

Overview of the floristic and taxonomic studies on Iridaceae Juss. in Bulgaria

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Abstract: According to the current data, the family Iridaceae is represented in Bulgaria by 30 species, grouped into 5 genera. The territory of Bulgaria, as part of the Balkan Peninsula, is characterized as a part of a secondary speciation center for the largest genera in the *Iris* and *Crocus*. In addition to widespread species, there are local and regional endemics that are of great conservation importance. Despite this fact, it is little known about the taxonomy and phylogeny of Iridaceae in Bulgaria. The main purpose of this publication is to present the degree of study of the family in Bulgarian literature, compared to the investigations in the Balkan Peninsula and the literature in the world. Inventory of the collections in the national herbaria SO, SOA and SOM have done. Most of the materials have been collected in the years of the active field studies on the Bulgarian flora – at the beginning of the 20th century until the taxonomical work on the family in vol. II of Flora Reipublicae Popularis Bulgaricae in the middle 60s. The lack of taxonomical investigations in Bulgarian flora justifies the need to reconsider the taxonomical structure of the Iridaceae family. Preliminary studies on species chorology have shown that the Bulgarian floristic literature's data are outdated and do not coincide with recent taxonomic changes in polymorphic paraphyletic and polyphyletic groups. On other hand, the decisions in the phylogenetic relationships between the polyphyletic taxa could be realized

using molecular techniques, which have not yet been implemented for the species in Bulgaria. The taxonomic complexity of the group and still scarce knowledge demands further studies in Bulgarian flora, including investigating phylogenetic relationships.

Keywords: Iridaceae, Bulgaria, *Iris*, *Crocus*, *Gladiolus*, *Romulea*, *Sisyrinchium*.

Introduction

Iridaceae is a well-defined family of about 2000 species and between 60 and 70 genera (Goldblatt et al. 2008). Despite the dynamic taxonomic treatments of the world's vascular flora in recent years (Angiosperm Phylogeny Group 1998, 2003, 2009, 2016), the Iridaceae family retains relative stability in terms of the volume in its taxonomic structure. According to the classical concepts of the volume of the family, the main genera distributed in the flora of Bulgaria are *Iris* L., *Crocus* L., *Gladiolus* L. and *Romulea* Maratti. So far, there is no consensus on the number of species in different genera and the taxonomic structure of polymorphic groups. Information about the family Iridaceae in Bulgaria can be found in floristic publications, conspects, and determination keys (Stojanov & Stefanov 1924, 1933, 1948; Stojanov et al. 1966; Delipavlov 2003; Assyov & Petrova 2012). Over the past few decades, the information for the species in our flora has been reduced to sporadic reports of new chorological data for the known taxa in the country (Georgieva 2000; Vladimirov 2007; Petrova et al. 2016, 2019). The main reason for the increased interest in the family is the lack of current studies on the morphology, taxonomy, and phylogeny of this group of higher flowering plants. Due to its close relations with the Mediterranean and Pre-Asian flora, our country appears to be a secondary active center of the Iridaceae family (Goldblatt 2000). Within the evidence for this, there is an elevated number of endemics (including a large number of local endemics) and a significant number of species with conservation status (Tan et al. 2007). Depending on the degree of threat, the species are classified under different natural conservation categories, conservation regimes, and sustainable use in regulatory and resource laws (Tab. 1).

In the taxonomic literature, the differentiation of species from the *Crocus* and *Iris* polymorphic groups is based on a small number of overlapping features. That is why the morphological traits are not sufficiently convincing to distinguish species. That is one of the reasons for the widespread use of molecular phylogenetic studies over the last 15 years, leading to taxonomic rearrangement of polyphyletic groups to elucidate the phylogenetic relationships between taxa (Petersen et al. 2008; Harpke et al. 2013; Mavrodiev et al. 2014).

Tab. 1 Conservation significance of species of the Iridaceae family in Bulgaria

| Taxon | Endemic | Red Data Book ¹ | BG Red List ² | IUCN Red List ³ | Biological Diversity Law Appendix No. | Medicinal Plants Law Appendix No. |
|--|---------|----------------------------|--------------------------|----------------------------|---------------------------------------|-----------------------------------|
| <i>Crocus tommasinianus</i> Herb. | Balc. | VU | VU | | 3 | |
| <i>Crocus chrysanthus</i> Herb. | | | | | 4 | 1 |
| <i>Crocus olivieri</i> J. Gay | | | | | 3 | |
| <i>Crocus pallasii</i> Goldb. | | | | | 4 | 1 |
| <i>Crocus veluchensis</i> Herb. | Balc. | | | | | |
| <i>Iris aphylla</i> L. | | CR | CR | | | |
| <i>Iris reichenbachii</i> Heuff. | Balc. | | | | | |
| <i>Iris pseudacorus</i> L. | | | | LC | | 1 |
| <i>Iris pumila</i> L. | | | | | | 1 |
| <i>Iris graminea</i> L. | | | | | | 1 |
| <i>Gladiolus imbricatus</i> L. | | | | | | 1 |
| <i>Gladiolus italicus</i> Mill. | | | | LC | | |
| <i>Gladiolus communis</i> L. | | | | | | 1 |
| <i>Gladiolus palustris</i> L. | | | | DD | 2, 3 | |
| <i>Romulea bulbocodium</i> (L.) Sebast. & Mauri | | | VU | | 3 | |
| <i>Romulea linaresii</i> ssp. <i>graeca</i> Bég. | | | VU | | 3 | |
| <i>Crocus</i> sp. div.* | | | | | 4 | |
| <i>Gladiolus</i> sp. div.* | | | | | 4 | |

¹ Vladimirov (2011), Ivanova (2011); ² Bancheva (2009), Ivanova (2009), Meshinev (2009), Petrova (2009); ³ Bilz (2011), Contu (2013), Kavak (2014). * - the whole genus

The high morphological similarity between closely related taxa in the Iridaceae is probably due to recent divergence and the subsequent radiation-adaptive process that results in species complexes (Goldblatt 1990; Chauveau et al. 2011). Hybridization and introgression, recently recorded in *Gladiolus* (Szczepaniak et al. 2016) and accompanied by polyploidy and chromosomal rearrangements in the genus *Crocus*, play an important role in the evolutionary history of the Iridaceae (Harpke et al. 2013). Such data, including hybrid imaging, also occur for genus *Iris* (Arnold et al. 1990).

Based on these facts, the taxonomy and phylogeny of Iridaceae species in Bulgaria should be supplemented and revised. This publication's main purpose is to review the known information about the family in Bulgaria until now.

Preliminary studies of Bulgarian species in Iridaceae

The study is based on the update made on the chorology and taxonomical structure for the species from the Iridaceae family in Bulgaria. Analysis of the existing data in the literature on this problem has been made. The herbarium collections in the national herbaria SO, SOA and SOM have been inventoried (Fig. 1).

Data analysis shows the presence of the following problems and white spots in the studies of the Iridaceae genera in Bulgaria:

The chorological information in the older deposited herbarium sheets is incomplete. Some of the specimens are irreversibly damaged, and some are inappropriate for determination and revision. For some critical taxa, the deposited specimens are single or simply lack (e.g., *Crocus pallidus*, *C. variegatus*, *Gladiolus palustris*, *Iris aphylla*, *Romulea* spp.). The number of species in the Bulgarian flora is unclear. The determination keys are outdated and unreliable. No taxonomical revisions have been undertaken. As a result, the status and priority names of the taxa are incorrect. Most of the specimens have been collected in the period of the active botanical field trips, starting from the 20s and ending to the 60s of the last century. The results of this research have been reflected in *Flora Reipublicae Popularis Bulgaricae* vol. II (Velchev & Radenkova 1964; Kovachev 1964; Radenkova 1964; Velchev 1964, 1964b). After this period, there has been a decline in interest, and only single materials have been found.

Status of the research on Iridaceae in the Bulgarian flora

The area of distribution of the family Iridaceae covers all continents and brings together species with different phytogeographical affiliations. The family has been treated taxonomically in Volume II of the multi-volume edition of *Flora Reipublicae Popularis Bulgaricae* (Velchev & Radenkova 1964), where the results of the active research in the group have been critically evaluated. A half of a century after, this information, compared to the recent knowledge in the family, is already obsolete and does not provide a comprehensive answer to the questions regarding biodiversity, taxonomic structure, and range of morphological and genetic intraspecific variation in the large groups of Iridaceae.

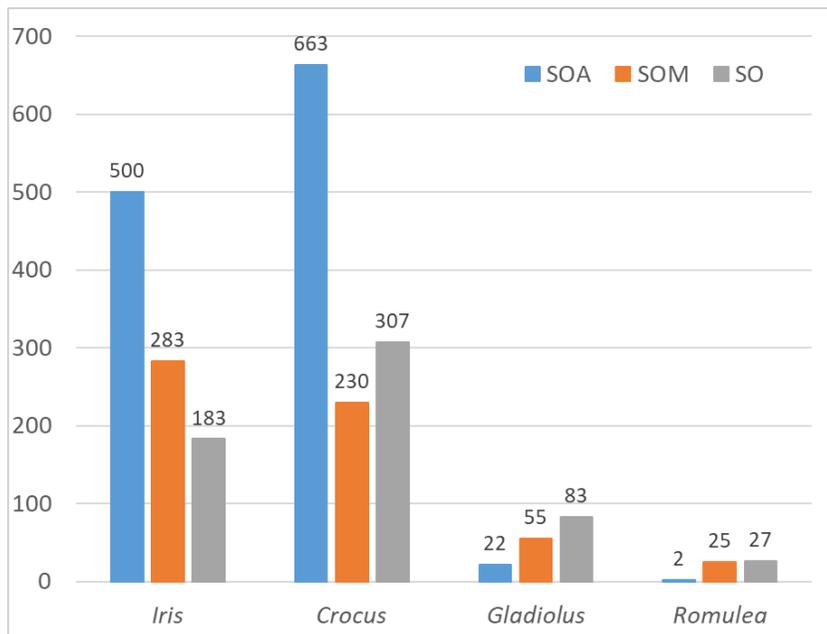


Fig. 1 Number of Iridaceae specimens deposited in the national herbaria SO, SOA, and SOM.

Genus *Iris* L. is represented in the global flora with 328 naturally occurring herbaceous, rhizomatous and bulbous, perennial species (WCVP 2020). The known representatives of this genus on the Balkan Peninsula are 19 (Euro+Med 2021). According to various authors, 9-10 wild and two cultivated species are distributed in the Bulgarian flora (Stojanov & Stefanov 1924, 1933, 1948; Stojanov et al. 1966; Delipavlov 2003; Assyov & Petrova 2012), representing the subgenera *Limniris* (Tausch) Spach and *Iris*. Unlike the members of subgenus *Iris*, the *Limniris* representatives have smooth beardless outer tepals (Rodionenko 1961; Mathew 1982). After the taxonomic work of the genus in Volume II of *Flora Reipublicae Popularis Bulgaricae* (Radenkova 1964), there are no further floristic taxonomic updates of the genus in the country.

The present taxonomy of the genus in the Bulgarian flora, e.g., in Delipavlov & Cheshmedzhiev (2003), is still based on classic taxonomical and morphological studies. The karyotypes studies of Bulgarian *Iris* accessions have been conducted (Popova & Cheshmedzhiev 1976). The morphological diagnostic features of *Limniris* have been analyzed (Raycheva & Stoyanov 2012). The morphologically similar species *I. sintenisii* and *I. graminea* (Fig. 2 A, B) have different anatomical characters and occur in different environmental conditions (Raycheva & Stoyanov 2013). Unfortunately, complete molecular phylogenetic studies have not been conducted in Bulgaria so far.

The taxonomy of the typical subgenus *Iris*, which comprises species with overlapping characters of the species used in keys, as a result of uncared delimitation morphological descriptions and hybrid relationships, remains controversial. *Iris aphylla* L. has been included in the Red Data Book of the Republic of Bulgaria as "critically endangered" (Ivanova 2011). The distribution of *I. aphylla* L. in Bulgaria remains unproven and needs to be confirmed. The overlapping morphological features without significant differences have accumulated conflicting chorological data for the other members in the subgenus, as well *I. suaveolens* Boiss. & Reut. (syn.: *I. mellita* Janka), *I. pumila* L. and *I. variegata* L.. *Iris ×germanica* L. (syn. *I. florentina* L.) has been considered as complex group taxa, with similar morphology (Mathew 1981, 1984a, 1991), and the problem in this group is probably similar in the flora of Bulgaria. The introgression in genus *Iris*, subgenus *Iris* causes a specific morphological variability. As a result, the clear determination of the species becomes difficult. Several taxa in Serbia are distinguished in the complex of *Iris ×germanica*, s.l. based on the karyotype and the chromosome number ($x=11$ and $x=12$) - *I. ×sambucina*, *I. ×trojana*, *I. croatica*, and *I. ×germanica*. The known hybrids of *I. reichenbachii* in Serbia until now are *I. ×kobasensis* Prodan and *I. ×seminaturalis* Niketić, Tomović & Šiljak-Yak. (Niketić et al. 2018).

The natural hybrids in the genus in Bulgaria have not been studied yet. During the last decades, the taxonomy of the genus *Iris* has changed. The molecular approaches have proved that *Iris* s.l. contains 23 separate genera (Mavrodiev et al. 2014).

Genus *Crocus* L. includes between 100 (Petersen et al. 2008; Harpke et al. 2013) and 235 taxa (species and subspecies) throughout its distribution range (Rukšāns

2017). According to the concept of the authors, the total number of species varies in very wide ranges. This could be traced to the successive studies in the genus during the last several decades (Mathew 1982, 1983, 1988, 2000a, 2000b; Rukšāns 2010, 2013). The genus is restricted to the Old World and extends longitudinally from the Iberian Peninsula and Morocco to Western China, and Poland south to the Caucasus region and the northern parts of the Arabian Peninsula (Mathew 1982, 1984b; Rukšāns 2010). The species of this genus exhibit intraspecific polymorphism in terms of morphology and chromosomal numbers, especially in the eastern parts of the Balkan Peninsula and Anatolia (Karamplianis et al. 2013). According to Feinbrun (1958), these latter areas outlined the center of origin of the whole genus. These species share habitats with the genus *Romulea* (Goldblatt 1971; Mathew and Brighton 1975). According to recent phylogenetic studies (Petersen et al. 2008; Seberg & Petersen 2009), the place of origin of *Crocus* is probably North Africa and the Iberian Peninsula, although only a small number of taxa are residents in these regions. According to the latest data, ten *Crocus* species are known in the Bulgarian flora (Assyov & Petrova 2012). Nine species in volume II of *Flora Reipublicae Popularis Bulgaricae* are included (Velchev 1964), and the taxonomy has not been changed until now. So far, at the micromorphological level, data on pollen morphology of already known species have been published in the Bulgarian literature, with no changes in the taxonomic structure of the genus (Uzundzhaliyeva & Popova 2012). Studies on variability within critical, polymorphic species (e.g., *C. biflorus*, *C. chrysanthus*, and *C. reticulatus*), including cytogenetic studies, are lacking. Evidence of the outdated taxonomic structure of the crocuses is the lack of revisions in the herbaria. As a result, deposited specimens of the same species are with different names, mainly taxonomic synonymous. At the same time, the genus has been a subject of intense research on a global scale, including the Balkan Peninsula, where several new species have been described over the last decade. Some of them have been supposed to be distributed in our country as well (Rukšāns 2013) – e.g., *C. speciosus* subsp. *ibrahimii* Rukšāns, *C. vaclavii* Rukšāns, *C. thracicus* Yüzb. & Aslan (Yüzbaşıoğlu et al. 2015) from northwestern Turkey. This is undoubtedly another fact that supports the need to study Bulgarian species of the genus. *Crocus chrysanthus*, which is widespread in the Balkan Peninsula to Romania, has high variability that shows no correlation with the occupied habitats, and the chromosome numbers within its range (Mathew 1999). This creates taxonomic problems that lead to different taxonomic treatments in the world's flora. As a result of studies within the Turkish populations of *C. chrysanthus* (Candan & Özhatay 2013), four subspecies have been identified and three new varieties have been described. Studies on the populations of *C. biflorus* subsp. *stridii* (Papan. & Zacharof) B. Mathew, place the taxon in the category "rare" and have been included in the Red Book of Greece (Mathew 1995). In recent decades, active botanical research in selected areas of the country has led to single reports of new localities and areas for *Crocus* species (Vladimirov 2007; Petrova et al. 2016). This fact is also an indication of reduced scientific interest and a lack of knowledge about species in Bulgaria. As a result of

revisions at the herbarium collections and field studies, new floristic records for the distribution of *C. pallasii* Goldb. have been added – Rhodope Mts. (Raycheva & Stoyanov 2018) and *C. pulchellus* Herb. - Sredna Gora (Stoyanov & Raycheva 2019). In contrast to studies on the chorology of the Bulgarian species, the surveys have been more extensive in the neighbouring Balkan territories. There is a large number of new data for the investigation of the range of species in Greece, Albania, Turkey, and Romania, including descriptions of new taxa (Randelović et al. 1990; Randelović et al. 2007; Tan et al. 2007; Rakaj 2009; Kerndorff & Pasche 1994, 2004; Kerndorff et al. 2013a, b; Rukšāns 2014; Miljković et al. 2016). Turkey is an especially rich territory of *Crocus* species. Because of more intensive investigation in the last decade, the number of new taxa there is about 50 new species (Erol et al. 2015). According to Yüzbaşıoğlu & Özhatay (2014), the number of new Turkish species is 73. Despite a large number of newly described species, for many of them, the membership of sections and series has not been clarified, since the performed phylogenetic analyses do not give a uniquely convincing answer to the related taxon relationships. The conventional morphological approach is not reliable enough to objectively distinguish taxa of polymorphic groups. Contributions to the elucidation of the evolutionary history in this group are the studies of ITS sequences from the inner and outer spacer sequences of conservative regions of the plastid DNA regions, pointed by Harpke et al. (2014, 2015) as useful and working phylogenetic markers in the genus. The phylogenetic analysis leads to the grouping of taxa with similar morphological characteristics, which hinders species differentiation. However, the description of new species as a process continues on a phytogeographical principle, e.g. *C. orphei* Karamplianis & Constantin., *C. danubensis* Kernd., Pasche, Randjel. & V. Randjel. A study resulting from the combination of molecular, morphological, and karyological data in *Crocus* section *Nudiscapus* sensu Mathew (1982), series *Reticulati* has offered a new, well-grounded concept for taxon rank (Harpke et al. 2014). The revision has shown that *C. reticulatus* Steven ex Adams is not a species with a wide range and heterogeneity, but is an aggregate of closely related species. To comply with the principle of monophyly, this polyphyletic group has been divided into several species. This calls for the revision of the Bulgarian taxa, perceived as *C. reticulatus*, according to the concept of Mathew (1982), which includes as synonyms in this taxon *C. variegatus* Hoppe & Hornsch. and *C. micranthus* Boiss.

Recent taxonomic changes in series *Reticulati* cast doubt about the presence of *C. reticulatus* s. str. in Bulgaria. Currently, *C. reticulatus* s. str. refers to Caucasus populations from which the species has been described, and the North and East Black Sea regions - Eastern Europe, Moldova, Georgia, and Russia. *Crocus danubensis* is distributed alongside the Danube River, the territory of Serbia, Bulgaria, and Romania. The distribution area of *C. variegatus* is the Pannonian Plain - from Hungary, Slovenia, Serbia, Bulgaria, reaching eastern Italy. The species status of the taxon has been restored recently as a result of DNA analysis (Harpke et al. 2014; Stoyanov et al. 2020).

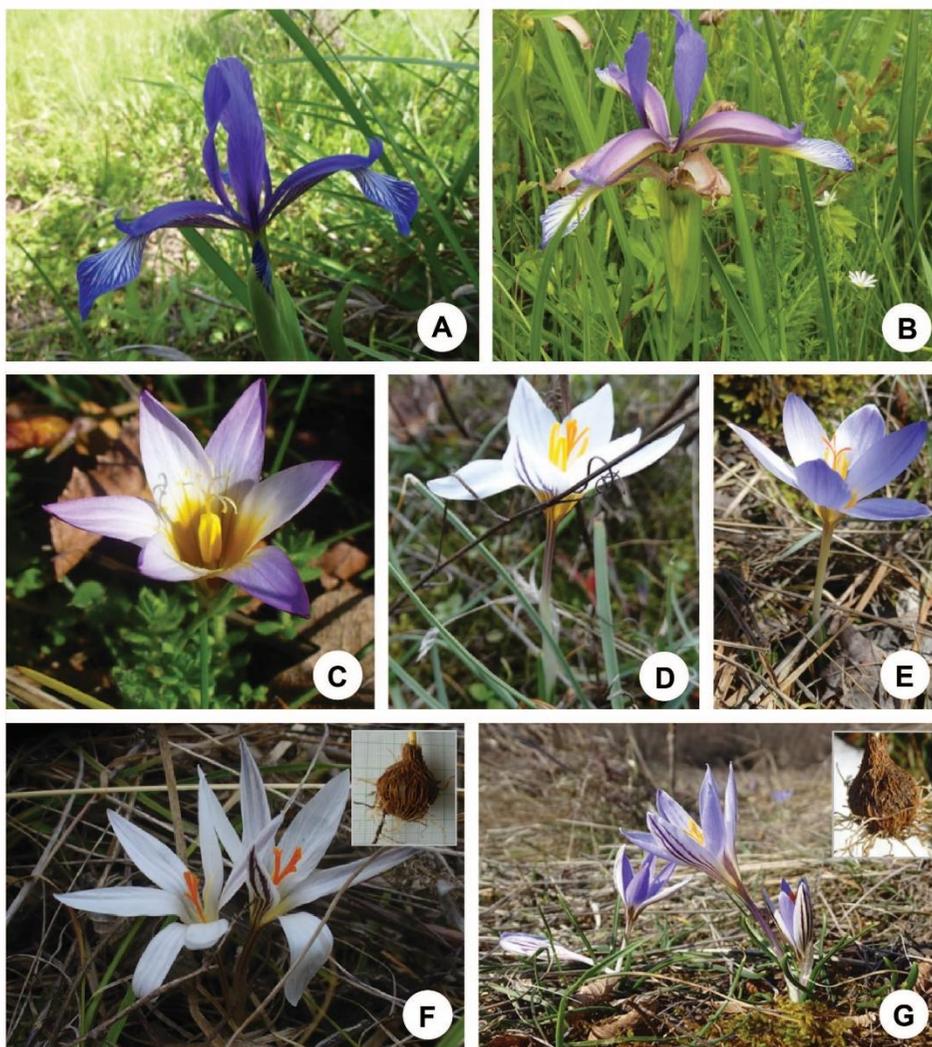


Fig. 2 Species cited in this publication: A - *Iris sinensisii*; B - *I. graminea*; C - *Romulea bulbocodium*; D - *Crocus adamioides*; E – *C. randjeloviciorum*; F - *C. danubensis*; G - *C. variegatus*. Photos: the authors (A-F), J. Barzov (G).

In the referent Bulgarian floristic sources, the taxa with reticulate corm tunica have regarded also as *C. reticulatus*. According to morphological and geographical data, as well as to preliminary field studies of the authors' team, *Crocus danubensis* (Fig. 2F) has localized in a previously known locality from the area of Besarbovo village (Ljubisavljević & Raca 2020), as well as in the area of Mechka village; *C. variegatus* (Fig. 2 G) – near Lakatnik (Stoyanov et al. 2020). It is necessary to analyze Bulgarian populations of both species to reveal definitive features with discrete character at an anatomical, morphological and molecular level. This could lead to taxonomical

decisions about the collected plants deposited as *C. reticulatus* in the national herbaria.

Samples collected in January 2018 in the Thracian Lowland have identified originally as *Crocus* cf. *adamii*. Morphological descriptions in the literature (Mathew 1982; Kerndorff et al. 2012; Rukšāns 2017) and repeated observations have changed the status of materials such as *Crocus adamioides* Kernd. & Pasche (Trifonov et al. 2019; Raycheva et al. 2021), which is a new taxon to the flora of Bulgaria – a part of the volume of the accepted *C. biflorus* complex until now (Fig. 2D).

Until now, data on the distribution of *Crocus adamii* Gay in the Balkan Peninsula have in question. The species has been reported for Serbia in the regions of Timok, Niš, and South Morava (Randjelović & Randjelović 1990). As a result of molecular-morphological studies, Harpke et al. (2017) has described the new well-differentiated species *C. randjeloviciorum* Kernd., Pasche, Harpke & Raca from the region of Eastern Serbia. This species shows closer phylogenetic relationships with *C. alexandrii* and *C. chrysanthus*, but not with *C. adamii*, which distribution area covers the territory from Anatolian Diagonal to Iran and Caucasian mountains. During a field trip, we found *C. randjeloviciorum* in the Western part of Bulgaria (Stoyanov et al. 2020). Recently, the species is located in the region of Bouchin Narrow, near Petrohan (Fig. 2 E). The species is likely much more widespread in Bulgaria. Additional studies in this polymorphic series *Biflori* Matthew would display what is the real distribution of the species in Bulgaria. Polyphyletic taxa still exist in the genus, since phylogenetic analyzes have been applied in a small number of polymorphic groups.

Genus *Gladiolus* L. has a center of origin in Africa and includes 311 species (WCVP 2020). The European *Gladiolus* representatives, although not a large group, show high similarity in the morphological features, which makes it extremely difficult to identify them. This is the reason why they have been listed as a problem taxonomic group in all European editions (Mifsud & Hamilton 2013). Genus *Gladiolus* includes five species naturally represented in Bulgaria (Assyov & Petrova, 2012). *Gladiolus palustris* Gaudin has conservational importance as a European endemic, included in Annex IIb of the Council Directive 92/43 EEC. The species is classified as endangered in the Bulgarian legislation as well as in many European Red lists. In the IUCN database, the species is in the DD category (Data Deficient) (Bilz, 2011). The species range starts from the North Alps, extends to France, Alsace, Germany, Czech Republic, Slovakia, Poland to the East with fragmented spots in Belarus, Ukraine, and Russia. To the south, it covers the Apennines in Italy to Eastern Austria and Hungary, where it extends to Western Bulgaria and Albania in the Balkans (Käsermann & Moser 1999; Euro + Med Plantbase 2006-2018). According to the latest summarized data, the distribution of *G. palustris* has been confirmed in Bulgaria for two floristic regions, and the sites are part of the NATURA 2000 network - Pirin Mt. and Rhodope Mts. (Petrova et. al. 2019). There is no confirmation for the other two regions (Rila Mt. and Slavyanka Mt.) since 1900 because of the difficulty in identifying the species (Bilz 2011). Recent studies have suggested hybridization and introgression of

sympatric populations of *G. palustris* and *G. imbricatus* in the Western Balkans (Szczepaniak et al. 2016).

Genus *Romulea* Maratti has African-Eurasian distribution. Initially, based on a species found in the vicinity of Rome, the genus has been assigned to the *Crocus* L. Maratti (1772) and later to the genus *Ixia* L. (Frignani and Iriti 2011). It has been accepted that genus *Romulea* has two areas of distribution and centers of differentiation. The first one is located in Sub-Saharan Africa, Socotra, and the Arabian Peninsula, where more than 70 species of *Romulea* have been found. The second one, comprising about 20 species, covers the Southern Mediterranean and Atlantic European provinces in the Holarctic Kingdom, according to a phytogeographic analysis (Takhtajan 1986; Marais 1980). In Volume II of *Flora Reipublicae Popularis Bulgaricae*, *R. bulbocodium* (L.) Seb. & Mauri (Fig. 2C) has indicated as one of the species native to Bulgaria. *Romulea linaresii* Parl has been reported as new to the Strandja region (Georgieva 2000). Subsequently, the species also has been indicated for the regions of the Southern Black Sea coast, the Southern Struma valley, and the Eastern Rhodopes (Assyov & Petrova 2012). There are a few specimens, deposited in the herbaria (Fig. 1). That determines the need for additional investigations on the genus *Romulea* in Bulgaria.

Genus *Sisyrinchium* L., with origin from South, Central, and North America, includes about 140 species, with two main centers - Mexico and South America. Species occur in diverse habitats - from natural (meadows, rocky sites), semi-natural (meadows, grasslands) to ruderal ones (Goldblatt et al. 2008; Chauveau et al. 2011). Data for about 11 species of the genus for Europe have been given by Parent (1980) as a result of the cultivation of some of them as ornamental species. *Sisyrinchium montanum* Greene has been referred to Balkan flora as a North American adventive species, localized in Romania in 1943 (Popescu & Boruz 2008), later on in Serbia (Randelović et al. 2010) and Bosnia and Herzegovina (Milanović et al. 2018). Data on the adventitious distribution of this species are also known in other countries of Europe (Ingram 1980; Kaplan et al. 2016; Dudáš et al. 2019). The species has been reported as new for the flora of Bulgaria for the first time by Kolev (1972) under the name *S. angustifolium* Mill. (syn.: *S. bermudiana* L., *S. graminoides* E.P. Bicknell), and it has been neglected for a long time. Later on, *S. montanum* (native in America) has been indicated for the floristic region of Belasitsa (Assyov & Petrova 2012). Herbarium sheets of this species have not been deposited in SO, SOA or SOM. As the species is adventive to the flora of Europe, in particular the Balkans, the information on its participation as a floral element is controversial. It has been cited as critically endangered for Serbia (Randelović et al. 2010). The CABI database indicates it as invasive for Europe, but, according to other authors, there is no evidence of invasive behavior (Patterson 1989).

Along with the species known in the Bulgarian flora, alien taxa have also been introduced (*Dierama pulcherrimum* (Hook.f.) Baker, *Iris* × *norrisii* (L. W. Lenz) C. Whitehouse, *I. pallida* Lam., *I. reticulata* M. Bieb., etc.), that are likely to exhibit the behaviour of adventive species. This assumption is provoked by the fact that the

movement of taxa of the Iridaceae family, atypical of the European and in particular of the Balkan flora, is consolidating and spreading at an intense rate. An example is *Freesia leichtlinii* subsp. *alba* (G.L.Mey.) J. C. Manning & Goldblatt - a subspecies with a natural range from North Africa, extending its habitats to the territory of Greece (Biel & Tan 2015).

All the data summarized above show that the Iridaceae family has studied poorly in Bulgaria. The data on the chorology and conservation value of the species with limited distribution, as well as the Balkan endemics, need to be updated.

The differentiation of species from the family is difficult because of the small number, often highly varying and overlapping diagnostic features, which leads to different taxonomic treatments. Because of this fact, the application of modern population-genetic, morphological and molecular-taxonomic methods would make it possible to clarify the case of the taxonomy.

The selectivity of molecular ISSR markers in Bulgarian populations has been demonstrated by a study of 7 species from Iridaceae – *Crocus flavus*, *Gladiolus italicus*, *Iris reichenbachii*, *I. germanica*, *I. pumila*, *I. sintenisii*. These preliminary studies have indicated that the seven ISSR primers used are selective for clear segregation of genera and differentiation of the *Iridoideae* and *Crocoideae* subfamilies (Raycheva et al. 2011). This approach would be useful in the rest of the critical taxa in Iridaceae.

A checklist of the species that recently occurred in Bulgaria (Appendix 1) shows the proven species of the family are 30, as follow: genus *Crocus* (12), *Gladiolus* (5), *Iris* (9), *Romulea* (2), and *Sisyrinchium* (1).

Conclusion

The complex morphological pattern and the active hybridization in the polymorphic genera *Iris*, *Gladiolus*, and *Crocus* are the reason for some difficulties in the determination of the species using the conventional morphological method. This is an obstacle to create working determination keys and a taxonomic structure based on kinship relationships. The sporadic studies conducted so far on the chorology and karyology of individual single populations of the species of the Iridaceae family in Bulgaria do not give a complete concept of the number of species, their chorology, as well as the range of morphological and genetic intraspecific variability. The herbarium collections are incomplete, and often with severely damaged specimens. The phylogenetic studies demonstrate the need for a review of the taxonomic structure of genera and the concept of species volume and boundaries in polymorphic aggregate in the genera *Iris*, *Crocus*, and *Gladiolus*. The dynamic taxonomic changes worldwide emphasize the need for updating and evaluation of the species composition and taxonomic structure of this group of flowering plants in Bulgaria.

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Appendix 1.

Checklist of the species occurred in Bulgaria. Synonyms are given in brackets. The distribution in the country given by numbers: 1 - Black Sea Coast, 2 - Northeast Bulgaria, 3 - Danube Plain, 4 - Forebalkan, 5 - Balkan Range, 6 - Sofia region, 7 - Znepole region, 8 - Vitosha region, 9 - West Frontier Mts., 10 - Strouma Valley, 11 - Belasitsa, 12 - Slavyanka, 13 - Mesta Valley, 14 - Pirin Mts., 15 - Rila Mt., 16 - Sredna Gora, 17 - Rhodopi Mts., 18 - Thracian Lowland, 19 - Toundja Hilly Plain, 20 - Strandja Mt. Subregions signed as c - central, w - western, n - northern, e - eastern, s - southern. Floristic elements are given in italic with abbreviations according to Assyov & Petrova (2012). Protected species by the Bulgarian Biodiversity Act are signed with “§”. Species listed in the Red Data Book are signed with RDB, and with the IUCN category in brackets. Species listed in IUCN red list marked with the same abbreviation and with the category in brackets. The doubtful data are signed with “?”. The rejected data are signed with “?”.

Genus *Crocus* L.

1. ***C. adamioides*** Kernd. & Pasche (*C. biflorus* auct. Bulg., non Mill.): 18, 19; **Bal**
2. ***C. chrysanthus*** Herb.: 1n, 2, 4, 5c, 5e, 6, 8, 9, 10, 11, 12, 14, 15, 16w, 17, 18, ?20; **Bal-Anat**
3. ***C. danubensis*** Kernd. Pasche, N. Randelovic & V. Randelovic (*C. reticulatus* auct. Bulg., p.p.): 3; **Bal**
4. ***C. flavus*** West. (*C. aureus* Sm.; *C. moesiacus* Ker Gawl.): widespread; **Eur-Pont**
5. ***C. olivieri*** J. Gay: 1, 7, 10, 11, 12, 13, 14s, 16w, 17, 18, 19; **Bal-Anat; §**

6. ***C. pallasii*** Goldb.: 1n, 2, 3, 4e, 5e, 6, 10,11, 17c, 18, 19; **Pont-Med**
7. ***C. pallidus*** Kitan. & Drenk. (*C. weldenii* Hoppe & Furnr.; *C. biflorus* subsp. *weldenii* (Hoppe & Fuernr.) K.Richt.): 1n, 2; **Bal**
8. ***C. pulchellus*** Herbert: 1n, 9, 10, 11, 12, 13, 17c, 17e, 18, 19, 20; **Bal-Anat**
9. ***C. randjeloviciorum*** Kernd., Pasche, Harpke & Raca: 5w, 7; **Bal**
10. ***C. tommasinianus*** Herb.: 3, 4w; **Pann-Bal.**; §, RDB (VU).
11. ***C. variegatus*** Hoppe & Hornsch. (*C. reticulatus* auct. Bulg., p.p.): 4w, 5w; **Pont-Med**
12. ***C. veluchensis*** Herbert: 4, 5, 7, 8, 9, 11, 12, 14, 15, 16, 17; **Bal**
13. *C. biflorus* subsp. *adamii* (J. Gay) K. Richt.: ?
14. *C. biflorus* subsp. *alexandri* (Ninic ex Velen.) B. Mathew: 7, 8
15. *C. reticulatus* Steven ex Adams: ?2, ?3, ?4w, ?5w, ?6; **Pont-Med**
16. *C. biflorus* Mill. subsp. *biflorus*: ?; **Med**

Genus *Gladiolus* L.

1. ***G. communis*** L.: widespread; **Med**
2. ***G. illyricus*** Koch: 5e, 9, 10, 11, 12, 13, 17; **Med-OT**
3. ***G. imbricatus*** L.: widespread; **Med**
4. ***G. italicus*** Mill.: 1, 2, 3, 10, 17e, 18, 19, 20; **Med**, IUCN (LC)
5. ***G. palustris*** Gaud.: 12, 14, 15, 17; **subMed**, IUCN (DD), §

Genus *Iris* L.

1. ***I. germanica*** L.: widespread; ? **Adv**
2. ***I. graminea*** L.: widespread; **Pont-Med**
3. ***I. pseudacorus*** L.: widespread; **Eur.**
4. ***I. pumila*** L.: widespread; **subMed**
5. ***I. reichenbachii*** Heuff.: 1s, 3, 4w, 5, 7, 8, 9, 10s, 12, 14, 15, 17; **Bal-Dac**
6. ***I. sibirica*** L.: 4w, 5w, 5c, 6, 7, 8, 12, 16, 19; **Pont-Sib**
7. ***I. sintenisii*** Janka (*I. urumovii* Velen.): 1, 2, 3, 4e, 5, 6, 7, 10e, 17c, 17e, 18, 19, 20; **Med**
8. ***I. suaveolens*** Boiss. & Reut. (*I. mellita* Janka): 1, 2, 10, 11, 12, 13, 14, 17e, 18, 19, 20; **Bal-Anat**
9. ***I. variegata*** L.; widespread; **subMed**
10. *I. aphylla* L.: ?17e, RDB, CR, §

Genus *Romulea* Mar.

1. ***R. bulbocodium*** (L.) Seb. & Mauri: 9, 10s, 14s, ?11, ?12, ?20; **Med**, §
2. ***R. linarsii*** Parl.: 1s, 10s, 17e, 20; **EMed**, §

Genus *Sisyrinchium* L.

1. ***S. montanum*** Greene: 10s; **Adv.**
2. *S. angustifolium* Mill.: ?11; **Adv.**