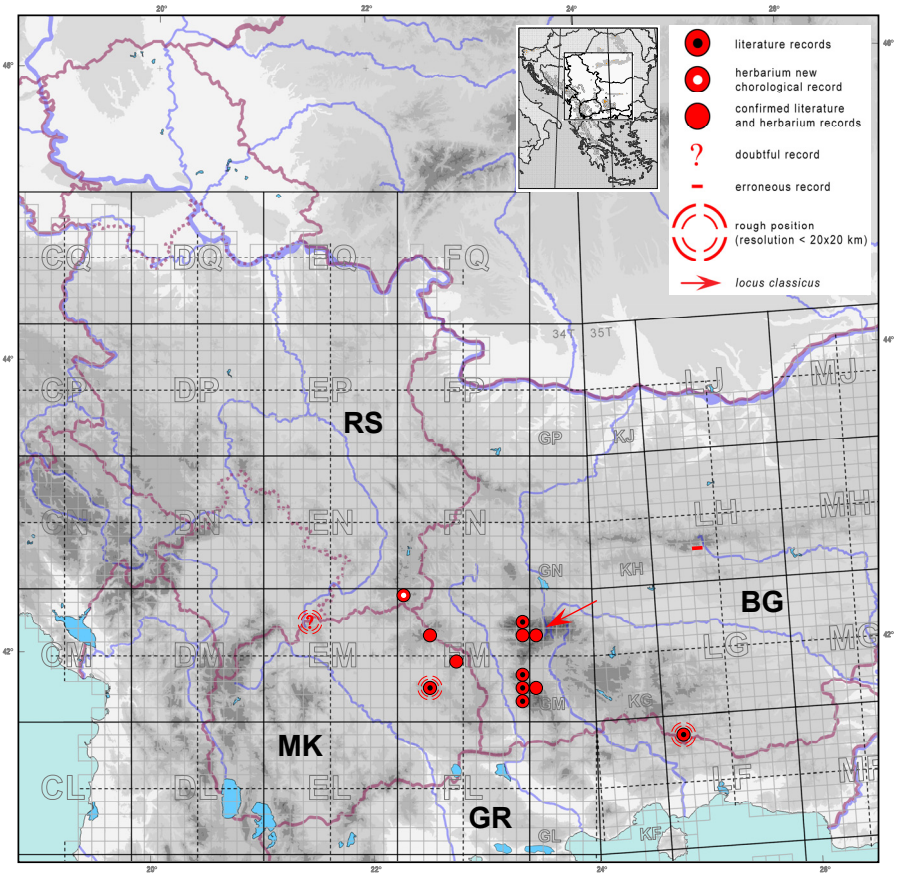

Fig. 4. Distribution of *Viola orbelica* Pančić in the Balkan Peninsula.

Serbian population of *V. orbelica* on Mt. Dukat was found on eroded silicate (quartz latite and schists) high-mountain pastures and rocky grounds of the vegetation class *Juncetea trifidi* Hadač in Klika et Hadač 1944, on the elevation between 1610 m and 1730 m a.s.l. Habitat of the species is dominated by the following plant taxa: *Viola orbelica* Pančić, *Deschampsia flexuosa* (L.) Trin., *Hypericum barbatum* Jacq., *H. maculatum* Crantz, *Cytisus eriocarpus* Boiss., *Cerastium arvense* L., *Rumex acetosella* L., *Agrostis canina* L., *Thymus longicaulis* C. Presl, *Ornithogalum orthophyllum* subsp. *kochii* (Parl.) Zahar., *Pilosella hoppeana* (Schultes) F.W. Schultz & Schultz Bip., *Genista sagittalis* L., *Anthoxanthum odoratum* L., *Festuca stricta* subsp. *sulcata* (Hack.) Pils, *Armeria rumelica* Boiss., *Scleranthus perennis* L., *Ranunculus sartorianus* Boiss. & Heldr., *Minuartia bulgarica* (Velen.) Graebn., *Trifolium velenovskyi* Vandas, *Allium melanantherum* Pančić, *Verbascum*

longifolium Ten., *Potentilla neglecta* Baumg., *Antennaria dioica* (L.) Gaertn, *Centaurea velenovskyi* Adamović, *Alchemilla bulgarica* Rothm., *A. flabellata* A. Kern., *A. lanuginosa* Rothm. Actually, *V. orbelica* is the most frequent on the edges of the rocky grounds, near the high-mountain tracking road (Fig. 5).

Fig. 5. – Habitat of *Viola orbelica* Pančić in Mt. Dukat (Beli Kamen) (photo G. Tomović).

Chromosome number

According to Valentine *et al.* (1968), unconfirmed chromosome number is 2n = 20, but Erben (1985) cited Merxmüller (1974) who counted $2n = 26$. Specimens of *V. orbelica* from Mt. Dukat are diploids with $2n = 2x = 26$ chromosomes. Chromosomes are small, 0.86 to 3.37 µm long (Fig. 6).

Population size and area of occupancy in Serbia

This plant occurs on restricted area of 0.15 km^2 (1500 × 100 m) between Bandera and Beli Kamen peaks in Mt. Dukat, from 1610 to 1730 m a.s.l. Population size is estimated to be less than 1000 mature individuals. The major threat for the only population of *V. orbelica* in Serbia is habitat destruction, since the large portion of the population is restricted to the edge of the high-mountain tracking road.

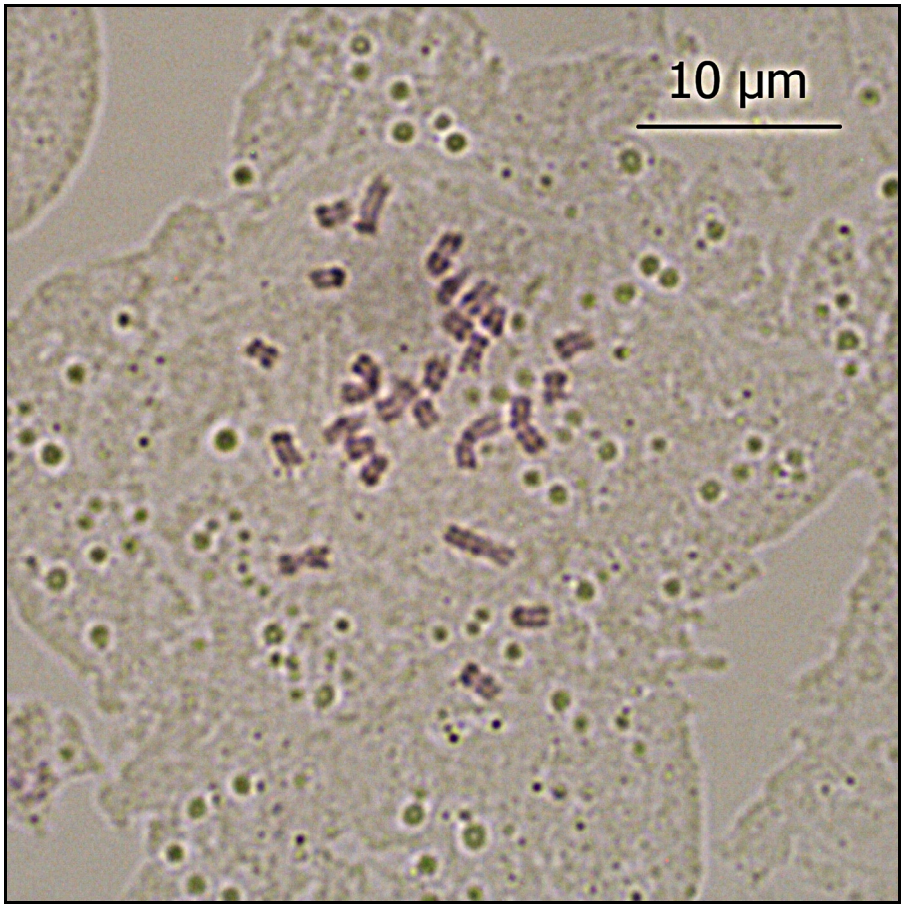

Fig. 6. – Metaphase chromosome plate of *Viola orbelica* Pančić *2n* = 26 (population from Mt. Dukat) (photo M. Lazarević).

Estimated threatened status in Serbia

According to the IUCN (2012) the threatened status in Serbia is critically endangered: CR B1ab(i,ii,iv)+2ab(i,ii,iv).

CONCLUSIONS

Viola orbelica represents new species for the flora of Serbia. Very restricted locality of this plant in Mt. Dukat in southeast Serbia is c. 30 km distant from the nearest known locality in the Republic of Macedonia (Mt. Osogovski Planini) and marks the northernmost limit of the species distribution range in the Balkan Peninsula. It is assumed that the species

also occurs in other mountains of southeast Serbia. Using the IUCN (2012) criteria, we estimated the threatened status of this species in Serbia as Critically Endangered. We consider that *V. orbelica* should be included in the next edition of the Red Data Book of the Flora of Serbia and strictly protected by the low in the near future.

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VIOLA ORBELICA (VIOLACEAE), НОВА ВРСТА У ФЛОРИ СРБИЈЕ

ГОРДАНА ТОМОВИЋ, БОЈАН ЗЛАТКОВИЋ, МАЈА ЛАЗАРЕВИЋ,
МАРЈАН НИКЕТИЋ

Р Е З И М Е

Током ботаничке екскурзије на подручју југоисточне Србије (Дукат планина) откривена је једна интригантна врста из рода *Viola* (*V.* sect. *Melanium*). Интензиван преглед и компарација са биљним материјалом из неколико хербарских колекција показали су да је у питању врста *V. orbelica* Pančić, која представља нову врсту за флору Србије. У раду је приказан детаљан опис, распрострањење, еколошке карактеристике, број хромозома и статус угрожености новооткривене врсте у флори Србије.