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A NEW SPONTANEOUS HYBRID BETWEEN THE CULTIVATED AND WILD *IRIS* SPECIES FROM SERBIA

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A new spontaneous wilding hybrid, *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. (*I.* ×*germanica* L. s.l. × *I. reichenbachii* Heuff.) from C Serbia was described and illustrated. The comparative analyses of the morphological characters and genome sizes were performed on *I.* ×*seminaturalis* and its parents. A new taxonomic review of the complex *I.* ×*germanica* s.l. was also presented.

Key words: vascular flora, *Iris* ×*seminaturalis*, *Iris* ×*germanica*, spontaneous hybrid, Serbia

INTRODUCTION

With six subgenera, 301 currently accepted species, 17 hybrids and 27 additional non-type subspecies (Barker & Govaerts 2018), genus *Iris* L. has

the richest taxonomic diversity in the family Iridaceae (Asparagales). Five subgenera are widespread in the Palaearctic, while subgenus *Limniris* (Tausch) Spach is distributed in the Holarctic. Most of the European species belong to the type subgenus with rhizomatous stocks and equitant ensiform leaves (Webb & Chater 1980). Representatives of the type section, *I.* sect. *Iris* (*Pogoniris*), are the most numerous among them. In the horticultural classification, they are included among the Bearded Irises (B), also comprising some related sections (Warburton & Gantz 1970).

Four native species from *I.* sect. *Iris* are presented in the Flora of SR Serbia: *I. pumila* L. (2n = 32), *I. suaveolens* Boiss. & Reut. (2n = 24), *I. variegata* L. (2n = 24) and *I. reichenbachii* Heuff. (2n = 24) (Stjepanović-Veseličić 1976). The lattermost species is widespread in a hilly-mountainous area and gorges of the Balkan Peninsula and SW Romania. It is 5–30(35) cm tall perennial, with keeled bracts, 1–2 usually yellowish (rarely violet to brownish-purple) flowers, and with hypanthial tube, much shorter than the perianth segments. According to horticultural classification, it belongs to the MDB class (Miniature Dwarf Bearded Irises) (Warburton & Gantz 1970). This plant inhabits dry pastures and rocky places, on different geological substrata, up to subalpine zone (Stjepanović-Veseličić 1976). The taxonomic status of a similar plant, *I. bosniaca* Beck, with broader falcate leaves, inflated bracts and longer filaments, is still doubtful and this name is currently not accepted (Barker & Govaerts 2018). However, from the karyological point of view, there are significant differences between these two plants (Siljak-Yakovlev *et al.* 2005). Hybridogenous potential of *I. reichenbachii* is confirmed by the existence of *I.* ×*kobasensis* Prodan (*I. reichenbachii* × *I. variegata*). According to Barker & Govaerts (2018), this is the only currently accepted natural Balkan hybrid, described and known from Bosnia and Herzegovina. However, *I. reichenbachii* is also a putative progenitor of hybridogenous *I. orjenii* Bräuchler & Cikovac from SW Montenegro (Bräuchler & Cikovac 2007).

In 2002, during the field excursion to the southern part of C Serbia, two unusual iris clusters were observed in a forest clearing within yellowish-flowering population of *I. reichenbachii* (Fig. 1). They were significantly higher, with more numerous and larger flowers, and with pale bluish falls and dark violet veins. Although it could be assumed that the plants were of hybrid origin, the second ancestral species was not observed in the habitat. By all accounts, this progenitor should have a violet to bluish flowers. However, in the Serbian flora there is no such native plant from *I.* sect. *Iris*. Fortunately, many ramet clusters of cultivated *I.* ×*germanica* L. s.l. were found at a nearby cemetery (Fig. 2). So, the origin of the new putative hybrid plant was initially presumed basis on intermediary morphological traits and adjacent habitats of *I. reichenbachii* and *I.* ×*germanica* s.l.

Fig. 1. – Parental species *Iris reichenbachii* Heuff. on natural habitat in C Serbia near Prokuplje (photo M. Niketić).

Fig. 2. – Parental plant *Iris* ×*germanica* L. s.l. (*I.* ×*trojanensis* group) on the cemetery near the natural habitat of *I. reichenbachii* in C Serbia near Prokuplje (photo M. Niketić).

Understanding the name of *Iris* ×*germanica*

Although the origin of *I.* ×*germanica* is uncertain, many authors consider it a synanthropic natural hybrid complex of Intermediate and Standard Tall Bearded Irises (IB, STB) from *I.* sect. *Iris*. It had been known in horticulture from ancient times as ornamental, aromatic and medicine plant, while it was introduced in plant science during the Renaissance era when Clusius described 28 varieties of 11 bearded irises (Wister 1978, Gessert 2010). It grows spontaneously in dry and stony soils, along the roads, borders, on cemeteries, in vineyards and former areas of cultivation, in the terraced fields, on beaches and in old abandoned house yards. It has been usually reported as ferals on the slopes near settlements, fortresses and ruins. According to Mathew (1981) it is a doubtful wilding, "propagated vegetatively and distributed locally giving the impression of having stable populations with a distinct geographical range" and appears never to occur in truly wild situations (Mathew 1984, 1991).

The plant was initially distributed in C and S Europe, the Mediterranean, and W Asia. It belongs to rhizomatous clonal perennial hybrids and in the flora of the British Isles more than 2/3 of such hybrids are infertile or slightly fertile (Preston & Pearman 2015). However, thanks to vegetative propagation that hybrids commonly occurred in areas without one or both parents (20% vs. 1% of nonclonal hybrids) [calculated here from (Preston & Pearman 2015)]. Thus, the range of *I.* ×*germanica* s.l. occupies a much larger area of potential places of origin (C Apennine and W Balkan Peninsulas). According to Prodan (1939), among the 1000 specimens of German Iris it is difficult to find one with fruit; even when found, they tend to have very few seeds. Furthermore, seedlings are difficult to obtain and are usually dwarfed (Dykes 1924), while malformations of the flowers are frequent (Dykes 1914). However, it is noticeable that in typical bluish flowered IB/STB forms fertility rises from west to east, and several ± fertile tetraploid clones (2n=48) have been found in SW Asia, causing many authors to consider them independent species.

Mathew (1981, 1984, 1991) was the first who lumped almost all IB and STB hybrid irises in *I.* ×*germanica* as "one of the number of hybrids of unknown and fairly ancient origin". His treatment is mainly accepted in some of the most prominent current floras and checklists, including a few dozen of described taxa, with the exception of the *I.* ×*florentina* group and modern cultivars (Henderson 2002, Barker & Govaerts 2018). According to them, all lineages arose through the hybridisation of blue Dalmatian and yellow Hungarian Irises, *I. pallida* L. and *I. variegata*. Subsequent backcrossing led to the appearance of different genetic and phenetic patterns, varying from one parental extreme to the other. However,

considering the karyological data and the results of experimental crossings, the possible origin of these strains seems to be much more complex.

For this complex, two main ancestral base numbers were reported. The first (x = 11) includes allotetraploid hybrids (2n = 44), which are usually reported in specimens having characteristics that are similar to those of Linnaean *I.* ×*germanica*. The second (x = 12) includes diploids (2n = 24) and polyploids (2n = 36, 48, 60). A new base number (x = 10, 2n = 40) was also recently reported from China (Yu *et al.* 2007), but this record requires verification. Based on the chromosome number and morphology, included plants can be separated into several groups of possible different origins. In any case, the hypothesis of *I. aphylla* L. as a possible ancestor (other parent is unknown) (Dykes 1924) was not confirmed experimentally and should be rejected.

I. ×*SAMBUCINA* GROUP (*I. pallida* s.l. × *I. variegata*)
Diploids (2n = 24)
IB to STB (infertile to fertile)

At the beginning of the 19th century, Paul de Bure showed in breeding experiments that several old STB irises actually represent hybrids of *I. pallida* and *I. variegata* (Mahan 2007). Compared to the type *I.* ×*germanica*, these hybrids often have yellower flowers, and their falls are more elongated with conspicuous deep violet to brownish-red veins on the entire surface. Conflict between blue and yellow colour often produces muddy or squalid shades (Dykes 1914). There are several old hybrid variants with various ratios of yellow and blue (*I.* ×*sambucina* L., *I.* ×*squalens* L., *I.* ×*lurida* Aiton, *I.* ×*neglecta* Hornem., *I.* ×*amoena* Hornem.), some of which some have been recognised as independent infertile or fertile hybrids (Colasante & Maury 2018).

According to Dykes (1914) almost innumerable variants between *I. variegata* and *I. pallida* have been observed. The area of the potential origin of these hybrids are limited to the Dinaric Alps in W Balkans and only one locality of the natural hybrid has been known to date. The plant was found by Degen on Mts. Velebit in 1912 (in the vicinity of Karlobag in NW Croatia). It was inspected by Dykes in 1913 (Dykes 1914a), and twenty two years later it was formally named and published as *I.* ×*rotschildii* Degen "(*illyrica* × *variegata*)" (Degen 1936). Twelve years ago, the same hybrid was rediscovered in the vicinity of Karlobag (Mitić *et al.* 2006) and karyologically investigated (Siljak-Yakovlev *et al.* 2008). This homoploid hybrid (2n = 24) includes an entire series of backcrossed morphologically intermediate individuals (a hybrid swarm), varying in combinations of yellow and blue colour. The investigated individuals, morphology pretty much corresponded to some of the mentioned cultivars (Warburton & Gantz 1970). The same situation had been also observed by

Dykes (1914a) on *locus classicus*. Concerning pollen morphology, *I.* ×*rotschildii* has a small amount of fertile pollen grains, similar to *I.* ×*sambucina* (Mitić *et al.* 2013). The diploid plant collected by F. Kušan in Mt. Dinara massif in Dalmatia, southeast of Mt. Velebit and identified as *I.* ×*squalens* (Warburton & Gantz 1970) can be a similar or same natural hybrid.

I. ×*TROJANA* GROUP (uncertain origin)
Allo- or autotetraploids (2n = 48), sometimes diploids (2n = 24)
STB (fertile to slightly fertile)

This is a very heterogeneous group, distributed mainly in the Orient, Levant and the Balkan Peninsula. Limb of the falls is bluish-violet, with a whitish to yellowish zone and a conspicuous violet venation limited to the lower 1/3 of the limb or slightly more. It comprises different old lineages that are poorly investigated and morphologically hardly distinguishable: *I.* ×*trojana* A. Kern. ex Stapf (NW Anatolia) (the oldest name), *I.* ×*cypriana* Foster & Baker (Cyprus), *I.* ×*germanica* nothovar. *askabadensis* Dykes (Turkmenia), *I.* ×*germanica* nothovar. *amas* Dykes (Anatolia: Amasia), *I.* ×*belouinii* Bois & Cornuault (Morocco and Algeria), *I.* ×*cypriana* Foster & Baker (Cyprus, Anatolia), *I.* ×*mesopotamica* Dykes (Orient, Syria, Israel). According to many authors, *I.* ×*trojana* and the last two above listed irises could be recognised as independent allotetraploid native species (Dykes 1924, Henderson 2002, Samad *et al.* 2016, Colasante & Maury 2018), and at the end of the 19th century they were used in the process of production of new cultivars by forced hybridisations with the original clones of *I.* ×*germanica* s.s. In comparison with them, representatives of this group are taller and larger, later flowering plants (June vs. May), with usually more scarious bracts (but almost herbaceous in some clones), an often larger yellowish zone on the limb of the falls, and usually bicolorous flowers. Similar STB tetraploid fertile irises, *I.* ×*altobarbata* A. E. Murray (Murray 1969), correspond to modern cultivars only (usually with strongly undulate falls) and should be excluded from this group.

It is interesting to note that the presence of this group in S, C and E parts of the Balkan Peninsula and Romania (Warburton & Gantz 1970, Aldén 1976, Blažek 2016) has been usually neglected. It is either native or was introduced from the East during the rule of the Ottoman Empire. With the exception of *I.* ×*varbossiana* K. Malý from Bosnia and Herzegovina and probably *I. hellenica* Mermygkas, Kit Tan & Yannits. from the Peloponnese in Greece, they are still unidentified and unnamed. Some of them were also observed as fertile plants in the wild (Warburton & Gantz 1970, Mermygkas *et al.* 2010).

Two ploidy levels were recorded for synanthropic *I.* ×*varbossiana*, with diploids (Simonet 1952) and tetraploids (Randolph & Mitra 1959).

According to Samad *et al.* (2016), tetraploid plants reported by Siljak-Yakovlev *et al.* (2005) for "*I.* ×*germanica*" from Bosnia and Herzegovina (probably corresponding to *I.* ×*varbossiana*) could be an autopolyploids. *I. mesopotamica*, on the other hand, could be an interspecific hybrid, and ribosomal RNA genes mapping enables the distinction between plants from the Balkans and *I. mesopotamica* (Abdel Samad *et al.* 2016). Diploids are also reported from Bulgaria (Popova & Češchmedjiev 1975, sub *I.* ×*germanica*). As there are no data on their fertility, and other features, finding and studying the diploid populations would bring us closer to resolving the origin of this group.

Although it is generally accepted that representatives of the *I.* ×*trojana* group possess a high rate of fertility, this has never been inspected *in situ*. It is known that *I.* ×*germanica* nothovar. *amas* produces only fertile pollen (Anonymous 1923) and, according to Mathew, various irises described as species in Turkey actually represent "sterile or near sterile clones which are propagated vegetatively".

I. CROATICA
Autotetraploids? (2n = 48)
STB (fertile)

Particular tetraploid, *I. croatica* Horvat & M. D. Horvat, is known as native fertile species in the area near the NW boundaries of distribution of *I.* ×*trojana* group, in the Peripannonian parts of NW Croatia and E Slovenia. The plant grows on clearings and margins in thermophilous pubescent oak forests. It has inflated herbaceous to slightly scarious bracts, very small bright basal area on the limb of the falls, and a karyotype that is similar to that of *I.* ×*cypriana* and *I. kashmiriana* Baker (Warburton & Gantz 1970). The plant was initially connected with *I. aphylla* (Horvat & Horvat 1962) and later was synonymised with *I.* ×*germanica* (Barker & Govaerts 2018), but Mitić *et al.* (2013) presumed that *I. croatica* could be an autotetraploid of *I. pallida* s.l. In our opinion, this tetraploid probably arose as a result of polyploidisation of backcrossed hybrid progenies between *I. pallida* × *I. variegata* (*I.* ×*rotschildii*), more similar to *I. pallida*. Since this single clone (Warburton & Gantz 1970) was discovered relatively late (Horvat & Horvat 1962), it may correspond to some artificial tetraploid cultivar from the *I.* ×*altobarbata* group that has escaped from cultivation, but this is less likely. It is expected that future molecular and karyological research will clarify the origin of this plant.

According to Barker & Govaerts (2018) the name *I. croatica* Horvat & M. D. Horvat is illegitime as a younger homonym of *I. croatica* Prodan (1931). However, on the cited page (15) Prodan (1931) actually published *I.* ×*cavarnae* Prodan (from *I.* ×*trojana* group), not *I. croatica*. The epithet *croatica* does not appear anywhere in Prodan's publication.

I. ×*GERMANICA* GROUP (*I. lutescens* s.l. × *I.* ×*trojana* s.l. or *I. croatica*)
Allotetraploids (2n = 20 + 24 = 44)
IB (infertile to slightly fertile)

Karyotype and chemotaxonomic analyses and experimental crosses indicated that 44-chromosome hybrids originated from tetraploid representatives of Standard Dwarf Bearded Irises (SDB) (2n = 40) and STB (2n = 48) (Mitra 1956, Werckmeister 1981, Williams *et al.* 2000, Colasante & Maury 2018). The first progenitors represent allotetraploid hybridogenous species distributed in the W Mediterranean. *I. lutescens* Lam., with pale yellow to violet flowers is most widespread. The few remaining species are endemic to Italy (Colasante & Maury 2018). Second progenitors from the *I.* ×*trojana* complex have E Mediterranean distribution. The occurrence of STB "*I. croatica*" (2n = 48) in NW Croatia and E Slovenia, not so far from that of tetraploid SDBs, led some authors to the conclusion that their natural hybrids arose in their former contact zone (Mitra 1956).

The original hybrid forms of *I.* ×*germanica* were of lower (intermediate) growth and subsequently have been selected for higher growth (Henderson 2002). In comparison with those from the *I.* ×*trojana* complex, the representatives of *I.* ×*germanica* group have slightly lower growth, less scarious bracts, usually concolorous flowers, an often smaller whitish zone on limb of the falls, and they rarely produce fruits and seeds. However, the emergence of new artificial hybrids has blurred the identification of these differences.

Although almost infertile, *I.* ×*germanica* is also considered the nomenclatural type of the genus. Its lectotype was designated from cultivated material from the garden of George Clifford III in Holland (Mathew ex Jarvis *et al.* 1993). Taking into account slight differences between *I.* ×*germanica* and *I.* ×*trojana* group, the suggested chromosome number of the original Linnaean plant (2n = 44) requires confirmation.

This group also comprises two pale white flowered cultivars (*I.* ×*florentina* L. and *I.* ×*albicans* Lange), *Iris* ×*biliottii* Foster (Anatolian Black Sea region), as well as *I.* ×*germanica* nothovar. *fontarabie* Dukes (Iberian Peninsula) and *I.* ×*kochii* A. Kern. ex Stapf (NW part of the Balkan Peninsula).

MODERN CULTIVARS (complex origin)
(2n = 32, 44, 48)
IB, STB (infertile to slightly fertile)

At the end of the 19th century, Sir Michael Foster started the process of target crossing of *I.* ×*germanica* (old clones) with tetraploid lineages of E Mediterranean *I.* ×*trojana* group, producing larger and more resistant

cultivars. Murray (1969) named all artificial tetraploid cultivars *I.* ×*alto-barbata*.

In the 20th century many other species from Europe and Asia were included into the breeding: *I. lutescens* Lam., *I. pontica* Zapal., *I. reichenbachii*, *I. imbricata* Lindl., *I. pumila*, *I. aphylla*, etc. For artificially created hybrids, Henderson (1993, 2002) proposed a new group name, *I.* ×*conglomerata* N. C. Hend., which should be distinguished from the complex *I.* ×*germanica* s.l. The emergence of thousands of new artificial hybrids led to a significant reduction of ancient cultivars, which remained mainly in rural gardens and habitats.

KEY FOR *I. PALLIDA* AND OLD HYBRID GROUPS OF *I.* ×*GERMANICA* S.L.

1a Limb of the fall with dark veins on the brighter background on the entire or nearly entire upper surface (similar to *I. variegata*) [*Limb of the fall obovate to obovate-spathulate. Limb of the standard strongly undulate. Unfertile to slightly fertile plants. 2n = 24*] . ***I. sambucina*** aggr.

1b Limb of the fall with brighter background limited to the basal part of the upper surface or absent 2

2a Bracts and bracteoles completely scarious at anthesis. Limb of the fall have no dark veins on a brighter background on the upper surface, rarely at the base. Limb of the standard strongly to moderately undulate. 2n = 24 [*Limb of the fall obovate. Fertile plants*] . . ***I. pallida*** s.l.

2b Bracts and bracteoles partially scarious or herbaceous at anthesis. Limb of the fall has dark veins on a brighter background in the basal part of the upper surface. Limb of the standard moderately to finely undulate. 2n = (24) 44, 48 3

3a Flowers generally bright pinkish- to lavender-violet (similarly to *I. aphylla*), concolorous. Limb of the fall obovate; brighter basal background occupies no more than 20(25)% of the upper surface. Bracts and bracteoles herbaceous to slightly scarious at anthesis, inflated. 2n = 48 [*Fertile plants*]. . . . . ***I. croatica***

3a Flowers generally less-saturated violet (more nuances), but rarely completely bright pinkish- to lavender-violet, concolorous or bicolorous. Limb of the fall obovate to obovate-spathulate; brighter basal background occupies (20)25–40(50)% of the upper surface. Bracts and bracteoles partially scarious at anthesis, rarely herbaceous and inflated. 2n = (24) 44, 48 4

4a Unfertile to slightly fertile plants, up to 70(80) cm height. Flowers usually concolorous. Brighter basal background of the fall limb occupies (20)25–30% of the upper surface; whitish, rarely suffused with pale yellow. 2n = 44 . . . . . ***I.*** ×***germanica*** aggr.

4b Unfertile to fertile plants, up to 100(120) cm height. Flowers usually bicolorous, with brighter standards. Brighter basal background of the fall limb occupies (25)30–40(50)% of the upper surface; usually suffused with pale yellow. 2n = 24, 48 . . . . . ***I. ×trojana*** aggr.

Iris pallida is included as parental species in all of the mentioned groups. Different taxonomic treatments of its entities led to a more intricate understanding of descendant *I.* ×*germanica* complex s.l. Three species have been separated from *I. pallida*: *I. cengialti* Ambrosi ex A. Kern., *I. illyrica* Tomm. ex Vis. and *I. pseudopallida* Trinajstić. Some of them are currently recognised at the rank of subspecies (Barker & Govaerts 2018), although Colasante & Maury (2018) presumed that they might be doubtful.

MATERIAL AND METHODS

The collected plant material is stored in the Herbarium of the Natural History Museum in Belgrade (BEO) and the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade (BEOU). Reproductive abilities of hybrid and parental individuals were monitored in the habitat over a period of ten years. They are vegetatively propagated and also monitored in *ex situ* culture.

Horticultural classification follows Warburton & Gantz (1970). Characters with no alternatives in identification keys are given in italics in square brackets.

The total nuclear DNA amount was assessed by flow cytometry according to Bourge *et al.* (2018) using fresh leaves of samples and internal standards, *Triticum aestivum* L. "Chinese Spring" (2C = 30.9 pg) (Marie & Brown 1993).

Small parts of leaves (approx. 0.5 cm²) of both internal standard and target species were simultaneously chopped using a razor blade in a plastic Petri dish with 600 µl of Gif nuclei-isolation buffer (Bourge *et al.* 2018): [45 mM MgCl2, 30 mM sodium citrate, 60 mM MOPS (4-morpholine propane sulphonate, pH 7), and 1% (w/v) polyvinylpyrrolidone 10,000, pH 7.2] containing 0.1% (w/v) Triton X–100 and supplemented with 5 mM sodium metabisulphite and RNAse (2.5 U/ml). The nuclei suspension was filtered through a 30 µm nylon mesh. The nuclei were stained with 100 µg/ml propidium iodide (PI), a specific DNA intercalating fluorochrome dye, and kept 5 min at 4°C.

DNA content of about 3,000 stained nuclei was determined for each sample using a Cyflow SL3, Partec, 532-nm laser cytometer (Munster, Germany). The samples comprised from one to three individuals, measured

separately. To check the reproducibility of values, two distinct measurements were performed for each individual.

The total nuclear DNA content was calculated based on the following formula: 2C DNA sample (pg) = (Sample 2C peak mean/Standard 2C peak mean) × Standard 2C DNA(pg).

RESULTS AND DISCUSSION

Two adjacent populations of natural *I. reichenbachii* and cultivated *I.* ×*germanica* s.l. were recorded in the southern part of C Serbia, together with two clusters of their putative hybrid. The observed mixture of phenotypic parental traits was the first evidence of the hybrid origin of the new plant. This was also supported by the investigation of DNA content, ploidy level determination and detected infertility. According to horticultural classification, it is included in Intermediate Tall Bearded Irises (IB).

Iris* ×*seminaturalis Niketić, Tomović & Šiljak-Yak., **hybr. nov.** (Figs 3-5)

[= *I.* ×*germanica* L. s.l. (*I.* ×*trojana* group) × *I. reichenbachii* Heuff.]

TYPE

C Serbia, Prokuplje, Gubetin, MGRS 34T EN488, marble, 320 m s.m., clearings in *Quercetum frainetto-cerridis* forest, coll. Niketić, M., Tomović, G., 30-Apr-2008, (holotype: BEO 00-64; isotype: BEOU 17470) (Fig. 6).

DESCRIPTION

Rhizome stout, horizontal, 20–50 cm long, branched, with uniform diameter. *Stems* straight, solid, (30–)40–45 × 0.3–0.4(–0.8) cm, green. *Leaves* equitant, ± ensiform; basal 8–25(–40) × 1.5–2(–3.5) cm, quite smooth, green, slightly glaucescent; cauline leaves 2–3(4) in the lower part of the stem, much smaller, ensiform to slightly falcate. *Inflorescences* with (1)2–3 particoloured odourless flowers, lower shortly pedunculate or lowermost usually aborted; lowermost peduncles up to as long as subtending bracts. *Bracts and bracteoles* 3.5–4(5) cm long, slightly keeled, greenish at first and becoming white to slightly brownish, with a scarious margin (2–3 mm wide) in the upper part. *Hypanthial tube* 1.5–2.5 cm long. *Falls* 5–7.5 × 3–4 cm, reflexed, with obovate limb, taper gradually to the claw; lower surface of the limb pale bluish-green; upper surface of the limb pale lilac to bluish-gray in the upper part (55–70%), with dark violet veins, and yellowish in the lower part (30–45%), with dark purplish veins, also with a beard of intensely orange multicellular hairs along proximal 1/4 of the middle vein; beard continues on the claw. *Standards* erect, creamy white to pale yellowish to pale lilac, with obovate to broadly obovate limb, 5–7.5 × 3.5–4 cm, and 0.7-1 cm long claw, spotted with dark purplish. *Stamens*

with whitish filaments, 0.6–0.8 cm long, and yellowish anthers, 1-1.3 cm long. *Ovary* 1–1.5 × 0.5 cm, subcylindrical. *Style* ca. 3 cm long, with pale yellowish petaloid branches, suffused with blue, dividing at apex into two short triangular and irregularly laciniate lobes. *Fruit*s were not detected.

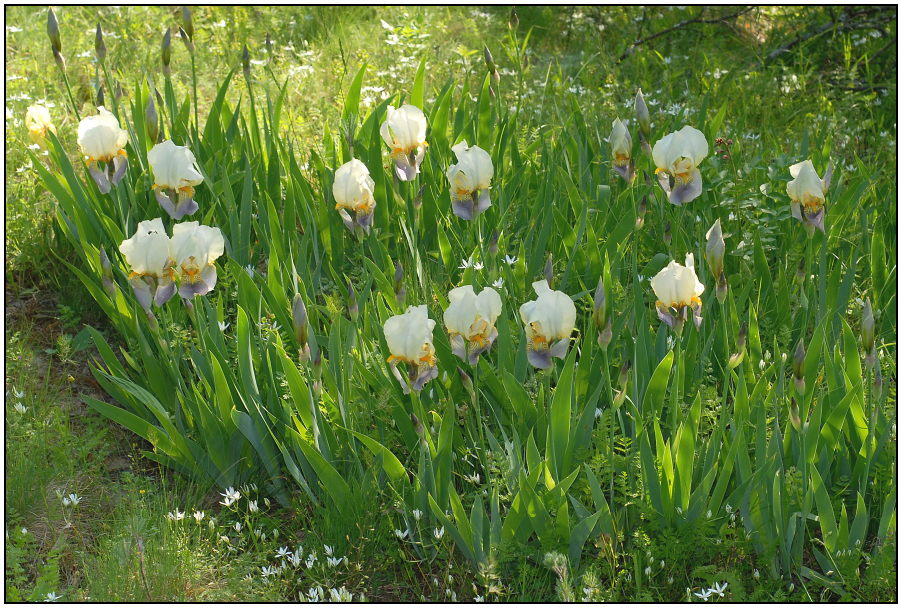

Fig. 3. – The new spontaneous hybrid *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. on natural habitat in C Serbia near Prokuplje (Gubetin) – habitus (photo M. Niketić).

DIAGNOSTIC CHARACTERS

The new iris is almost certainly an F1 hybrid between *I. reichenbachii* and *I.* ×*germanica* s.l. (*I.* ×*trojana* group) and has intermediate morphology between parental taxa. It is much taller than *I. reichenbachii*, with larger particoloured ± pale lilac to bluish-gray flowers (vs. yellowish) and slightly keeled, partially scarious bracts. In comparison with *I.* ×*trojana* it has lower growth, less scarious bracts and smaller and fewer flowers with pale lilac to bluish-gray falls (vs. more intense purplish-violet) which have a larger yellowish basal part on the limb.

Iris ×*seminaturalis* also resembles several other hybrid IB irises (cultivated or natural). It is very similar to the bicolorous cultivar *I.* ×*conglomerata* 'Progenitor', registered in 1951 (Sekerka *et al.* 2013), which also emerged in F1 generation by way of crossing *I. reichenbachii* with another cultivar, *I.* ×*conglomerata* 'Shining Waters'. Our hybrid differs by pale lilac to bluish-gray falls (vs. more intense purplish-violet) with distinctly larger yellowish basal part on the limb, also by ± uniformly coloured standard limb (without purplish-violet midvein).

Fig. 4. – The new spontaneous hybrid *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. on natural habitat in C Serbia near Prokuplje (Gubetin) – flowers (photo M. Niketić).

Recently described *I. orjenii* (Bräuchler & Cikovac 2007) has *I. pseudopallida* (from *I. pallida* group) and *I. reichenbachii* as putative parent species. *Iris* ×*seminaturalis* is recognisably different from *I. orjenii* and has narrower leaves, less scarious bracts, particoloured ± pale lilac to bluish-gray flowers (vs. concolorous, white to very pale yellow) and obovate limb of the fall (*vs.* obovate to spathulate).

In relation to *I. rotschildii* (*I. pallida* × *I. variegata*) it has obovate pale lilac to bluish-gray limb of the fall (vs. obovate-spathulate and purplish-violet) with darker veins only in the basal part of the upper surface (vs. on the entire upper surface, similar to *I. variegata*).

DNA CONTENT AND CHROMOSOME NUMBER

Total nuclear DNA assessed by flow cytometry confirmed the hybrid origin of individuals. They are described as hybrids according to their morphology, which is intermediary between *I.* ×*germanica* s.l. and *I. reichenbachii*. The parents are diploid (*I. reichenbachii*, 2n = 24, 2C = 12.76 pg) and tetraploid (*I.* ×*germanica* s.l., 2n = 48, 2C = 24.96 pg) while the hybrid has genome size of 2C = 19.38 pg corresponding to triploid plant (2n = 36).

Fig. 5. – The new spontaneous hybrid *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. on natural habitat in C Serbia near Prokuplje (Gubetin) – flower (photo M. Niketić).

Fig. 6. – *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. – holotype (BEO 00-64).

REPRODUCTION

Despite the population monitoring since 2008, fertile individuals have not been found at the investigated locality to date, either in the newly described hybrid or the progenitors. Accordingly, it can be concluded that *I.* ×*seminaturalis* is probably a sterile triploid of F1 generation. Therefore, introgression with the parental taxa probably does not occur. The lack of fruits in fertile *I. reichenbachii* could be explained by population decreasing in conditions of permanent reforestation and shading of habitat, since there are currently only a few individuals remain. Fruits are also not seen in parental hybrid *I.* ×*germanica* s.l., but the production of pollen grains has been noted. Consequently, it can be assumed that *I.* ×*germanica* s.l. is probably the male parent, while *I. reichenbachii* is the female. The new hybrid has been successfully propagated vegetatively in garden conditions.

DISTRIBUTION AND ABUNDANCE

So far, *I.* ×*seminaturalis* is known only from one locality in the surroundings of Prokuplje in C Serbia. Only two adjacent clusters were found within the population of *I. reichenbachii*.

ADDITIONAL SPECIMENS EXAMINED

C Serbia, Prokuplje, Gubetin, MGRS 34T EN488, marble, 320 m s.m., clearings in *Quercetum frainetto-cerridis* forest, coll. Niketić, M., Tomović, G., 01-May-2002 (BEO 82881; *15442* BEOU).

HABITAT AND ECOLOGY

The new hybrid inhabits the thermophilous variant of Balkan climatogenic deciduous forests of Hungarian and Turkey oak, *Quercetum frainetto-cerridis* Rudski (1940) 1949 subass. *pubescentosum* Jovan. The plant grows in the lowland-colline belt (320 m s.m.) on brown soil and on carbonate bedrock (marble). It appears in small patches of forest clearing, together with its parental species, *I. reichenbachii* (Fig. 7). In conditions of permanent reforestation, the plant grows in more sunny forest environments with remains of steppic fescue pastures, *Sanguisorbo-Festucetum valesiacae* Danon 1960 subass. *koelerietosum macranthae* Diklić 1962. The herbaceous-subshrub layer of the forest includes: *Cytisus hirsutus* L., *Genista tinctoria* subsp. *ovata* (Waldst. & Kit.) Arcang., *Festuca valesiaca* Schleich. ex Gaudin, *Sanguisorba minor* Scop., *Koeleria macrantha* (Ledeb.) Schult., *Ornithogalum kochii* Parl., *Fragaria vesca* L., *Poa bulbosa* L., *Medicago rigidula* (L.) All., *Veronica prostrata* L., *Veronica jacquinii* Baumg., *Lathyrus sphaericus* Retz., *Ajuga chamaepitys* (L.) Schreb., *Dictamnus albus* L., *Ranunculus psilostachys* Griseb., *Anchusa officinalis* L., *Euphorbia epithymoides* L., *Onobrychis alba* (Waldst. & Kit.) Desv., *Verbascum banaticum* Schrad., *Melampyrum arvense* L., *Cerastium brachypetalum* Desp. ex Pers., *Potentilla recta* L. subsp. *pilosa* (Willd.) Rchb. f. ex Rothm., *Potentilla astracanica* subsp. *pirotensis* (Borbás) Soják,

Teucrium chamaedrys L., *Geranium columbinum* L., *Draba muralis* L., *Orlaya grandiflora* (L.) Hoffm., *Muscari neglectum* Guss. ex Ten., *Lamium purpureum* L., *Stellaria media* (L.) Vill., *Scorzonera hispanica* L., *Neotinea tridentata* (Scop.) R. M. Bateman, *Orchis purpurea* Huds., Pridgeon & M. W. Chase, *Helianthemum salicifolium* (L.) Mill., etc.

Fig. 7. – Parental species *Iris reichenbachii* Heuff. (on the left) and the new hybrid *I.* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. (on the right) on natural habitat in C Serbia near Prokuplje (photo M. Niketić).

ORIGIN AND TAXONOMIC POSITION

In addition to *I. reichenbachii*, another parental plant belongs to *I.* ×*germanica* complex. Judging by the number of chromosomes (2n = 48), it may be considered to be a member of *I.* ×*trojanensis* group, but due to unresolved taxonomy, definitive identification has not been performed. This group includes some old clones that are traditionally cultivated in rural areas of Serbia and the Balkans. Observed plants, cultivated in a nearby village cemetery, have recently become pollen donors in crossing with wild individuals of *I. reichenbachii*. This type of spontaneous crossing between the decorative plants and indigenous species in their habitat is very rare – e.g., in *Larix* Mill. (Meirmans *et al.* 2014). So far, crossings between autochthonous and agricultural plants (or trees from plantations) have been recorded much more often (Dostálek 1984, Ammitzbøll & Jørgensen 2006, Zaharieva & Monneveux 2006, Schierenbeck & Ellstrand 2009, Paffetti *et al.* 2018).

Iris ×*seminaturalis* is most similar to the hybrid iris cultivar, *I.* ×*conglomerata* 'Progenitor'. However, in addition to *I. reichenbachii*,

some other species (*I. pallida*, *I. variegata*, *I. kashmiriana*) as well as 18 hybrids has been involved in artificial crossing, including four tetraploids from *I.* ×*trojanensis* group.

Hybridogenous *I. orjenii* (*I. pseudopallida* × *I. reichenbachii*) is somewhat related to *I.* ×*seminaturalis*, but this diploid plant (2n = 24) may have arisen as a result of homoploid hybrid speciation.

Another potentially related taxon could be *I.* ×*kobasensis*. According to Barker & Govaerts (2018) it is a natural hybrid between *I. reichenbachii* and *I. variegata*. However, it was erroneously described as an intermedial species between *I. lutescens* ["*I. virescens*"] and *I. reichenbachii* ["*I. bosniaca*"] from Bosnia and Herzegovina (Mt Vitorog) (Prodan 1935). Furthermore, according to the original description, the plant is not so different from *I. reichenbachii*, and no similarities with *I. variegata* were indicated. Consequently, the hybrid origin of this plant (actually most probably *I. reichenbachii*) has to be rejected.

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НОВИ СПОНТАНИ ХИБРИД ИЗМЕЂУ КУЛТИВИСАНЕ И ДИВЉЕ ВРСТЕ ПЕРУНИКЕ ИЗ СРБИЈЕ

МАРЈАН НИКЕТИЋ, ГОРДАНА ТОМОВИЋ, SONJA SILJAK-YAKOVLEV

Р Е З И М Е

Нови спонтани самоникли хибрид, *Iris* ×*seminaturalis* Niketić, Tomović & Šiljak-Yak. (*I.* ×*germanica* L. s.l. × *I. reichenbachii* Heuff.) из централне Србије, описан је и илустрован у овом раду. Компаративна анализа морфолошких карактера, величине генома и дедукција броја хромозома обављени су на узорцима *I.* ×*seminaturalis* и њених прогенитора. Такође је дат нови таксономски осврт на комплекс *I.* ×*germanica* s.l.