

Research Article

***Sisyrinchium rosulatum* (Iridaceae), a new alien species to the Bulgarian flora**Stoyan Stoyanov¹, Vladimir Vladimirov^{1,2,*}, Svetlana Bancheva^{2,1} and Georgi Gogushev³¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Akad. G. Bonchev Street, bl. 23, 1113 Sofia, Bulgaria²Botanical Garden, Bulgarian Academy of Sciences, Okolovrasten pat, 22, 1415 Sofia, Bulgaria³Regional Forest Directorate Blagoevgrad, 2 Vasil Koritarov St., 2700 Blagoevgrad, Bulgaria

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OPEN ACCESS**Abstract**

Sisyrinchium is one of the largest genera in Iridaceae, comprising more than 200 species and native to Hawaiian Islands, Temperate and Subtropical America to Falkland Islands. In Bulgaria, the genus was first reported in 1972 with the species *S. angustifolium*. In 2020, during field work near the Oshtava village, Kresna district (SW Bulgaria), a species of *Sisyrinchium* was discovered. The taxon was identified as *S. rosulatum*. The aim of the present article is to report *S. rosulatum* as a new species to the Bulgarian flora and to provide data about its main distinguishing morphological characters and its distribution and habitats in Bulgaria. The genome size of the species, obtained from the Bulgarian accessions, is $1C = 1.00\text{--}1.08$ pg which is congruent with earlier data from elsewhere. Based on field observations, it can be inferred that *S. rosulatum* is a naturalized, yet non-invasive alien species in the Bulgarian flora. A careful examination of the previously collected herbarium material of *Sisyrinchium* from Bulgaria revealed all specimens belong to *S. rosulatum*.

Key words: Bulgaria, genome size, naturalized alien species, neophyte, new record**Introduction**

Sisyrinchium L. contains 204 accepted species and its native range comprises Hawaiian Islands, Temperate and Subtropical America to Falkland Islands (POWO 2022). It is regarded as one of the largest and most taxonomically complicated genera in Iridaceae due to the common occurrence of polyploidy, often overlapping morphological variation and difficulty to find reliable distinguishing characters between the species (Tacuatiá et al. 2012, 2017; Shin et al. 2016; Burchardt et al. 2018). The genus is still understudied in Europe, mainly due to the difficult identification of the species and its limited and occasional occurrence. The number of species and their distribution have not been fully clarified yet. According to the Euro+Med PlantBase (World Checklist of Selected Plant Families 2010), the genus *Sisyrinchium* is represented in the European flora with four species – *S. angustifolium* Mill., *S. californicum* (Ker Gawl.) Dryand., *S. montanum* Greene, and *S. septentrionale* E.P. Bicknell. In POWO (2022),

four more species of this genus are included as introduced in Europe – *S. micranthum* Cav., *S. platense* I.M. Johnst., *S. rosulatum* E.P. Bicknell and *S. striatum* Sm. There is also a significant discrepancy between the two databases in terms of the distribution of *Sisyrinchium* taxa by countries.

Similarly, the data on the taxonomic identity of *Sisyrinchium* species in Bulgaria is unclear and dubious. So far, two reports of *Sisyrinchium* taxa, new to the Bulgarian flora, have been published. First, Kolev (1972) reported *S. angustifolium* from the region of village Eleshnitsa (currently Belasitsa), Petrich district. Later, Gogushev (1999), apparently overlooking Kolev's article, reported *Sisyrinchium* as a new genus for the Bulgarian flora from the area of Petrich town, especially from the vicinity of Kolarovo village, which is near the location of the Kolev's gathering. In fact, due to the complexity of the genus, Gogushev was unable to identify the species and noted that further studies are needed. Without any evidence of additional research, the name "*Sisyrinchium montanum*" appears in Dimitrov (2001) with a distribution in the Valley of River Struma (*Southern*) floristic region which comprises the lands of both Belasitsa and Kolarovo villages. Consequently, *S. montanum* was accepted by the later authors (Assyov and Petrova 2012; Raycheva et al. 2021; Dudáš 2022).

In this article, *Sisyrinchium rosulatum* is reported for the first time for the Bulgarian flora as a naturalized alien species. Additionally, the previous records and specimens of *Sisyrinchium* in Bulgaria have been studied and revised.

Materials and methods

Plant material was collected from two localities in the Valley of River Struma (*Southern*) floristic region of Bulgaria. Morphological features of *S. rosulatum* were obtained from the examined personal gatherings and compared with data from relevant literature (Bicknell 1899; Cholewa and Henderson 2002) and with specimens of *Sisyrinchium* kept in the Bulgarian herbaria SO, SOA and SOM (acronyms according to Thiers 2021+). The collected specimens were deposited in SOM. Data on the populations and habitats of the species are based on the authors' personal observations.

The genome size of *S. rosulatum* from Bulgarian accessions was measured by CyFlow SL Green flow cytometer (PARTEC, Germany), equipped with a green (532 nm) solid-state laser. Fresh leaves from plants collected from the field were used for the study. *Pisum sativum* "Kleine Rheinländerin" (1C = 4.38 pg, Greilhuber et al. 2007) was applied as an internal standard. The plant material was treated with the extraction and staining kit "CyStain® PI Absolute P" (SYSMEX) following the protocol prescribed by the manufacturer. The samples were then measured at a rate of 10–20 nuclei per second, with a total of 5 000 nuclei per run. Five replicates were done for each sample and only measurements with CV below 5% were taken into account.



Figure 1. *Sisyrinchium rosulatum*. A, inflorescences with flowers and young capsules; B, flower. Photographs by S. Stoyanov.

Results and discussion

In 2020, during field work near Oshtava village, Kresna district (W foothills of Pirin Mts), an unknown species of *Sisyrinchium* was discovered. By its slender annual habitus it clearly differed from the so far reported for the Bulgarian flora *S. angustifolium* and *S. montanum*, both being densely caespitose perennials. After consulting the relevant literature (Bicknell 1899; Cholewa and Henderson 2002), the taxon was identified as *S. rosulatum*, a new alien species to Bulgaria. Field survey in the area of Kolarovo village (Petrich district), from where Gogushev (1999) reported a record of the genus *Sisyrinchium* in Bulgaria, revealed that *S. rosulatum* is present in the same locality. Additionally, Kolev's specimen, gathered in the region of Belasitsa village (Petrich district) and reported as *S. angustifolium* (Kolev 1972), was carefully examined and identified as *S. rosulatum* too. Thus, recent field studies and revision of the previously collected herbarium material from the genus in Bulgaria showed that both *S. angustifolium* and *S. montanum* had been erroneously reported and, in fact, only *S. rosulatum* is present in the Bulgarian flora.

Taxonomy

***Sisyrinchium rosulatum* E.P. Bicknell, Bull. Torrey Bot. Club 26: 228.1899 (Figure 1)**

Herbaceous annual with thin tufts, or occasionally short-lived rosulate perennial, yellowish green when dry. Stems 10–15(–25) cm, compressed, usually 2–3 in a tuft, up to 10 in more vigorous individuals, rarely simple, glabrous, with 1–2 nodes. Leaf blades 15–50(–70) × 1–2.5 mm, linear-lanceolate, glabrous, straight, acuminate, sparsely scabrous on margins. Inflorescences terminal, with 2–5 pedunculated flowers, spathes 15–25(–30) mm long, conspicuously compressed, keeled, glabrous, usually entire, with thin, 0.2–0.3 mm wide, scarious margins. Perianth urceolate-campanulate basally, spreading in upper half, tepals 5–10 mm long, acute to aristate, spreading



Figure 2. Comparison of *S. rosulatum* (A) and *S. montanum* (B). Photographs by G. Gogushev (A) and S. Stoyanov (B).

part pale lavender, tinged purple at base, urceolate part yellowish to ochroleucous with purple patterns or strips, hairy outside, filaments connate basally, occasionally to 1/2 their length, ovary ovate, up to 1 mm long, green, patent-hairy. Capsules 2–3 mm, globose, tan with purplish sutures, pedicels spreading to arcuate. Seeds 0.5–1 mm, $\pm$ globose to slightly compressed, black, surface rugulose to finely alveolate.

In addition to its life form, *S. rosulatum* differs from *S. angustifolium* and *S. montanum* in a number of other characters. In *S. rosulatum*, perianth is urceolate-campanulate basally (vs perianth stellate-rotate, widely spreading from base in *S. angustifolium* and *S. montanum*), tepals are 5–10 mm, pale lavender in spreading part (vs tepals 7.5–12.5 mm, pale blue in *S. angustifolium* and 9–14.5 mm, dark bluish-violet in *S. montanum*) (Figure 2), filaments are connate to 1/2 of their length and capsules are 2–3 mm (vs filaments connate $\pm$ entirely and capsules 4–7 mm in *S. angustifolium* and *S. montanum*).

Sisyrinchium rosulatum is morphologically closest to the South American species *S. micranthum*. A detailed comparison between the two species is presented by Shin et al. (2016). Both species have a one-year life cycle, perianth urceolate-campanulate basally and filaments connate basally. *Sisyrinchium micranthum* is distinguished by its wider (3–6 mm) ensiform leaves, wider capsules (ca. 5 mm) and larger seeds.

Genome size

Sisyrinchium rosulatum belongs to a taxonomically intricate group of species in which polyploidy is a common phenomenon (e.g., Shin et al. 2016; Tacuatiá et al. 2017). Therefore, data about the ploidy level may be helpful for the correct identification of taxa. The determination of the herbarium material from the Bulgarian accessions was confirmed by measuring the genome size of the plants by flow cytometry. The obtained values are in the range $1C = 1.00\text{--}1.08$ pg, which is congruent with earlier data for *S. rosulatum* and corresponds to a tetraploid chromosome number ($1C = 0.98$ pg, $2n = 4x$, Tacuatiá et al. 2017).

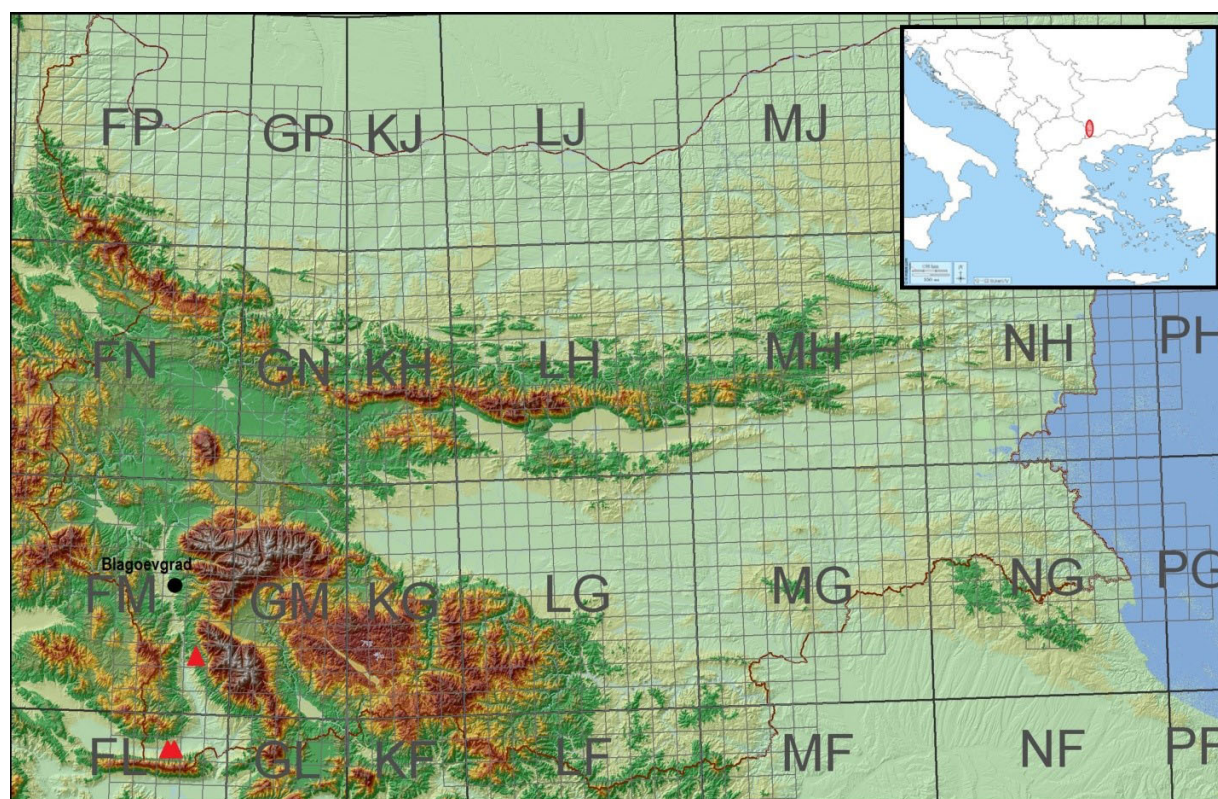


Figure 3. 10×10 km UTM distribution map of *Sisyrinchium rosulatum* in Bulgaria: red triangles represent the currently known localities of the species.

Distribution

In Bulgaria, the species has been recorded in three locations in the south-western part of the country so far, all situated in the Valley of River Struma (Southern) floristic region: north of Belasitsa and Kolarovo villages and south of Oshtava village (for the exact localities, see “Examined specimens” below) (Figure 3). It seems that Kolev’s gathering from 1970 (Kolev 1972), apart from being the first for Bulgaria, turned out to be the earliest record of *S. rosulatum* in Europe, although at that time the species was erroneously identified as *S. angustifolium*. Apparently, in the area of the villages of Belasitsa and Kolarovo it has been naturalized for more than 50 years.

Sisyrinchium rosulatum is native to North America – SE USA and NE Mexico (Cholewa and Henderson 2002; POWO 2022). However, it has been introduced and naturalized to other parts of the world, e.g., South Europe, South and South-East Asia, Madagascar, Caribbean Islands, and Nova Caledonia (POWO 2022). In Europe, the first report of *S. rosulatum* was from France (Parent 1977). Afterward, the species was reported for the Iberian Peninsula (Verloove and Sánchez Gullón 2012) and Italy (Nicoletta and Ardenghi 2013). It has been only recently reported for the first time for the Balkan Peninsula from Albania (Gjeta et al. 2020).

Examined specimens

Author-collected specimens: Bulgaria. Valley of River Struma (Southern): South of Oshtava village, Kresna district, in a wet meadow of the alliance

Cynosurion cristati, 690 m, 41.78142°N; 23.22613°E, 25 June 2020, S. Stoyanov, V. Vladimirov, S. Banchева (SOM 177433, 177434); *loc. ibid.*, in wet places along the outflow of a small artificial pond, 690 m, 41.78167°N; 23.22635°E, 3 July 2020, S. Stoyanov, V. Vladimirov, S. Banchева (SOM 177435); Valley of River Struma (Southern): North of Kolarovo village, Petrich district, in wet to waterlogged meadows, 270 m, 41.37689°N; 23.11246°E, 28 June 2020, G. Gogushev (SOM 177436). *Revised specimens*: Eleshnitsa village, Petrich district, Kodzha Orman [big forest] locality, wet meadow, 12 June 1970, I. Kolev (SOA, sub *S. angustifolium*).

Habitats and populations

In Bulgaria, *S. rosulatum* is commonly present in moist grasslands or at least temporarily wet sites. According to its species composition, the habitat near Oshtava village belongs to the class *Molinio-Arrhenatheretea* and to alliance *Cynosurion cristati* as evidenced by numerous diagnostic and constant species – *Cynosurus cristatus*, *Festuca pratensis*, *Holcus lanatus*, *Lolium perenne*, *Lotus corniculatus*, *Plantago lanceolata* and *Trifolium repens*. Other accompanying species have been recorded as well: *Bromus arvensis*, *Centaureum erythraea*, *C. pulchellum*, *Eleocharis palustris*, *Holoschoenus vulgaris*, *Juncus bufonius*, *Moenchia mantica*, *Orchis elegans*, *Trifolium campestre*. A small water reservoir is constructed in close proximity, and it is most likely that moistening of the meadow is provided underground by this reservoir, as well as by the surface runoff during its overflow.

A similar vegetation type is found in the locality near the villages of Belasitsa and Kolarovo where the meadows are more or less waterlogged, which is due to the abundant water reserves retained in the deluvial deposits at the northern foot of Belasitsa Mountain, as well as to the water supply from the numerous streams in the region. This high moisture storage provides the habitat with an almost year-round hygro- to mesophilic conditions. Co-occurring species recorded there were *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Equisetum palustre*, *Festuca pratensis*, *Holcus lanatus*, *Juncus conglomeratus*, *J. effusus*, *Phragmites australis*, *Trifolium hybridum*, *T. pratense*, *T. repens* etc. (Kolev 1972).

The population of *S. rosulatum* near Oshtava village occupies an area of 0.2 ha. In 2020, about 400 individuals were counted in the locality. The wet meadows near the villages of Belasitsa and Kolarovo are about 5 ha and hold a population of several thousands of individuals. Part of the latter locality falls within the Topilishte Protected Site declared for the protection of the rare fern species *Osmunda regalis*.

In its native range, the species grows on roadsides, stream banks, in old fields and other disturbed areas, and wet areas bordering woods (Cholewa and Henderson 2002).

Alien status

Sisyrinchium rosulatum is considered a naturalized alien species in the Bulgarian flora. It was already established in natural habitats in Bulgaria in 1970, which is more than 50 years ago (Kolev 1972). Apparently, it was introduced to the country several decades ago and since then it has maintained its populations without any human assistance. The species propagates by seeds and may form locally abundant populations. However, in the known Bulgarian populations, so far the species has not demonstrated any obvious negative impacts on the native biodiversity mainly due to its small size and annual life cycle. It seems the species is not very competitive since it usually prefers places with somewhat sparser vegetation. No convincing evidence for invasive behaviour has been reported from other European countries either (cf. Parent 1977; Verloove and Sánchez Gullón 2012; Nicoletta and Ardenghi 2013; Gjeta et al. 2020).

It can be inferred that *Sisyrinchium rosulatum* is an alien, yet non-invasive (with somewhat high spreading potential) species in the Bulgarian flora which should be further studied especially for its impact on native biodiversity.

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Authors’ contribution

All authors equally participated in the research conceptualization, field work and data collection, writing and editing of the manuscript, VV – flow cytometry measurement.

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