Ornithogalum sibthorpii Greuter (Asparagaceae), a species overlooked in Croatia

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Abstract – *Ornithogalum sibthorpii* (Asparagaceae) is an early flowering species, with populations scattered across the Balkan Peninsula and Turkey. It inhabits rocky places and clearings, open habitats, parks and marginal parts of wetlands. Based on the known distribution, habitat preferences and literature records for the Balkan Peninsula, it was hypothesised that this species might be distributed in Croatia as well. To confirm this, herbarium material was revised, and field investigations were organized. The first report confirmed that *O. sibthorpii* is widespread along the eastern Adriatic coast, reaching the inland Dinaric region too. To present the currently known localities in Croatia, a distribution map is provided. Detailed morphological and leaf anatomy descriptions are given. Morphological affinities with similar species, *O. excapum* and *O. refractum*, are also briefly discussed, and an identification key is given. All Croatian populations of *O. sibthorpii* proved to be diploids with chromosome number 2n = 18.

Keywords: anatomy, geophytes, karyology, morphology, Ornithogalum

Introduction

The genus *Ornithogalum* L. is characterized by a wide ecological tolerance and pronounced morphological variability. According to Stevens (2001) it is one of the largest genera in the Asparagaceae family, with approximately 160 species (300 taxa). It is distributed in Africa, Europe and Southwest Asia (Speta 1998, Martínez-Azorin et al. 2013). Global databases record nearly 120 species with distributions in the Mediterranean region and in the rest of Europe (Govaerts 2019, Euro+Med 2006–2019). According to Nikolić (2019a, b) in the Croatian flora there are 19 *Ornithogalum* species, three stenoendemic, and 10 species belonging to the subgenus *Ornithogalum*.

Several discrete European *Ornithogalum* groups are differentiated based on flowering time, that is, species flowering in late winter/early spring, spring, and in late spring/ early summer. One of the species that flower in late winter/ early spring is *Ornithogalum sibthorpii* Greuter. It is a rare and scattered species, distributed in the western part of Turkey and in the Balkan Peninsula, in the north reaching Dobrugea in Romania (Sibthorp and Smith 1809, Baker 1873a, b, Markgraf 1932, Radenkova 1964, Zahariadi 1966, 1977, 1980, Diklić 1975, Landström 1989, Speta 1990, Rat and Barina 2017, Rat 2019). It prefers karst, rocky ground, clearings and open habitats (Zahariadi 1980), and belongs to the group of *Ornithogalum* species that have underground scape, bearing from one to many flowers on the shortened inflorescence. Taxonomically significant characters compared with morphologically similar species in the investigated region (*O. exscapum* Ten. and *O. refractum* Kit. ex Schltdl.) are the more or less pronounced pulvinus, refracted pedicels at anthesis and bulbs without bulbils (Zahariadi 1980, Landström 1989).

There is one literature reference indicating that *O. sib-thorpii* is distributed in Croatia. Ascherson and Graebner (1905–1907: 249) stated that *Ornithogalum nanum* Smith in Sibth. et Smith (today synonym of *O. sibthorpii*) is recorded

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in Ljubuški and Stolac in Bosnia and Herzegovina, near to the Croatian border. Furthermore, for general distribution they cited the Adriatic region which covers an important part of the Croatian coast. For that reason, herbarium revisions and field trips were organized, with the aim of providing new data about the potential distribution of the species in the western part of the Balkan Peninsula i.e. Croatia, and to complement it with biological and ecological descriptions.

Materials and methods

Field trips were organized during season in 2015 and 2016. Plant material was collected for morphological, anatomical and karyological analysis (Tab. 1). Voucher specimens are kept in the herbarium collections ZAGR and BUNS. Herbarium materials of *O. sibthorpii* were revised in BEO, BEOU, BP, BUNS, K, MKNH, SOM, SARA, W, WU, ZA, ZAGR and ZAHO. Virtual collections G, GZU and OXF were accessed as well. Herbaria acronyms follow Thiers (2019). A distribution map of *O. sibthorpii* in Croatia was produced in QGIS software ver. 3.10.

Observations and morphological analyses included qualitative and quantitative description of bulb, leaves, scape and inflorescence with flowers. Ten fresh plants were collected and pressed for analysis. All observations were performed on fresh material using a Leica M205C binocular stereomicroscope, while measurements were carried out using Digimizer image analysis software ver. 4.2.6.0.

Anatomical investigations included a description of leaf structure. Cross sections were cut from the middle part of the leaf blade, using Leica CM 1850 cryostat, at -20 °C with a cutting interval of 60 nm. Observations and measurements were performed using a ZEISS light microscope AxioVision A2, equipped with a ProgRes Speed XT^{Core5} camera and CapturePro v.2.8.8 image analysis software.

For chromosome counting and analyses, 10 bulbs were planted in pots, and young root tips were collected, treated with 0.5% colchicine solution for 1 h at room temperature, and then fixed with a fresh solution of ethanol and glacial acid (3:1) for storage. To visualize metaphase chromosomes, root tips were hydrolysed with 1M HCl at room temperature, and then stained with Schiff's reagent. Standard squash technique was used for preparation of slides (Jong 1997). Image analysis and measurements of chromosomes were completed using KaryoType 2 software (Altinordu et al. 2016).

Results

Ornithogalum sibthorpii Greuter, Boissiera 13: 160 (1967) (Fig. 1)

Type – "In Arcadia, et prope Abydum, Martio florens", Smith loc. cit. (OXF!; file name: Sib-0793).

Synonyms – Ornithogalum nanum Smith in Sibth. et Smith, Fl. Graec. Prodr. 1: 230 (1809); Fl. Graec. (Sibthorp). 4: 28, t. 333 (1823); non O. nanum (Burm. f.) Thunb., Prodr. Fl. Cap. 62 (1794).

Morphological description (based on Croatian material, Fig. 1) – Bulb hypogeal, ovoid, $12 - 15 \times 0.8 - 20$ mm, with-



Fig. 1. *Ornithogalum sibthorpii* (Croatia, material from Brgat locality): a – ascapose form with prominent pulvinus (bulb without outer tunics), b – scapose form with small pulvinus, c – flower, d – gynoecium, e – seed micromorphology. Scale bar: a-d – 1 mm, e – 200 μm.

Tab. 1. List of examined plant materials of Ornithogalum sibthorpii in Croatia.

Locality	Latitude / Longitude	Collecting date	Collector(s)	Herbarium voucher number
Croatia, Brgat (South Dalmatia)	42°38'45.06" 18°09'30.63"	28.03.2015	M. Rat	BUNS-2-1151
Croatia, Nin (North Dalmatia)	44°14'48.58" 15°10'38.10"	23.04.2016	S. Bogdanović, V. Šegota, Z. Ljubešić	ZAGR-41135
Croatia, Donji Lapac (Lika)	44°31'36.82" 15°58'44.48"	06.05.2016	S. Bogdanović	ZAGR-55520 ZAGR-55521
Croatia, Sukošan (North Dalmatia)	44°02'59.10" 15°24'17.05"	05.04.2018	M. Pandža, M. Milović	ZAGR-46301



Fig. 2. Leaf "U" cross section of *Ornithogalum sibthorpii* (Croatia, material from Brgat locality). Scale bar: 50 μm.

out bulbils; outer tunics light brown to brown. Leaves numerous, glabrous, longer than inflorescence, up to 3.5 mm wide, canaliculate, with median white stripe on the adaxial side. Scape short, mostly hypogeal, $1 - 5 \text{ cm} \log 1$. Inflorescence up to 20 mm long, corymbose, with 1 - 8 (12) flowers. The overall length (scape + inflorescence) is ca 5 cm. The lower pedicels refracted, $10 - 25 \text{ mm} \log 3$, with the pulvinus at the base. Bracts shorter than or equal to pedicels. Outer tepals $16 - 20 \times 4 - 7 \text{ mm}$, with abaxial green stripe 4 - 6 mm wide. Inner tepals $15 - 21 \times 5 - 8 \text{ mm}$, with ab-

axial green stripe 3 - 6 mm wide. Filaments 7 - 11 mm long; anthers 2.5 - 5 mm long. Ovary elongated to rounded, $5 - 6 \times 3.5 - 5.5$ mm, with 6 prominent ribs; style 4 - 6 mm long, longer than or equal to ovary. Seeds globose, 1 - 2 mm in diameter, black, luminous, with reticulate testa.

Phenology – Flowering and fruiting time from March to June.

Leaf anatomy – Leaf has typical "*umbellatum*-type", that is "U" shape on transverse cross section. Leaf blade is canaliculate, on abaxial side with 3 – 6 ribs. Longitudinal white stripe visible on the adaxial side is a consequence of noncontinuous palisade tissue in the central part. Visible on the cross section are 1-layered epidermis, 1-layered palisade tissue and mesophyll. Vascular bundles are arranged in two lines: larger bundles are in the centre of mesophyll, and smaller ones along palisade tissue of abaxial side. Other than vascular bundles, mesophyll contains large and small cavities, later usually with raffid crystals (Fig. 2).

Karyology – For karyological studies, bulbs were available from localities Brgat, Donji Lapac and Nin. All investigated individuals were diploid, 2n=2x=18 (Fig. 3a). Combined chromosome formula is 2n=2x=6m + 12sm, and Stebbins karyotype asymmetry degree is 2A. Two chromosome pairs are long, three of medium length and four pairs are small chromosomes. Karyotype is characterized with chromosomes that have more or less gradual transition in size (Fig. 3b). Two pairs are metacentric, while others are submetacentric, with total haploid chromosome length 59.01±0.89 µm (Tab. 2). Satellited chromosomes were not detected.



Fig. 3. Mitotic metaphase chromosome plate of *Ornithogalum sibthorpii* (Croatia, from Brgat locality): a – metaphase plate; b – karyo-gram (2*n*=2*x*=18). Scale bar: 10 μm.

Tab. 2. Chromosome parameters for *Ornithogalum sibthorpii* in Croatia. Ten individuals are analyzed (2n=2x=18): four from Donji Brgat, three each from Nin and Donji Lapac. Number of analyzed metaphase plates is 40 in total (four per individua). Abbreviations: L – long arm length, S – short arm length, TAL – total absolute length, TRL – total relative length, m – metacentric, sm – submetacentric.

Chromosome pairs	L (μm)	S (μm)	TAL (µm)	TRL (%)	Туре
Ι	6.20±0.72	5.13±1.03	11.32±1.39	10.51+8.68=19.19	m
II	5.96 ± 0.48	3.12±0.65	9.08 ± 0.84	10.10+5.29=15.38	m
III	5.41±0.55	2.08 ± 0.51	7.49 ± 0.97	9.17+3.52=12.69	sm
IV	4.52±0.99	2.18 ± 0.50	6.71±1.30	7.66+3.70=11.36	sm
V	4.17±0.71	2.01±0.54	6.18 ± 0.98	7.07+3.41=10.47	sm
VI	3.80±1.25	1.67 ± 0.41	5.47±1.27	6.44+2.83=9.27	sm
VII	3.04±0.51	1.87 ± 0.58	4.92±0.99	5.15+3.18=8.33	sm
VIII	2.82 ± 0.58	1.54±0.29	4.36±0.62	4.78+2.61=7.39	sm
IX	2.10 ± 0.82	1.39 ± 0.51	$3.49{\pm}1.18$	3.55+2.36=5.91	sm

Distribution and habitat - First herbarium specimens that confirmed presence of O. sibthorpii in Croatia were found in the collection of Ivo Horvat (ZAHO), and date back to the first half of the 20th century. Since then this species was omitted in collections, most probably due to the short vegetation period and early spring flowering time. However, herbarium revision of collections in GZU, W and WU revealed that the species was recorded earlier, but sparse data led to its neglect in Croatia. Recent field trips expanded the known distribution range of O. sibthorpii in Croatia. It is spread from the southeast of the eastern Adriatic cost to the central Dalmatian region in the west, and in the north the central Dinaric region, which is the only continental location in Croatia. According to all the data gathered, a distribution map was created (Fig. 4). O. sibthorpii inhabits dry, clear, open habitats, dry hillsides as well as anthropogenic areas and bare surfaces. The estimated altitude in Croatia ranges from sea level up to 1000 m. In other regions of the Balkan Peninsula habitats and altitude are similar (Radenkova 1964, Rat et al. 2014, Rat and Barina 2017, Rat 2019).

Examined specimens (*specimina visa*) – Croatia: Lika, Krbava – Donji Lapac, Bare, u nižem vlažnijem dijelu polja, 07.06.1958, I. Horvat s.n. (ZAHO-41129, ZAHO-41130); Makarska (Dalmat.), 17.04.1931, M. Salzmann s.n. (GZU-057466); Orebić (Dalmatien), 16.04.; 19.04.1930, M. Salzmann s.n. (GZU 057466); Süddalmatien, Halbinsel Pelješac: zw. Orebić u. Sattel östl. des, Mte. Vipera, 16.04.1930, J. Eggler s.n. (GZU 103693); Süddalmatien, Halbinsel Peljesac: Kräuterfluren u. Macchie westl. von Orebic., 14.04.1930, J. Eggler s.n. (GZU 103694); Dalmatien, Gravosa [Dubrovnik], 10.04.1933, K. Ronniger s.n. [sub *Ornithogalum exscapum* Ten.] (W, Herbarium Karl Ronniger 5580); Dalmatia, in vineis pr. Spalato [Split], 04.1870, Pichler s.n. [sub *Ornithogalum umbellatum* L. (Vis.)] (WU, Herbarium Kerner).

Identification key for *O. sibthorpii* and morphologically similar species in Croatia

- 1. Bulb with numerous bulbils (>10) outside of tunics; bracts equal to or longer than pedicels O. *refractum*

Discussion

This species has been known since the pre-Linnean period. Buxbaum (1728) described it for the first time from the region around Istanbul (Constantinopolium) in Turkey and he also provided the first iconography of the species. It was overlooked by Linne, but later botanists who investigated Greece and the eastern Mediterranean, Sibthorp and Smith, described Ornithogalum nanum as new species. Ornithogalum nanum Smith in Sibthorp and Smith (1809) was



Fig. 4. Distribution map of Ornithogalum sibthorpii in Croatia.

described as: "O. nanum, corymbo simplici paucifloro glabro scapo longiore, bracteis ventricosis scariosis, foliis linearibus numerosis". The locality is cited as "In Arcadia, et prope Abydum, Martio florens", with reference to Buxbaum (1728). Abydum is ancient name for the region close to today's Cannakalle in Turkey, and Arcadia is an ancient region in Peloponnese, but also in Antalya (Turkey) (Stearn 1967). Because of this, the precise locus classicus is not known. A detailed description of the species was published later (Sibthorp and Smith 1823). Herbarium specimen marked as type material is deposited in OXF herbarium, under the file number Sib-0793. Since O. nanum is a later homonym of O. nanum (Burm. f.) Thunb., replacement name O. sibthorpii was published by Greuter (Greuter and Rechinger 1967), in honour of John Sibthorp (1758–1796) who collected plant material (Stearn 1984).

Although in most of the relevant databases (e.g. The-PlantList, Euro+Med Checklist) *O. sibthorpii* is synonymised with *O. sigmoideum* Freyn et Sint. we retain viewpoint of Landström (1989), later supported by the results of Speta (1990), that *O. sibthorpii* is a good species which should not be underestimated without systematic investigations.

The results of field trips organized in 2015 and 2016 confirmed our assumptions that O. sibthorpii is distributed in Croatia, and these data were presented by Rat and Bogdanović (2016) at the 5th Croatian Botanical Symposium. Based on this report and deposited herbarium specimens in ZAGR herbarium (ZAGR-55520, 55521), Nikolić (2019a, b) included it in the national list of vascular flora. Historical data about distribution in Italy (Parlatore 1857) however have to be revised, since this record was included in distribution data for the species O. mutabile De Not., which is in meantime synonymised with O. exscapum by Peruzzi and Passalacqua (2002) and Garbari et al. (2003). Landström (1989) summarized differential morphological characters of O. sibthorpii and O. exscapum, describing O. sibthorpii with shorter pedicels, larger flowers, longer styles and anthers. An analysis of the material from Croatia shows that the additional differential qualitative morphological characters for these two species are: pedicel shape (in O. sibthorpii refracted at anthesis, in O. exscapum ascending to deflexed), visible pulvinus in O. sibthorpii, and absence of pulvinus in O. exscapum. Another informative parameter is bract/pedicel length ratio; in O. sibthorpii bract and pedicel are almost of the same length, while in O. exscapum bract is about half the length of the pedicel.

Comparative morpho-anatomical and cytotaxonomical studies of *Ornithogalum* species that belong to the group of small plants (overall length up to 10 cm) with hypogeal scape, including *O. sibthorpii*, have been done to clearly describe morphologically similar species in the area of Turkey and the Balkan Peninsula (Zahariadi 1962, 1965, 1977, Speta 1990, 2000). Both authors recognized three separated species: *O. sintenisii* Freyn, *O. sigmoideum* and *O. sibthorpii*. In addition, Speta (1990) described two new species, *O. saginatum* Speta and *O. plutullum* Speta, first from Romania and Moldavia, and later from the Balkan Peninsula.

Moreover, Landström (1989) recognized two ecotypes (montane and lowland) of O. sibthorpii and compare them to the intermediate type, localised for the region of İstanbul (Turkey). These ecotypes are confirmed in Croatia as well, since for plants collected at higher altitudes in the continental region (Donji Lapac, Lika, Dinaric area, 900 m a.s.l.) the flowering time is in June. In Croatia, two distinct morphotypes of O. sibthorpii are observed: ascapose and scapose. The ascapose form (Fig. 1a) is characterized by a short scape, and the inflorescence is more or less sessile on the ground, with numerous flowers; pulvinus is strong and prominent. The scapose form (Fig. 1b) is differentiated with a scape up to 5 cm long, and an inflorescence with only a few (1 to 2) flowers (Fig. 1b). Comparing our results with those of Speta (1990) who investigated O. sibthorpii and related species in the eastern part of the Balkan Peninsula, we can confirm that both morphotypes are mostly present in the same population. Going further, he indicated that it is not unusual for two or more species from this group, that are morphologically indivisible, to coexist in one locality.

In the Balkan Peninsula, *O. sibthorpii* could be easily misidentified with *O. refractum*. The most informative discriminate characteristic is a bulb without bulbils in *O. sibthorpii* (Landström 1989, Rat et al. 2014). It is not unusual to find both species in close localities, when in addition to bulb parameters flower characteristics can contribute to species identification (Rat et al. 2014). Furthermore, according to Rat et al. (2017) seed testa micromorphology can be used as a taxonomically important character for the differentiation of *O. sibthorpii* from other *Ornithogalum* species. Globose seeds are typical only for *O. sibthorpii* (Fig. 1e) and *O. refractum*, while, former is differentiated as species with rather small seeds.

Cytological review by Cullen and Ratter (1967), with doubtful discussion on plant material, reported three main cytotypes for O. sibthorpii - O. sigmoideum group, in the region from Caucasus to Italy, with numerous aloploid se-20, 28). They did not undertake any morphological measurements, and it was impossible for them to compare morphotypes and cytotypes or to discuss in detail obtained results. Nonetheless, they recognized only two species, O. sigmoideum and O. sintenisii, while for them O. sibthorpii was a synonym of O. sigmoideum. An opposite view was presented by Speta (2000), who included both morpho--anatomical and cytological reports for 18 species that belong to this group. He recognized all species based on taxonomical characters, including the karyotypes described. It is clear from his results that O. sibthorpii is separated from all other species, with 2n = 14, 28 chromosome complements, while in O. sigmoideum they are 2n = 20, 24. Chromosome arrangement 2n = 18 was documented also by Speta (1990) in an unresolved discussion in short notes. In addition to these species, for O. sintenisii chromosome number 2n = 12 is recorded. Diverse chromosome numbers (2n = 14, 16, 18, 24, 28) for *O. sibthorpii* were recorded and documented from the Balkan Peninsula, by other authors as well. For Bulgaria, Markova (1972) reported three different chromosome numbers (2n = 14, 16, 28), while Lungeanu (1971) reported 2n = 18, 24 for Romania.

Following evolutionary patterns in different Ornithogalum groups, it can be recognized that in almost all subgenera there are several groups or complexes that are characterized with "high morphological variability" and extensive chromosome numbers among samples. By now, for most of them, systematic studies have been undertaken and it has been confirmed that every complex (i.e. O. tenuifolium in Africa and O. umbellatum in Europe) includes "good species" and "transitional forms" (cytotypes and morphotypes) that exist in nature thanks to vegetative reproduction (Stedje and Nordal 1984, 1987, van Raamsdonk 1985, van Raamsdonk and Heringa 1987, Andrić et al. 2015). Stedje and Nordal (1984) concluded that rapid evolutionary processes in Ornithogalum, visible as cytotype differentiation, do not express their changes in morphological characters, but do indicate the evolutionary progress in the taxon. This statement evidently can be accepted for the O. sibthorpii related group that was studied in this research, taking into consideration that many similarities were observed.

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