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Cytotaxonomical remarks on *Allium serbicum* (Amaryllidaceae), a misappreciated species of the Balkan flora

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Abstract

Allium serbicum is a critical and unappreciated species described from Serbia (Balkan Peninsula), and usually treated as a synonym of *A. pallens* or *A. tenuiflorum*. Based on living and herbarium material it was possible to clarify its morphology, karyology, leaf anatomy, pollen grain and seed testa microsculpturing, ecology and chorology of this very peculiar and rare endemic species. It is diploid with $2n = 16$ chromosomes, and seems taxonomically quite isolated, since it is morphologically well differentiated from the other species of *A.* sect. *Codonoprasum*, with very specialized ecological requirements, being a glareicolous plant localized exclusively on calcareous screes. In addition, a second-step lectotypification, a detailed description, a new iconography, distribution map and conservation status are also provided.

Key words: Allioideae, karyology, leaf anatomy, lectotypification, pollen grain, seed testa, Serbia

Introduction

Within cytotaxonomical investigation on the genus *Allium* Linnaeus (1753: 294) in the North Balkan Peninsula, a rare and almost unknown species of the sect. *Codonoprasum* Reichenbach in Mössler (1827: 538) is examined. It was described as *Allium serbicum* by Visiani and Pančić (1865: 479) from some localities of Serbia, where it usually grows in rocky mountain habitats. Previously, only a single herbarium sheet with three specimens of this species preserved in the Pančić herbarium at BEOU, as well as its iconography published in the protologue was known. Because of this very limited information, *A. serbicum* was treated in the literature (Hayek 1933, Ritter-Studnička 1970, Tatić 1975, Stearn 1978, Gregory *et al.* 1998, Govaerts *et al.* 2005–2014, Anačkov 2009, Raab-Straube 2023, POWO 2024) as a rather critical species, usually considered as a synonym of *A. pallens* Linnaeus (1762: 427) or sometimes of *A. tenuiflorum* Tenore (1811–1815: 165). It should be noted that some authors (Niketić 2014, Stojanović *et al.* 2017, Niketić & Tomović 2018) believe that *A. serbicum* should be accepted as a species morphologically distinct from *A. pallens* and other allied taxa. In order to improve the knowledge on this critical species, field surveys were carried out to verify its current range, as well as its morphological and karyological peculiarities, leaf anatomy, ecology and microsculptures of the seed testa and pollen grains, and conservation status.

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Materials and methods

The morphological analysis on *Allium serbicum* were carried out on living plants coming from some Balkan localities, which are cultivated in the Botanical Garden of Catania. Quantitative and qualitative features were measured and scored on 10 individuals. Only a few individuals were used for the investigations as the populations are truly rare and very impoverished. Comparative investigation with allied taxa was based on personal data, as well as on herbarium material (see Table 1). The examined herbarium specimens are preserved in CAT, BEO, BEOU, BUNS and ZAGR. The herbarium acronyms follow Thiers (2024). Examination was made under a Zeiss Stemi SV8 stereomicroscope at 6–64× magnification.

TABLE 1. Main diacritic morphological characters of *Allium serbicum* and compared species**.**

Character	*A. serbicum*	*A. pallens*	*A. tenuiflorum*
Outer bulb coat	fibrous, grey-brown	coriaceous, whitish	coriaceous, blackish-brown
Stem height (cm)	(13)18–40	(15)20–70(90)	10–40
Leaf sheaths coverage stem	1/3–1/2	1/2	1/2–2/3
Leaf blade in section	slightly semi-cylindrical	semi-cylindrical	pentagonal
Leaf blade spongy tissue	compact	fistulous	compact
Leaf blade ribs	without	many	5
Leaf blade width (mm)	1–2	1.5–3	0.8–1.2
Spathe valves directions	divaricated to reflexed	divaricated	divaricated
Longer spathe valve length (cm)	2.5–3	2–12(15)	2.5–7
Shorter spathe valve length (cm)	0.5–1.5	1–6(10)	1.5–4
Longer spathe nerves	5	7–10	7
Shorter spathe nerves	3	6	5
Inflorescence number flowers	10–20(25)	more than 50	10–50
Pedicel length (mm)	ca. 5	5–25	20–25
Tepal colour	white to pinkish-white	white	pinkish-white
Tepal tinged	pink	no	purplish
Tepal length (mm)	4.8–5.3	4–4.5	4.5–5.2
Tepal width (mm)	2.3–2.4	1.2–1.5	2.0–2.2
Outer stamen filament length (mm)	1.8–3.5	2.5–4	1.5–1.7
Inner stamen filament length (mm)	1.8–3.5	2.5–4	2–2.5
Annulus length (mm)	0.7–0.8	1	1–1.3
Anther colour	white, tinged with pink	yellow	yellow
Anther size (mm)	1.4 × 0.8	1.2–1.3 × 0.8–1.0	1 × 0.8
Anther apex	rounded	rounded	apiculate
Ovary shape	globose	ellipsoid	cylindrical to obovoid
Ovary surface	tuberculate above	smooth	tuberculate above
Ovary length (mm)	2.3	3–4	3–3.2
Capsule shape	subglobose	subglobose to ovoid	subglobose to obovoid
Capsule size (mm)	3.8–4 × 4–4.2	4–4.5 × 4–4.5	4–4.5 × 3.5–4.5

For the leaf anatomy, leaf blades of maximum size in their optimal vegetative development taken from cultivated plants were used. Leaf cross-sections were fixed in fresh Carnoy solution (3:1 absolute ethanol and glacial acetic acid) and embedded in paraffin; the transverse sections were doubled stained with ruthenium red and light green.

Karyological analyses were performed on mitotic plates from root tip cells of cultivated bulbs, pre-treated 3h with a 0.3% (*w/v*) colchicine water solution at room temperature, fixed 12 h in Carnoy and stored in 70% ethanol. Then, root tips were hydrolyzed in 1N chloridic acid for 7 minutes at 60°C and stained in Schiff reagent. Microphotographs of good quality metaphase plates were taken using a Zeiss Axioskop 2 microscope equipped with a high-resolution

digital camera. The somatic chromosome number and karyotype details were studied in 10 spread metaphase plates from different individuals; mean values were used for the karyotype characterization. Metaphase observations and chromosome measures were made using the image analysis systems Zeiss AxioVision 5.1. Karyotyping was worked out by a specific software CromoLab© 1.1 (Brullo 2002) for the recognition of homologues, couple ordering, chromosome classification and karyotype formula based on centromere position (Levan *et al.* 1964, Tzanoudakis 1983). Karyotype symmetry indices followed Paszko (2006) and Peruzzi & Eroðlu (2013). For mean karyomorphometric parameters see Table 2.

Seed testa micromorphology was performed on mature and dry material from type locality using a scanning electron microscope (SEM) JEOL JSM 6460 LV at the University Center for Electron Microscopy in Novi Sad (UCEM-NS), according to the protocol reported by Stork & al. (1980), while terminology of the seed coat sculpturing follows Barthlott (1981, 1984) and Gontcharova *et al.* (2009).

Pollen morphology was examined according to Huynh (1975), Punt *et al.* (2007) and Hesse *et al.* (2009). Dry pollen of herbarium material (BUNS) coming from the type locality, was mounted on stubs without any preparation and observed using a scanning electron microscope (SEM) JEOL JSM 6460 LV. The device SCD 005 Ion Sputter Coater (BALL-TEC) was used to prepare the sample.

Results

Taxonomic treatment

Allium serbicum Visiani & Pančić (1865: 479, Tab. 22, fig. 1). Figs. 1, 2, 4

Type:—SERBIA. Mokra gora Serb. merid. in rupestrib. calcareis, Jul, *J. Pančić s.n.* (lectotype: BEOU 11931!), second-step designated here (Fig. 2).

—*A. tenuiflorum* Hayek (1932: 57) non Tenore (1811: 165)

—*A. pallens* Stearn (1978: 1619) non Linnaeus (1762: 427)

Bulb ovoid, 13–14 × 7–8 mm, outer tunics fibrous, grey-brown, inner tunics membranaceous, whitish. Stem erect, (13)18–40 cm tall, glabrous, cylindrical, covered by leaf sheaths for 1/3–1/2 of total length. Leaves 3(−4), green, glabrous, blade not rigid, slightly semi-cylindrical, compact, undulate at margine, without ribs, 10−20 cm long, 1−2 mm wide. Spathe persistent, with two valves unequal, opposite, divaricated to reflexed at flowering, with the appendage longer than the inflorescence, the larger one 5-nerved and 2.5−3 cm long, the smaller one 3-nerved and 0.5−1.5 cm long. Inflorescence compact, globose, up to 2 cm in diameter, 10−20(25)-flowered; pedicels erect, subequal, ca. 5 mm long. Perigone campanulate, with tepals equal, white to pinkish-white, tinged with pink, elliptical, rounded at the apex, 4.8−5.3 × 2.3−2.4 mm, with midrib purplish-green. Stamens included in the perigone or slightly exerted, with simple filament, white, subequal, 1.8−3.5 mm long, connate below into an annulus 0.7−0.8 mm high; anthers white tinged with pink, oblong, 1.4 × 0.8 mm, rounded at the apex. Ovary globose, markedly contracted at the base, greenish-yellow, tuberculate above, 2.3 × 2.1−2.2 mm; style white, ca. 1 mm long. Capsule 3-valved, subglobose, green, 3.8−4 × 4−4.2 mm.

Distribution and ecology:—According to literature data (Visiani & Pančić 1865, Anačkov 2009, Stojanović *et al.* 2017) and field investigation *Allium serbicum* is localized in few stands of West Serbia and in one nearby place of East Bosnia and Herzegovina (Fig. 3). It grows in calcareous screes (Fig. 4) more or less stabilized from 500 up to 900 m of altitude, in habitats characterized by several endemic species such as *Pseudo-fumaria alba* (Miller, 1768: no. 3) Lidén (1986: 32) subsp. *leiosperma* (Conrath, 1888: 50) Lidén (1986: 32), *Reichardia macrophylla* Visiani & Pančić in Pančić (1874: 460), *Stachys anisochila* Visiani & Pančić (1870: 13), *Scabiosa fumarioides* Visiani & Pančić (1865: 466), *Micromeria croatica* (Person, 1806: 130) Schott (1857: 93), and some other species with wider range *Achnatherum calamagrostis* P. Beauvois (1812: 19), *Athamanta turbith* (Linnaeus, 1756: 14) Brotero (1804: 435), *Clinopodium thymifolium* Kuntze (1891: 516), *Satureja montana* Linnaeus (1753: 568), *Epilobium dodonaei* Villars (1779: 45), *Aurinia corymbosa* Grisebach (1843: 271) etc.

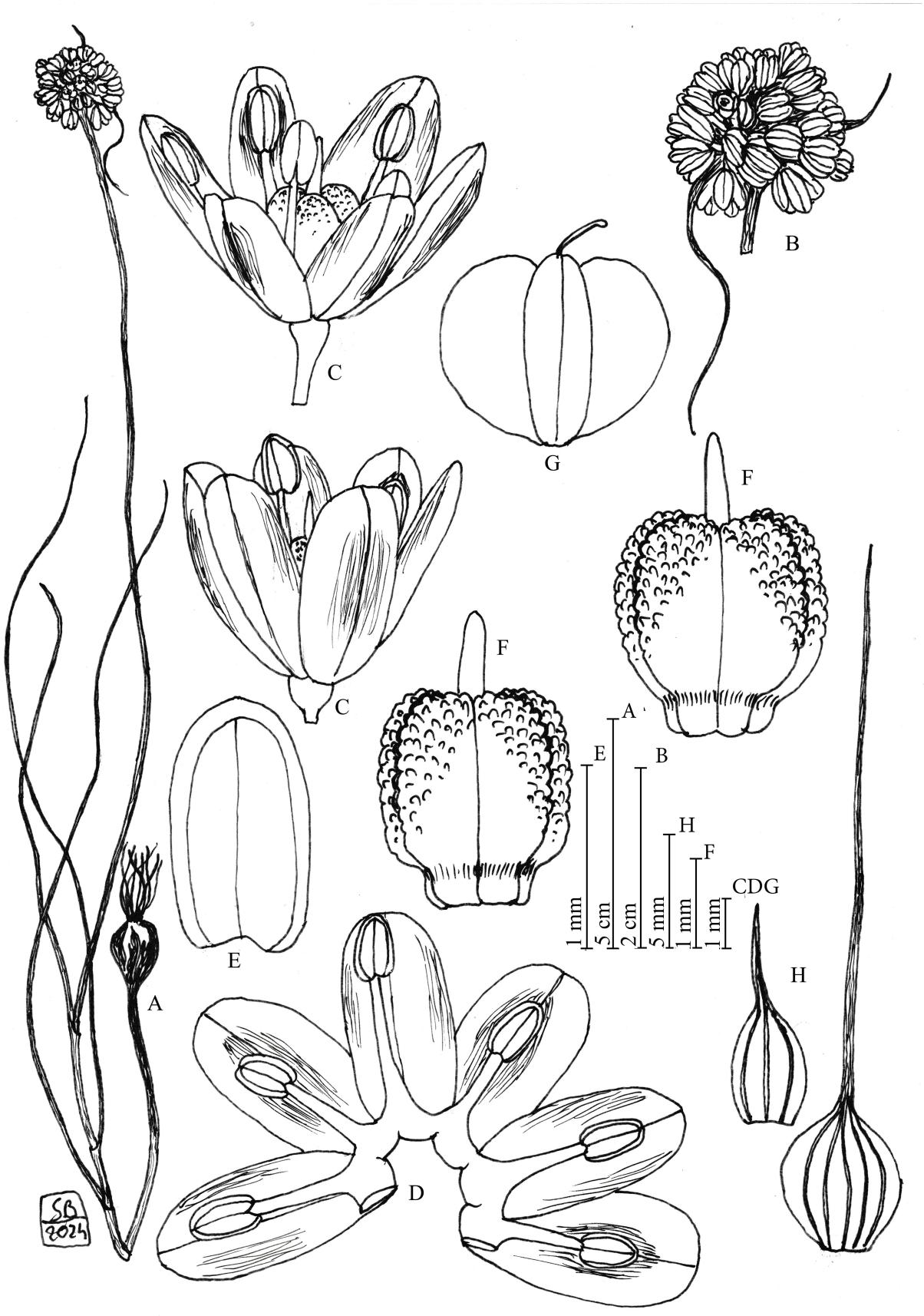

FIGURE 1. Diagnostic features of *Allium serbicum*. **A.** Habit. **B.** Inflorescence. **C.** Flowers. **D.** Open perigone and stamens. **E.** Anther. **F.** Ovaries. **G.** Capsule. **H.** Spathe valves. Illustration by S. Brullo based on living material from type locality.

FIGURE 2. Lectotype of *Allium serbicum* is indicated by black star from BEOU 11931!.

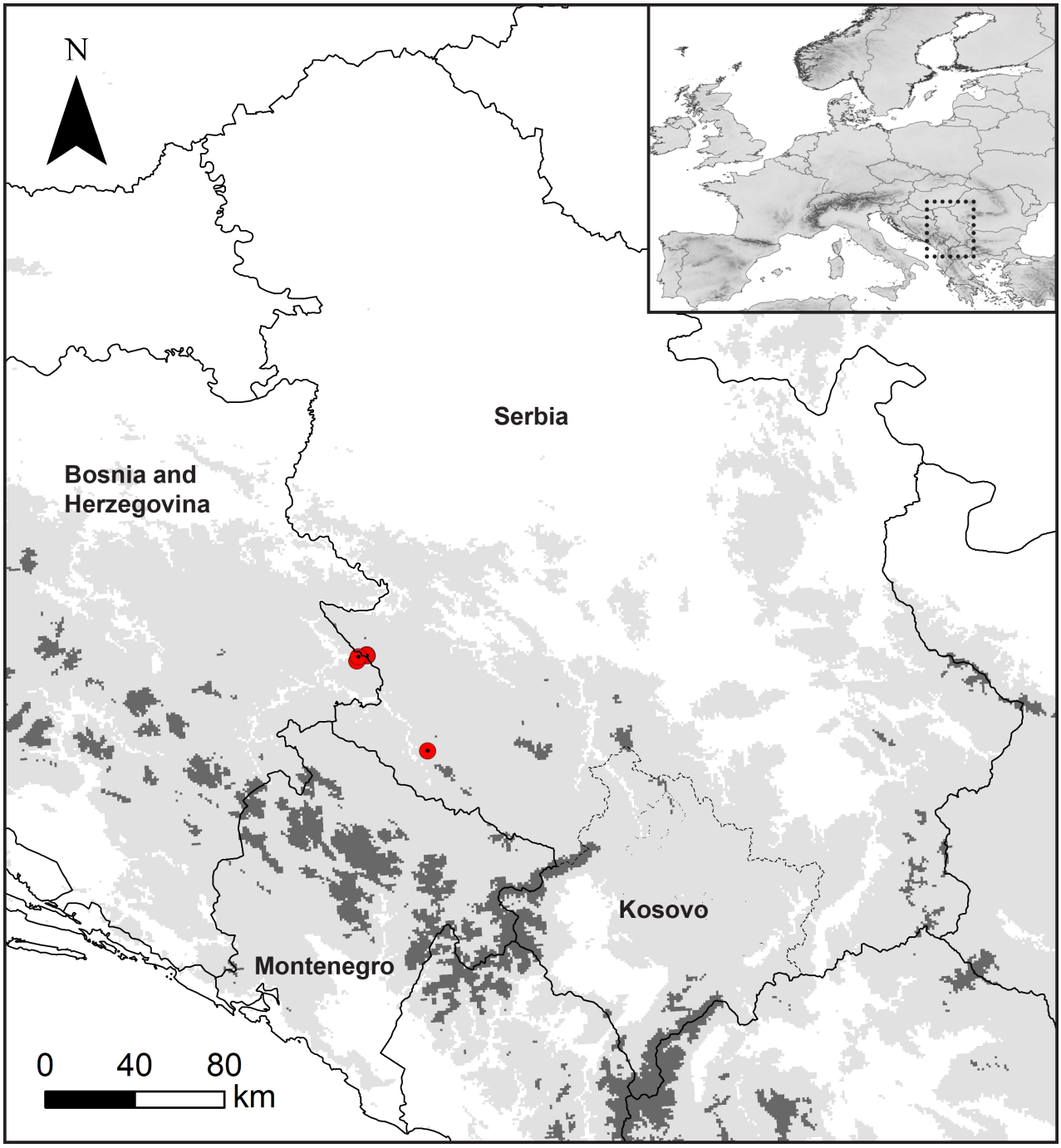

FIGURE 3. Distribution map of *Allium serbicum* (red dots) on the Balkan Peninsula.

Leaf anatomy:—The leaf cross section of *Allium serbicum* shows almost semi-circular outline markedly undulate at the margin. The epidermis shows regular small cells, covered by a well-developed cuticle. The surface of the leaf is covered with needle-like waxy structures. The stomata are numerous and distributed along the whole leaf perimeter, they are partially indented and framed by a thickened wax ring. The palisade tissue is arranged in two well distinct stratifications, the external one is constituted of elongated unistratified cell uniformly distributed in the whole perimeter, the inner one is constituted of small and rounded pluristratified cells (2−3 layers). The spongy tissue is compact, constituted by cell of different size, with many secretor canals under the palisade tissue. The vascular bundles are 10 (in the largest leaves), 7 of which variable in size are distributed in the adaxial face, while only 3 of small-size are in the abaxial face (Fig. 5).

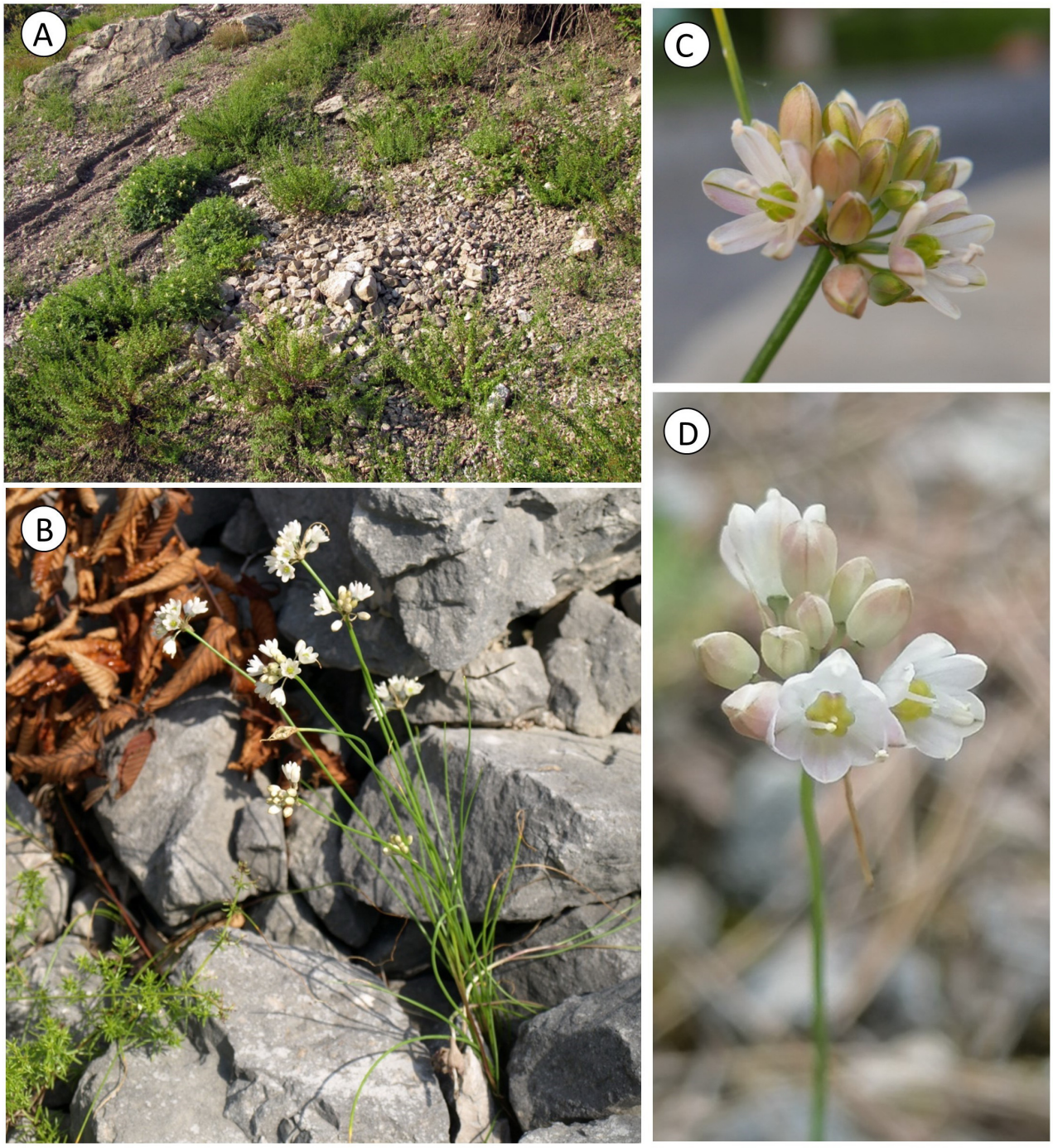

FIGURE 4. Phenological features of *Allium serbicum* from Beli Rzav (Serbia). **A.** Natural habitat. **B.** Individuals in natural habitat. **C.–D.**Inflorescences. (Photos A, C by B. Zlatković, B by G. Anačkov, D by U. Buzurović).

Karyology:—*Allium serbicum* has a diploid chromosome number, with $2n = 16$. Its chromosome complement (Fig. 6A) is characterized by nearly metacentric chromosomes, with two submetacentric pairs. Specifically, the karyogram (Fig. 6B) shows the chromosomes arranged in five typical metacentric (*m*) pairs (I, II, III, V, VI), one meta-submetacentric (*msm*) pair (IV), having an arm ratio between 1.30 and 1.67 (Tzanoudakis *et al.* 1983), and two submetacentric (*sm*) pairs (VII, VIII). Microsatellites were detected on the short arms of the meta-submetacentric pair and of one submetacentric pair (Fig. 7). Thus, the chromosome formula can be expressed as $2n = 2x = 16$: 10 m + 2 msm^{sat} + 2 sm + 2 sm^{sat}. Chromosomes have on average a total length varying from 12.4 ± 1.3 µm of the longest one to 6.5 ± 0.5 µm of the shortest one, with a mean length (MCL) of 9.03 ± 1.8 µm and a length coefficient of variation (CV_{CL}) of 19.86. Chromosome arm ratio (AR) shifts from 1.07 to 2.26, with a mean centromeric asymmetry (MCA) equal to 14.12%. The relative chromosome length ranges from 8.58% to 4.49% (Table 2).

TABLE 2. Karymorphometric features of *Allium serbicum*. Mean values ± standard deviation resulted from 10 good metaphase plates of distinct individuals from Dobrun-Razdoline. Abbreviations: Type = chromosome nomenclature according to Levan *et al.* (1964) and Tzanoudakis (1983); sat = satellited; TCL = total chromosome length; MCL = mean chromosome length; MAR = mean AR; MCI = mean CI; D-value = difference between total L and total S; CV_{CL} = coefficient of variation of chromosome length; CV_{CI} = coefficient of variation of the centromeric index; M_{CA} = mean centromeric asymmetry.

Chrom. No.	Long arm *L* (µm)	Short arm S (µm)	Total length *CL=L+S* (µm)	Relative length *RL=CL/TKL* (%)	Arm ratio *AR=L/S*	Centromeric index *CI=S/CL×100*	Type
1	6.50 ± 0.5	5.89 ± 0.8	12.39 ± 1.3	8.58 ± 0.4	1.10	47.52	m
2	6.07 ± 0.5	5.69 ± 0.7	11.75 ± 1.2	8.13 ± 0.2	1.07	48.37	m
3	5.86 ± 0.6	5.43 ± 0.8	11.30 ± 1.4	7.80 ± 0.2	1.08	48.10	m
4	5.51 ± 0.4	5.09 ± 0.6	10.59 ± 1.0	7.33 ± 0.3	1.08	48.01	m
5	5.14 ± 0.5	4.47 ± 0.4	9.60 ± 0.7	6.65 ± 0.2	1.15	46.51	m
6	4.99 ± 0.5	4.51 ± 0.3	9.51 ± 0.8	6.59 ± 0.3	1.11	47.48	m
7	5.42 ± 0.9	3.71 ± 0.8	9.26 ± 1.5	6.38 ± 0.5	1.46	40.06	msm^{sat}
8	5.20 ± 0.7	3.63 ± 0.6	8.83 ± 1.3	6.10 ± 0.5	1.43	41.14	msm^{sat}
9	4.60 ± 0.6	4.20 ± 0.7	8.80 ± 1.2	6.09 ± 0.6	1.10	47.70	m
10	4.47 ± 0.7	4.03 ± 0.6	8.50 ± 1.3	5.87 ± 0.7	1.11	47.44	m
11	4.35 ± 0.6	3.70 ± 0.7	8.05 ± 1.3	5.55 ± 0.5	1.17	46.01	m
12	4.04 ± 0.7	3.39 ± 0.4	7.43 ± 1.1	5.12 ± 0.3	1.19	45.64	m
13	5.20 ± 0.7	2.45 ± 0.4	7.73 ± 1.2	5.36 ± 0.9	2.12	31.75	sm^{sat}
14	5.08 ± 1.0	2.25 ± 0.3	7.41 ± 1.4	5.14 ± 1.0	1.85	35.15	sm^{sat}
15	4.50 ± 0.5	2.44 ± 0.2	6.94 ± 0.5	4.82 ± 0.4	1.85	35.15	sm
16	4.23± 0.6	2.24 ± 0.1	6.47 ± 0.5	4.49 ± 0.3	1.89	34.63	sm

TCL: 147.94 ± 18.4 µm; **MCL**: 9.03 ± 1.7 µm; **MAR**: 1.29; **MCI**: 42.87 ± 6.4; **D-value**: 18.04 µm

Symmetry indices**:** **CV_{CL}**: 19.86; **CV_{CI}**: 15.01; **MCA**: 14.12; **Stebbins' Cat.**: 2A

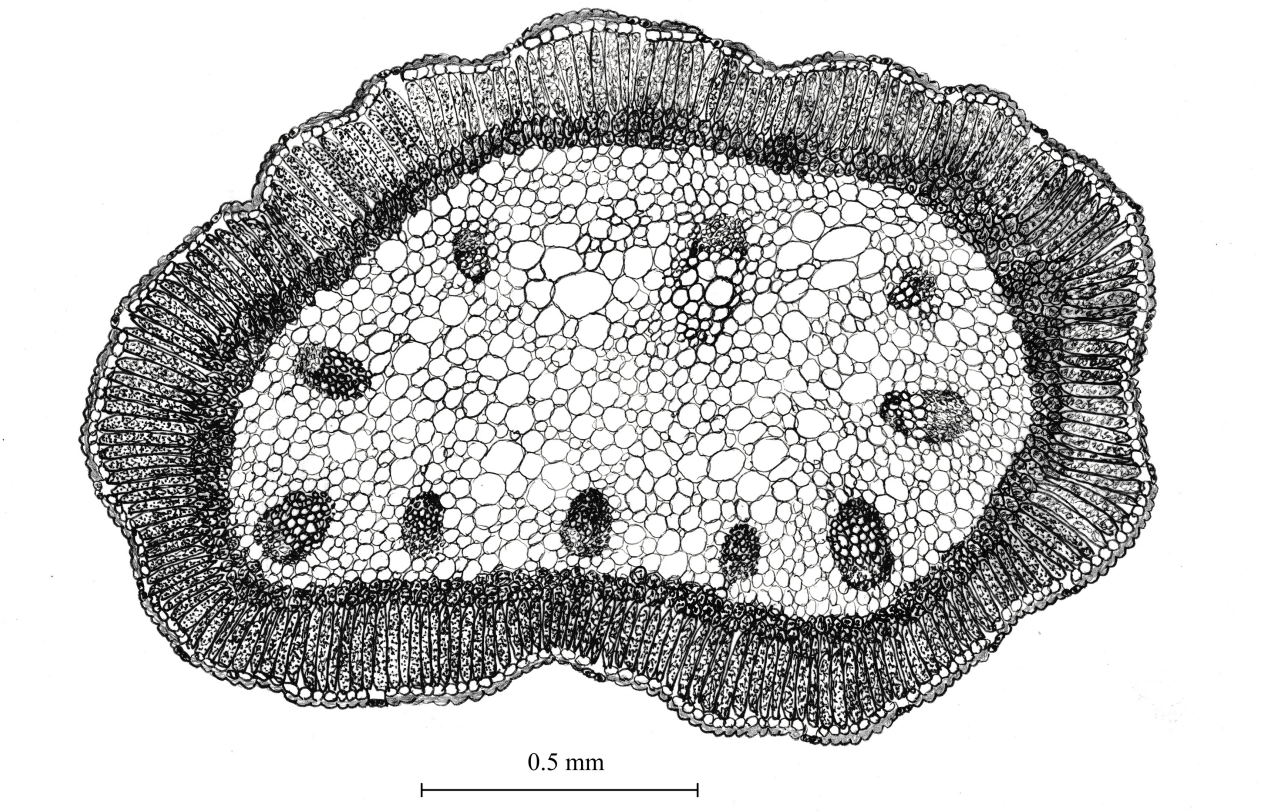

FIGURE 5. Leaf cross section of *Allium serbicum* from the type locality. Drawn by S. Brullo.

Seed testa micromorphology:—In the genus *Allium* the seed morphology provides useful systematic characters at various taxonomic levels (Celep *et al.* 2012, Salmeri *et al.* 2016, Lin & Tan 2017, Baasanmunkh *et al*. 2021, Yusupov *et al.* 2022). From this literature, it is clear that the seed testa ornamentations are conservative and stable features, which provide important information on this genus also from a phylogenetic point of view. The seeds of *A. serbicum* show a semi-ovoid outline (3.2–3.6 × 1.8–1.9 mm), with a rugose surface (Fig. 8A). Observations under SEM at high magnification (800×) show irregularly polygonate testa cells, 27–43 μm long. The anticlinal walls appear flat and straight, covered by strap-like sculptures forming a widened intercellular region. The anticlinal walls of the seedbed are characterized by a short connection in the form of the U-Ω connection model according to Fritsch *et al.* (2006). A similar form of undulation is also present in taxa that prefer thermophilic and xerophilic habitats (Anačkov 2009). The periclinal walls are slightly raised, with several granulose verrucae, distributed along the edges or sometime in the whole surfaces (Fig. 8B). These ornamentations are very similar to those ones observed in other species of *Allium* sect. *Codonoprasum* (Češmedžiev & Terzijski 1997, Neshati & Fritsch 2009, Celep *et al.* 2012, Brullo *et al.* 2013, Salmeri *et al.* 2016, Özhatay *et al.* 2018).

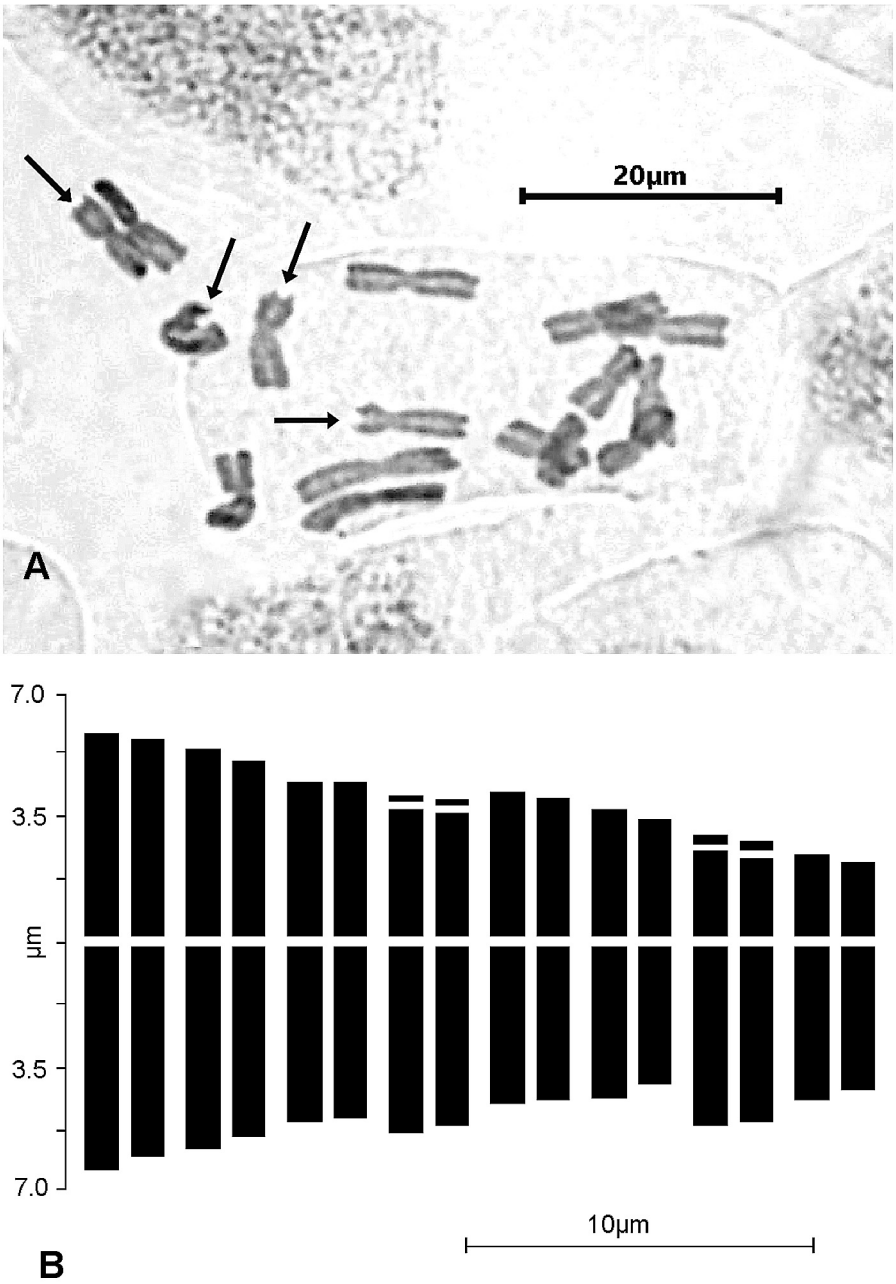

FIGURE 6. Chromosome complement (2*n* = 16) of *Allium serbicum*. Mitotic metaphase plate from type locality (**A**), arrows indicate satellited chromosomes, and karyogram (**B**).

Pollen grain micromorphology:—According to SEM investigations, the pollen grains are monad, monosulcate, and heteropolar with bilateral symmetry (Fig. 8C). The pollen shape is peroblate, with 29.40–32.00 µm long axis (LA) and 16.40–20.80 µm long short axis (SA). The sulcus is quite narrow and extends from distal to proximal portion of the pollen grain and the exine ornamentation is rugulate (Fig. 8D). According to the palynological investigation on the genus *Allium* (Nair & Sharma 1965, Diez 1987, Güler & Pehlivan 2006, Bogdanović *et al.* 2008, Neshati *et al.* 2009, Koçyiğit 2014, Yildiz 2023), *A. serbicum* shows a pollen morphology very similar to other taxa of the sect. *Codonoprasum*.

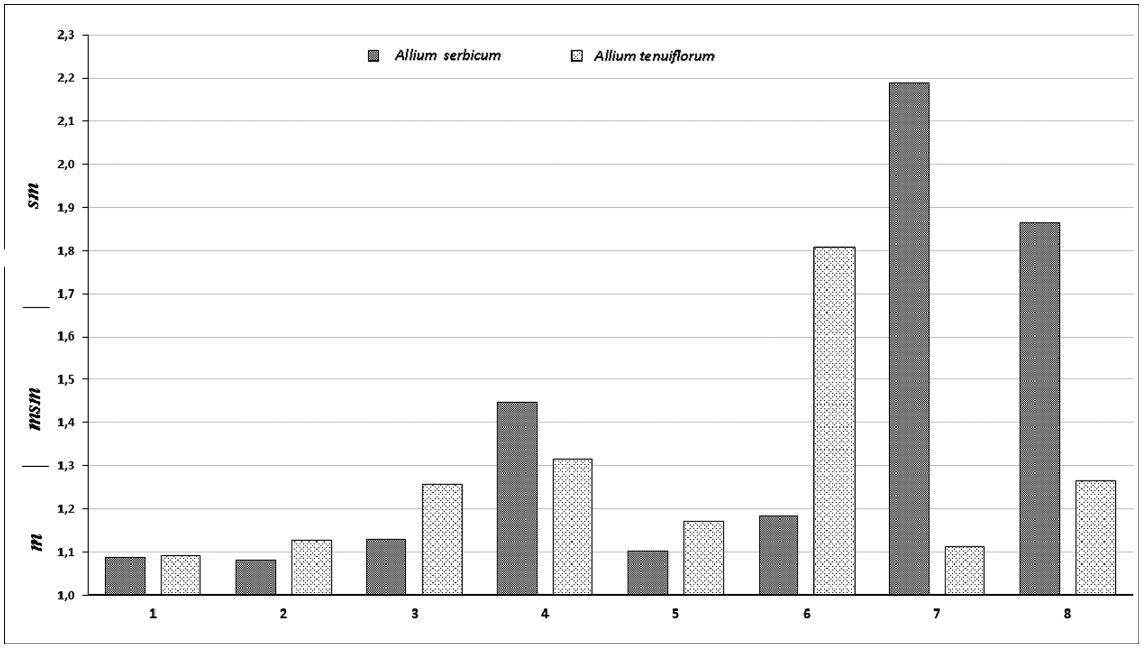

FIGURE 7. Comparison of karyotype structure between *Allium serbicum* and *A. tenuiflorum* based on the arm ratio of chromosome pairs ordered by decreasing size.

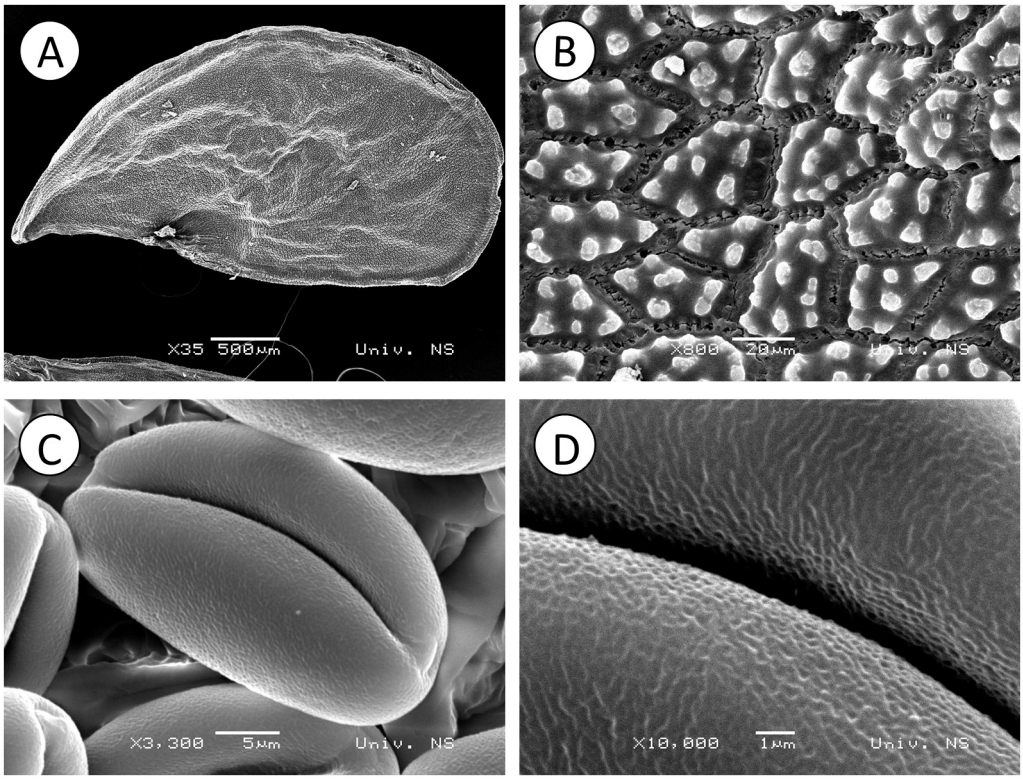

FIGURE 8. SEM micrographs of the seed coat and pollen grain of *Allium serbicum*. **A.** Seed (dorsal face, ×35). **B.** Seed coat (dorsal face, ×800). **C.** Pollen grain (equatorial view, ×3.300). **D.** Exine ornamentation (×10.000). Photos from material coming from the type locality (BUNS).

Conservation status:—Based on field investigations, *Allium serbicum* is currently know from seven locations where it grows in populations usually constituted by a few mature individuals or smaller groups, arranged in small tuffs dispersed over large scree surfaces. Extent of Occurrence (EOO) values were calculated for the species, which is 547.3 km^2, while the Area of Occupancy (AOO) value is 8 km^2. Namely, these values would have been different and lower if the microhabitat had not been found in the valley of the Mileševka River in 2009. The assessment was carried out according to standard criteria in the application made for the IUCN categorization of plant taxa for Serbia (Niketić 2022). Namely, intensive monitoring of the main population, located at the *locus classicus*, revealed a drastic reduction of the population, ranging between 30 and 50% for the previous 10 years. For the next period, given the established protection measures, it is expected that the reduction in the next 10 years will be between 10 and 20%, and maybe even less. The total number of mature individuals does not exceed 1000 and often not all individuals have formed fruits and seeds. There are also negative effects of expansion in the environment, especially fluctuations in the size of the space and occupied area, as well as the number of mature individuals. The habitat colonized by this species are quite natural and do not seem influenced by human activities except the removal of stones for construction. Since the implementation of protection measures, it has been observed that the population decline is so pronounced. According to the IUCN (2024), this species for its rarity, low number of mature individuals and restricted population distribution should be included in the category as CR: B2b(i,ii,iii,iv,v)c(i,ii,iv).

Additional specimens seen:—**BOSNIA AND HERZEGOVINA.** Dobrun-Razdoline, 43º45′28″N, 19º24′04″E, 507 m, 7 August 2022, *S. Bogdanović s.n.* (ZAGR!); Dobrun-Razdoline, in cult., 16 June 2023, *S. Brullo s.n.* (CAT!). **SERBIA.** West Serbia: Beli Rzav Gorge, entrance to the gorge, 43º46′27″N, 19º24′44″E, 527 m, July 2003, *B. Zlatković s.n.* (BUNS!); Mokra Gora, Kotroman, Beli Rzav Gorge, entrance to the gorge, 22 October 2004, *B. Zlatković s.n.* (BUNS!); Mokra Gora, Beli Rzav Gorge, srubs on limestone, limestone screes, 43º46′25″N, 19º27′44″E, 514 m, 27 July 2014, *G. Anačkov s.n.* (BUNS!); Mokra Gora, Beli Rzav Gorge, 43º46′28″N, 19º27′39″E, 523 m, 5 July 2015, *G. Anačkov s.n.* (BUNS!); Mokra Gora, Beli Rzav Gorge, 43º46′40″N, 19º27′39″E, 513 m, 31 July 2009, *G. Anačkov & B. Božin s.n.* (BUNS!); Prijepolje, Mileševka Gorge, on rocks, 43º21′45″N, 19º43′50″E, 657 m, 29 July 2009, *G. Anačkov & B. Božin s.n.* (BUNS!).

Taxonomic relationships:—From the taxonomic point of view, *Allium serbicum* must be treated as a species morphologically quite isolated from the other species of *A.* sect. *Codonoprasum*. For its globose inflorescence, it was attributed by various authors (Stearn 1978, Gregory *et al.* 1998, Govaerts *et al.* 2005–2014, Raab-Straube 2023, POWO 2024) to *A. pallens* species usually linked to synanthropic habitats and showing a wider Mediterranean distribution. According to Brullo *et al.* (2003a), *A. pallens* is well differentiated from *A. serbicum* in having a robust and bigger size, leaves 4–6, fistulous and semi-cylindrical, spathe valves longer, 6–10-nerved, inflorescence bigger, with more than 50 flowers, pedicels unequal 5–25 mm long, perigone with smaller tepals (4–4.5 mm long), usually white, stamens always exerted from perigone, anther yellow, shorter (1.2–1.3 mm long), ovary ellipsoid, smooth, longer (3–4 mm), capsule bigger (4–4.5 mm). Besides, all populations of *A. pallens* are characterized by a tetraploid chromosome number with $2n = 4x = 32$ (Brullo *et al.* 2003a). As concerns the relationships between *A. serbicum* and *A. tenuiflorum*, species by some authors considered synonyms (Hayek 1933, Tatić 1975, Anačkov 2009), they represent two completely distinct taxa. Indeed, based on the investigation carried out on *A. tenuiflorum* by Brullo *et al.* (2003b, 2013), it clearly differs from *A. serbicum* for habit, inflorescence, spathe valves, floral morphology, leaf anatomy, seed testa micromorphology and karyotype. In particular, *A. tenuiflorum* is characterized by outer bulb tunics coriaceous, blackish-brown, leaves 4–6, 5-ribed, manifestly pentagonal in cross section, spathe valves longer with more nerves (5 in smaller one, 7 in longer one), inflorescence lax and hemispherical, tepals narrower and apiculate, anthers yellow, shorter (1 mm), apiculate at the apex, annulus longer (1–1.3 mm), ovary more or less cylindrical, longer (3–3.2 mm), capsule longer (4–4.5 mm). Besides, the seed testa of *A. tenuiflorum* shows more elongated cells (50–61 µm long), with anticlinal walls depressed, covered by dense and regular strip-like sculptures, and periclinal walls highly convex, with markedly tubercles arranged in 1–2 rows. Both species have a diploid chromosome complement, with $2n = 16$, but the karyotype structure is rather different (see Brullo *et al.* 2013 Fig. 3C, D). In fact, *A. tenuiflorum* shows a more symmetric karyotype (Fig. 7), mainly characterized by median chromosomes and only one submetacentric pair (*vs.* 2 *sm* pairs in *A. serbicum*). Moreover, many populations of *A. tenuiflorum* revealed the presence of supernumerary chromosomes, with 0 up to 6 B-chromosomes (Brullo *et al.* 2003b, 2013).

On the whole, *A. serbicum* due to some its morphological peculiarities, especially regarding the leaf anatomy, type of inflorescence and floral traits, seems to have no close taxonomical relationships with Mediterranean species of the *A.* sect. *Codonoprasum*. For globose inflorescence, and short spathe valves with few ribs, it shows only some weak affinity with the species belonging to group of *A. staticiforme* Smith in Sibthorp & Smith (1809: 225), but it differs significantly from these ones in having large tepals exceeding 4 mm in length and stamens included in the perigone (Brullo *et al.* 1995, 2017, Koçyiğit *et al.* 2023).

Previously, Clementi *et al.* (2015) designated as lectotype of *A. serbicum* the illustration cited in the protologue by Visiani & Pančić (1865, Tab. 22, fig. 1), preferring it to the isotype belonging to original material kept in Pančić's herbarium mentioned by themselves "Mokra gora Serb[ia] merid[ionalis] in rupestrib[us] calcareis, Jul[io], *J. Pančić s.n.* (BEOU 11931!)", since in their opinion it was not preserved in good conditions. The choice of this lectotype is not in conformity with the Article 9.12 of the ICN (Turland *et al.* 2018) and therefore must not be followed. Applying the Article 9.17 of the ICN, the specimen at the left of the herbarium sheet preserved at BEOU 11931! is here chosen as a lectotype of *A. serbicum* in second-step typification (Fig. 2).

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