Disentangling the taxonomic structure of the *Allium paniculatum* species complex in central and eastern Europe using molecular, cytogenetic and morphological tools

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Abstract: *Allium* section *Codonoprasum* includes a group of evolutionarily young taxa with unclear taxonomic boundaries, evolutionary relationships and complex synonymy. The most complicated taxon in this section is *A. paniculatum*, which until recently was considered to be the most widespread and morphologically most variable species, with a distribution extending from the Mediterranean area to central Europe and the Pontic region. A recent taxonomic study has shown that true *A. paniculatum* is a morphologically and genetically distinct species occurring in southern Ukraine and southern Russia. The clarification of its taxonomic identity questioned the taxonomic identity of populations referred to as *A. paniculatum* in other parts of Europe, including populations in central (Slovakia, Hungary, northeast Serbia) and the western part of eastern Europe (Romania, Ukraine), from where four other species of this complex (i.e. *A. fuscum*, *A. fussii*, *A. marginatum* and *A. podolicum*) were described and later frequently merged as synonyms with *A. paniculatum*. Here, the diversification within this complex in the abovementioned region is explored, using various biosystematic methods (morphometry, leaf anatomy and epidermal micromorphometry, karyology, estimation of genome size and GC content using flow cytometry, and molecular fingerprinting using Amplified Fragment Length Polymorphisms). By this means the existence of three well-separated population groups were revealed in the material studied, taxonomically corresponding to *A. fuscum*, *A. marginatum* and the group *A. paniculatum* + *A. podolicum*. The genetic patterns indicate that populations of at least some of these taxa might have survived in their current ranges or in neighbouring areas during the last glacial and the early Holocene. *Allium fuscum* is a species that inhabits shaded rocky outcrops in the wide neighbourhood of the Iron Gate area (south-western Romania, north-western Bulgaria and northeast Serbia). *Allium marginatum* is a species closely related to *A. fuscum*, inhabiting dry grasslands and dry oak forests in the Pannonian and Transylvanian Basins. Two eastern-European taxa, *A. podolicum* and *A. paniculatum*, which are very similar in morphology, genome size and genetic markers, are considered to be conspecific and named *A. paniculatum*. This species inhabits zonal steppe and forest steppe regions in eastern Europe from western Ukraine to southern Russia. In addition, a taxonomic and nomenclatural treatment, as well as a key for identifying the recognized species of the *A. paniculatum* complex in the region studied, are presented.

Keywords: *Allium paniculatum*, Amplified Fragment Length Polymorphisms, chromosome number, epidermal micromorphology, GC content, genome size, leaf anatomy, morphometrics, polyploidy, taxonomy

Introduction

With about a thousand accepted species, the genus *Allium* L. belongs to the largest monocot genera (Govaerts et al. 2021, WCVP 2022). However, despite intensive research, there are still many gaps in our knowledge of the infrageneric classification and evolution of this genus (Friesen et al. 2006, Li et al. 2010). The overall morphological similarity of *Allium* species, which is further complicated by the loss or even lack of many diagnostic characters on dry specimens (Don 1827, Brullo 2009), as well as the frequent occurrence of polyploidy (Peruzzi et al. 2017, Duchoslav et al. 2020, Han et al. 2020), obscure the morphological distinctiveness of many taxa (Ascherson & Graebner 1905, Hanelt et al. 1992, Mathew 1996, Gregory et al. 1998, Fialová et al. 2014). Therefore, there is an urgent need to use a comprehensive biosystematic approach to overcome these obstacles.

The synthesis of available data on the European representatives of the genus *Allium* in Flora Europaea (Stearn 1980) provides the basis for further biosystematic research, but also reveals an untenably broad concept for many species. This is especially the case in *A*. sect. *Codonoprasum* Reichenb., which consists of a set of morphologically similar, diploid and polyploid bulbous species, characterized by ribbed, glabrous or hairy leaves with a semicylindrical to flat outline, two-valved persistent spathe with unequal valves longer than the pedicels, a campanulate perigon with simple stamens and an ovary with inconspicuous nectaries (Stearn 1980, 1981, Brullo et al. 1994, 1996, 1997a, b, c, 2001, 2003, 2008, Friesen et al. 2006, Salmeri et al. 2016). The centre of diversity of this section is located in the Mediterranean region, particularly Greece and Turkey, but extends to other parts of Europe, North Africa and the Middle East (Vvedensky 1935, Wilde-Duyfjes 1976, Stearn 1981, Kollmann 1984, 1986, Pastor & Valdes 1985, Özhatay 1990, Brullo et al. 1996, 1998, 2001, 2014, Hanelt 1996). Since the publication of the synthesis of the genus *Allium* in Flora Europaea (with 22 species belonging to *A*. sect. *Codonoprasum*, Stearn 1980), there has been a great increase in taxonomic and biosystematic research on section members, which has resulted in dozens of newly described species (e.g. Brullo et al. 1997b, c, 2003, 2014, Tzanoudakis & Tan 2000, Jauzein et al. 2002, Bogdanović et al. 2008, 2009, 2011, Kalpoutzakis et al. 2012, Koçyiğit & Özhatay 2012, Tzanoudakis & Trigas 2015, Koçyiğit et al. 2016, Jandová et al. 2017, Özhatay et al. 2018, Galanos & Tzanoudakis 2019, Ioannidis & Tzanoudakis 2022) and taxonomic reassessments of a number of previously described species (e.g. Brullo et al. 1991, 1994, 1996, 1998, 2008, Salmeri et al. 2016). However, this effort has not resulted in a better integration of regional taxonomic concepts. Although the taxonomy of *A*. sect. *Codonoprasum* is summarized in several recent regional floras and monographs (e.g. Brullo et al. 2001, Jauzein & Tison 2001, Seregin 2007, Anačkov 2009, Cheshmedzhiev 2011, Aedo 2013, Tison & Foucault 2014, Brullo & Guarino 2017), they are not entirely consistent and the taxonomic status of many described taxa remains unresolved.

Allium paniculatum L. is apparently the most striking example of a wrongly interpreted species in this section (Salmeri et al. 2016). Linnaeus (1759) description of this species includes a short protologue with only a few diagnostic characters, which could be applied to a wide range of species within this section. Although he subsequently partially clarified the description of this species, he also states that it occurs in many regions, including Austria, Italy, Siberia and the Orient (Linnaeus 1762). Unfortunately, this led

to the misinterpretation of this species in subsequent taxonomic treatments and floras (e.g. Ascherson & Graebner 1905, Jávorka 1925, Jordanov 1964, Wendelbo 1971, Stearn 1978, 1980, Garbari 1982, Kollmann 1984, 1986, Pastor & Valdes 1985, Dostál 1989, Oprea 2005, Aedo 2013). Moreover, many of the subsequently described species were accepted as intraspecific taxa or only as synonyms of *A. paniculatum.* As a result, *A. paniculatum* was regarded as one of the most widespread species in this section (informal "*A. paniculatum* complex"), with a wide morphological range of locally distinguishable forms (Wilde-Duyfjes 1976, Stearn 1978, 1980, 1981). The decisive progress in clarifying the taxonomic identity of *A. paniculatum* was made by Salmeri et al. (2016), who collected and studied living material of this species from its locus classicus in southern Ukraine, based on the lectotypification of the species by Wilde-Duyfjes (1973). Taken together, these results clearly show that "true" *A. paniculatum* is morphologically and molecularly separated from the other taxa in this section and its distribution is more limited, restricted to southern Ukraine and southern Russia (Salmeri et al. 2016), where it inhabits steppes and forest steppes (Bordzilovsky 1950, Omelchuk-Myakushko 1979).

However, the clarification of the taxonomic identity of *A. paniculatum* questions the taxonomic identity of populations referred to as *A. paniculatum* in other parts of Europe. This also applies to populations in central Europe and the western part of eastern Europe (Slovakia, Hungary, Romania, Ukraine, Moldova, Serbia; e.g. Jávorka 1925, Polívka et al. 1928, Zahariadi 1966, Dostál 1989, Marhold & Hindák 1998, Čeřovský et al. 1999, Dobrochaeva et al. 1999, Somogyi 1999, Ciocârlan 2000, Oprea 2005, Cheshmedzhiev 2011, Király et al. 2011, Ghendov 2015), from which four other species in this complex (i.e. *A. fuscum*, *A. fussii*, *A. marginatum* and *A. podolicum*) are described or reported to occur. However, most of these taxa were ignored or considered to be synonyms or reclassified into infraspecific taxa of *A. paniculatum*.

Allium fuscum Waldst. et Kit. was described from rocky calcareous sites in Băile Herculane in the Banat region, Romania (Waldstein & Kitaibel 1808). Some authors consider *A. fuscum* to be a valid species (Regel 1875), but often in a broader sense, which frequently resulted in the assignment of plants of related species with brownish and brown-green perigon to this taxon (e.g. Reichenbach 1828, Stearn 1980, Garbari 1982, Kollmann 1984), whereas several other authors accepted it only as a synonym of *A. paniculatum* (e.g. Kerner 1878, Wilde-Duyfjes 1976) or treated it as infraspecific taxon within *A. paniculatum* (e.g. Boissier 1882, Ascherson & Graebner 1905, Soó 1972, Stearn 1980, Kollmann 1984, 1986). Brullo et al. (1996) study of both living and herbarium collections of *A. fuscum* from and around the locus classicus reveals that it is morphologically well differentiated from related taxa and occurs as a rare chasmophyte in south-western Romania and north-western Bulgaria. However, the confusion over the identity of many populations of plants of the *A. paniculatum* complex with brownish perigon still persists (e.g. Oprea 2005, Cheshmedzhiev 2011, Ghendov 2015). Moreover, Kerner (1878) describes another species related to *A. fuscum*, namely *A. fussii* Kerner, from rocky outcrops in the Eastern Carpathians, Romania. Zahariadi (1966) considers this species to be an altitudinal variety of *A. fuscum*, while Soó (1972) and Ciocârlan (2000) treat it as a subspecies of *A. paniculatum* or *A. fuscum*, respectively. Brullo et al. (1996) argue that *A. fussii* should be considered a separate species due to several characteristics that distinguish it from *A. fuscum*, but no recent biosystematic data are available.

Allium marginatum Janka was described from dry grasslands on slopes of a hilly area in north-western Transylvania, Romania, based on plants with white petals with brownish to purple midrib and petal margins (Janka 1884). Later on, this species was incorporated into *A. paniculatum* at various infraspecific taxonomic levels (e.g. Ascherson & Graebner 1905, Soó 1972, 1973) or considered to be a mere form of *A. fuscum* (Zahariadi 1966). Consequently, this species almost disappeared from central-European checklists and floras, including those for Romania (Ciocârlan 2000, Oprea 2005) and Hungary (Király et al. 2011), from where Janka (1884) reports its occurrence. In some other recent taxonomic sources from central Europe it is referred to as *A. paniculatum* subsp. *marginatum* (Janka) Soó (e.g. Király 2007, Bartha & Király 2015) or accepted as an independent species (Bartha et al. 2022). In fact, its taxonomic status, diagnostic morphological characters and distribution are currently unclear.

Allium podolicum Błocki ex Racib. was published by Raciborski & Szafer (1919), referring to Błocki's specimens collected from dry grasslands in the Podolia region, western Ukraine (Stearn 1978). This species is described as having narrow leaves and pink perigons, similar to the true *A. paniculatum* (Salmeri et al. 2016). Nevertheless, *A. podolicum* is included in (eastern) European floras (Bordzilovsky 1950, Omelchuk-Myakushko 1979, Stearn 1980, Czerepanov 1981, 1995, Dobrochaeva et al. 1999, Oprea 2005), ignored by Vvedensky (1935), or considered to be a synonym of *A. paniculatum* (Zahariadi 1966, Seregin 2007). Given that the ranges of *A. podolicum* and *A. paniculatum* appear to overlap and the ambiguous morphological differences between these two species (Bordzilovsky 1950, Omelchuk-Myakushko 1979, Dobrochaeva et al. 1999, Salmeri et al. 2016), it is questionable whether to distinguish *A. podolicum* as a separate species.

Here, the pattern of diversification within the taxonomically challenging *A. paniculatum* complex in central and the western part of eastern Europe is studied. Recent molecular investigations (ITS and plastid DNA) indicate that *A.* sect. *Codonoprasum* is a monophyletic group of taxa, which diversified recently (Friesen et al. 2006, Salmeri et al. 2016, Han et al. 2020), but also reveals the limitations of ITS and/or plastid DNA markers for resolving relationships among closely related species (Salmeri et al. 2016, K. Vojtěchová et al., unpublished results). Moreover, there are no population-based molecular studies encompassing genetic markers with high resolution allowing one to evaluate potential variation within species or species complexes within this section. Therefore, in addition to classical approaches (morphometry, anatomy), flow cytometry (FCM; Kron et al. 2007) and Amplified Fragment Length Polymorphisms (AFLP; Vos et al. 1995) were used to answer the following questions. (i) Does the variation in genetic structure, genome size, GC content, chromosome number, leaf anatomy and morphology within the *A. paniculatum* complex in central and the western part of eastern Europe allow the delimitation of the above-mentioned species or indicate a need for taxonomic reappraisal? (ii) Which morphological characters can be used to discriminate between taxa? (iii) What is the current distribution of the eventually redefined taxonomic units?

Material and methods

Plant material and definition of taxonomic groups

Due to the problem of identifying taxa of the *A. paniculatum* group, a special approach to determining the plants sampled was employed. The determination was based on the descriptions given in the original species descriptions or studies dealing with their taxonomy (Waldstein & Kitaibel 1808, Kerner 1878, Janka 1884, Raciborski & Szafer 1919, Brullo et al. 1996, Salmeri et al. 2016) and their reported distribution in regional floras. This investigation was based on plant material from the core distribution areas of the taxa studied in central and eastern Europe (Fig. 1A). Specifically, an effort was made to collect populations from or close to the type localities. *Allium fuscum* was sampled in the Banat region in south-western Romania (Waldstein & Kitaibel 1808, Brullo et al. 1996), with one additional locality in south-eastern Serbia. The locality where the type of *A. fussii* was collected (Kerner 1878) was visited, but no plants of the *A. paniculatum* complex were found. Populations of the *A. paniculatum* group occurring in the Pannonian and Transylvanian basins are reported under ambiguous names in national Floras and taxonomic literature (*A. fuscum*, *A. marginatum*, *A. paniculatum*). The samples from populations in this region were treated as one group for which the name *A. marginatum* is assigned below. The population from the locality Sucutard near Cluj, Transylvania, Romania, which can be considered the type locality of *A. marginatum* (see below for details), is also included in this group. *Allium podolicum* was sampled in Podolia region, western Ukraine (Ascherson & Graebner 1905, Raciborski & Szafer 1919, Stearn 1978, Dobrochaeva et al. 1999). *Allium paniculatum* was collected from localities in Zaporizhia region, southern Ukraine, considered to be the locus classicus of this species (Salmeri et al. 2016). In summary, four taxonomic groups were included in the analyses: *A. fuscum*, *A. marginatum*, *A. podolicum* and *A. paniculatum*.

In total, 248 living plants (or seed from different individual plants) from 39 populations were collected in the field, transported, potted and cultivated under identical environmental conditions (open site, water supplied by natural rain, occasional watering in dry summer periods) in the experimental garden of Palacký University in Olomouc, Czech Republic (coordinates: 49°34'32.1"N, 17°16'59.8"E). Cultivated plants were used in all subsequent analyses. The list of all the populations studied with localities is provided in Supplementary Table S1. Voucher specimens were deposited in the Herbarium of Palacký University in Olomouc (OL).

Molecular methods

Total genomic DNA was extracted from ~70 mg of fresh leaves following the CTAB protocol (Doyle & Doyle 1987) with minor modifications. The final DNA pellets were dissolved in 70 μl of distilled water. The quality of isolated DNA was checked using agarose electrophoresis and the DNA concentration measured using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, USA).

Twenty-four populations, with one to six individuals per population, were AFLP fingerprinted (Table 1); 15 individuals were replicated in order to determine the error rate, and two blanks (DNA replaced with water) were included to test for contamination. The AFLP procedure followed the protocol of Vos et al. (1995) with modifications
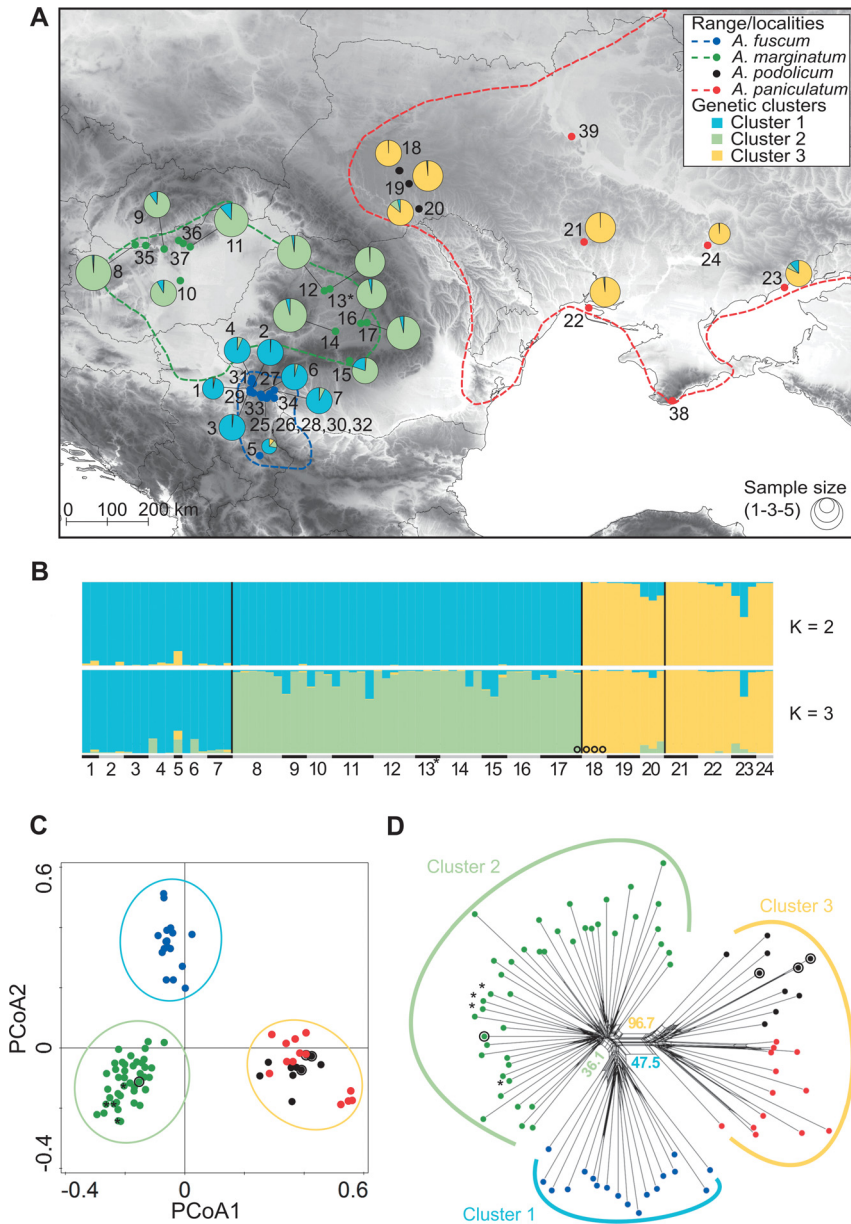
Fig. 1. Results of molecular analyses of 84 individuals of the *Allium paniculatum* complex based on 544 AFLP loci. (A) Sampled populations of the four taxonomic groups (dots) and spatial visualization of Bayesian assignment probabilities of the genetic clusters in the analysed populations (pie charts), based on STRUCTURE clustering for K = 3. The size of each pie chart represents the number of individuals analysed. The distributions of the three distinguished species based on current knowledge are outlined in the map (dashed lines). (B) Bar plot showing Bayesian assignment probabilities of each individual using the software STRUCTURE for K = 2 and 3. Numbers below the plots are population IDs (for population coding see Supplementary Table S1). (C) Principal coordinate analysis (PCoA) using Jaccard's similarity coefficient. The first and the second axis explained 11.1% and 6.2% of the entire variation, respectively. (D) Neighbor-Net diagram. Colours of dots in A, C, D indicate taxonomic groups (see also inset in A; blue, *A. fuscum*; green, *A. marginatum*; black, *A. podolicum*; red, *A. paniculatum*); ellipses in C and thick lines in D mark AFLP (genetic) clusters for K = 3 as visible in B (light blue, cluster 1; light green, cluster 2; light yellow, cluster 3), triploids are marked by a black traced circle. Samples from type locality of *A. marginatum* (population no. 13) are marked by *. Bootstrap support (in %) for the three main groups is reported in D.

Table 1. Genetic diversity (D) and frequency-down-weighted marker values (DW) for the populations of the four taxonomic groups analysed using AFLPs. For each taxonomic group the mean±SD of each variable was calculated. Taxonomic groups that do not differ in D or DW are indicated by the same letter (Bonferroni corrected multiple paired comparisons using PermANOVA). n – number of individuals per ploidy investigated using AFLP. RO – Romania, SK – Slovakia, HU – Hungary, UA – Ukraine. For detailed information on localities see Supplementary Table S1.

Taxonomic group	Population ID	Country	n	Population ploidy composition (2n)	D	DW
A. fuscum	1	RO	2	2x	0.20	10.25
	2	RO	3	2x	0.18	7.70
	3	RO	3	2x	0.16	6.76
	4	RO	3	2x	0.20	7.86
	5	RS	1	2x	–	–
	6	RO	3	2x	0.18	6.92
	7	RO	3	2x	0.14	8.14
Mean±SD				–	0.18±0.02[a]	7.94±1.25[a]
A. marginatum	8	SK	6	2x	0.14	5.60
	9	HU	3	2x	0.17	6.70
	10	HU	3	2x	0.17	8.21
	11	HU	5	2x	0.16	5.30
	12	RO	5	2x	0.16	5.47
	13	RO	4	2x	0.15	6.30
	14	RO	5	2x	0.16	7.03
	15	RO	3	2x	0.16	7.32
	16	RO	4	2x	0.16	5.80
	17	RO	4/1	2x+3x	0.15	5.31
Mean±SD					0.16±0.01[a]	6.30±0.99[b]
A. podolicum	18	UA	0/3	2x+3x	0.14	8.78
	19	UA	4	2x	0.16	8.06
	20	UA	3	2x	0.18	8.56
Mean±SD					0.16±0.02[a]	8.46±0.37[a]
A. paniculatum	21	UA	4	2x	0.05	6.56
	22	UA	4	2x	0.17	7.73
	23	UA	3	2x	0.20	9.58
	24	UA	2	2x	0.10	10.01
Mean±SD					0.13±0.07[a]	8.47±1.61[a]

described in Schönswetter et al. (2009). In addition, 0.25 U of polymerase was used in the preselective and selective amplifications and 0.4 U for the NED-labelled primer combination. Twelve selective primer combinations were initially screened. Finally, the three primer combinations for selective PCR (fluorescent dye in brackets) were EcoRI (FAM)-ACA/MseI-CATG, EcoRI (VIC)-ACG/MseI-CAAC and EcoRI (NED)-AAC/MseI-CAGG. Purification and visualization of PCR products were done as described in Schönswetter et al. (2009).

All electropherograms were visualized in Genographer 1.6.0 (Montana State University, USA) for fragment scoring. Problematic profiles were removed. The remaining ones were scored using Peak Scanner 2.0 (Applied Biosystems, Foster, USA) using default peak detection parameters. Automatic binning and scoring were performed using RawGeno 2.0.1 (Arrigo et al. 2009) in R 2.15.2 (R Core Team 2012), with the following settings: scoring range = 100–500 bp, minimum intensity = 80 relative fluorescence units, minimum

bin width = 1 bp and maximum bin width = 1.5 bp. Fragments with a reproducibility of less than 85% based on a single sample comparison were discarded. The error rate (Bonin et al. 2004) was calculated using RawGeno 2.0.1. Fragments present or absent in only one individual were removed from the data set.

Bayesian non-hierarchical clustering was used to define AFLP groups in STRUC-TURE 2.3.2.1 (Pritchard et al. 2000) using a Markov chain Monte Carlo (MCMC) algorithm and an admixture model with correlated allele frequencies. Ten replicates of each K = 1–24 (upper value corresponding to the number of populations analysed) were used to determine the stability of the results. The burn-in of 100,000 generations followed by 1,000,000 additional generations of MCMC chains (Falush et al. 2007) were run on Metacentrum VO infrastructure (https://metavo.metacentrum.cz). The output files were evaluated using the PophelperShiny script (Francis 2017) in R to determine the optimal number of clusters (K) based on the second order rate of change of the likelihood function with respect to K (ΔK; Evanno et al. 2005) and to generate graphical outputs for selected Ks.

Principal coordinate analysis (PCoA) based on the square root Jaccard distance matrix between individuals was calculated in Canoco 5 (ter Braak & Šmilauer 2012). A Neighbor-Net network based on uncorrected P distances was calculated using SplitsTree 4 (Huson & Bryant 2006). To evaluate the support for the major branches of the Neighbor-Net network, a bootstrap analysis with 1,000 pseudoreplicates was performed.

Genetic diversity (D), determined as the average number of pairwise differences between genotypes (Kosman 2003), was calculated using AFLPdat (Ehrich 2006). To quantify the number of rare markers without setting an arbitrary threshold, frequency-down-weighted marker values (DW) were calculated according to Schönswetter & Tribsch (2005) in AFLPdat (a population with only one individual was excluded from the calculations). The average of individual values obtained for a population was used in order to diminish the effect of differences in sample size following Ehrich et al. (2007). Permutational ANOVA (pANOVA) was used to test for differences between taxonomic groups, based on the Euclidean distance measure (Anderson 2001). The significance was computed by permutation of group membership, with 999 replicates in Past 4.06 (Hammer et al. 2001). The relationship between D and DW, and geographic coordinates (latitude, longitude) was estimated using Pearson correlation for all populations regardless of taxonomic assignment and separately for subsets of populations.

Analysis of molecular variance (AMOVA; Excoffier et al. 1992; implemented in Arlequin 3.5.1.2; Excoffier & Lischer 2010) was performed to determine the distribution of genetic variance within and between taxonomic groups and populations. A three-level AMOVA was used to determine the distribution of genetic variance within and between taxonomic groups and populations. A two-level AMOVA was used to determine the distribution of genetic variance among and within populations of each taxonomic group, with additional analysis merging *A. paniculatum* + *A. podolicum*. To test the significance of isolation-by-distance, we performed Mantel test on the matrix of genetic distances (using Slatkin's linearized F_{ST}) and the matrix of geographical distances between populations with 999 random permutations, using PASSAGE 2 (Rosenberg & Anderson 2011). This was done separately for each taxonomic group, the group *A. paniculatum* + *A. podolicum* and for all populations regardless of taxonomic assignment.

Flow cytometry and chromosome numbers

The DNA ploidy level (relative genome size, RGS; Suda et al. 2006) and genome size (absolute genome size, AGS; Greilhuber et al. 2005) were estimated using flow cytometry. Samples were prepared according to the protocol described by Duchoslav et al. (2010) and were run on the following flow cytometers using two fluorochromes: (i) Partec PAS (Partec GmbH, Münster, Germany) – propidium iodide (PI) + RNAse (both 50 µg·ml^{-1}); (ii) BD Accuri C6 (BD Biosciences, San Jose, USA) – PI + RNAse; (iii) Partec CyFlow ML (Partec GmbH) – 4,6-diamidino-2-phenylindole (DAPI, 5 µg·ml^{-1}). *Secale cereale* L. 'Daňkovské' (2C = 16.19 pg; Doležel et al. 1998) served as a primary internal standard; other internal standards were calibrated against *Secale cereale*, i.e. *Triticum aestivum* 'Saxana' (2C = 34.24 pg) and *Pisum sativum* 'Ctirad' (2C = 8.75 pg). In general, only histograms with coefficient of variation (CV) less than 5% were accepted (except for population no. 38 with CV > 5%). For each sample, fluorescence intensity of 3,000 and 5,000 particles was recorded for the RGS and AGS estimations, respectively. For RGS estimations, separate plants or mixed samples of up to 4 plants per population were measured. The sample measurements for AGS were averaged per plant over three repetitions on different days and if the between-day variation in AGS exceeded 2%, the measurement was repeated (Doležel et al. 2007). The GC content was determined following Šmarda et al. (2008), based on the comparison of parallel measurements with both fluorochromes. Chromosome numbers were counted following the protocol of Duchoslav et al. (2010). These chromosome counts served as reference material for the estimates obtained using FCM.

Morphology, epidermal micromorphology and leaf anatomy

Morphometric analyses were performed on 111 individuals from 21 populations. A total of 42 morphological characters (23 quantitative, 19 qualitative) were mostly measured using an adjustable ruler or calliper, or scored on fresh material, and six additional ratios were calculated (for survey see Supplementary Table S2). Leaves were studied when they started to senescence, tepals and ovaries in the period of stigma receptivity and stamens just prior to the maturity of the anther and the release of pollen. During flowering, position of the valves of the spathe was recorded. Generative parts, as well as the capsules and seeds, were studied on three randomly selected flowers or fruits per plant. Size measurements of these traits were made from their digitized scans in ImageJ 1.50i (Rasband 2021). Finally, the leaf blades were preserved in 70% ethanol for a subsequent anatomical study.

The transverse sections of the leaf blades were made manually using a razor blade and then stored temporarily in glycerol. The epidermal characters (Supplementary Table S2) were investigated by direct observation of epidermal slides of the central part of the leaf blade. To remove the mesophyll, boiling for 20 minutes at 100 °C in 80% lactic acid was used. Subsequently, the epidermal part of the leaf was peeled off and a microscopic preparation was made. Using a digital camera Olympus DP70 on an Olympus BX60 microscope (Olympus Corp., Tokyo, Japan), 10 randomly positioned images were taken of each sample. The densities of epidermal cells and stomata were determined per image area and extended cells/stomata were only counted on two sides of the field of view. The resulting value was converted to 1 mm^2 of leaf surface. The character and distribution of micropapillae was observed on the leaf margins and the density of clavate papillae per 2 mm

of leaf calculated. The classification of types of the leaf margin is a modified version of that proposed by Mifsud & Mifsud (2018).

Three datasets were prepared and used in the analyses. (i) Matrix 1 – complete dataset including all 111 individuals as OTU and all primary morphological and derived characters measured on leaves, scape and flowers. (ii) Matrix 2 – a subset of 74 individuals with characters measured/recorded on capsules and seeds and 77 individuals measured or scored for epidermal characters on leaves. These characters were studied on a limited number of individuals in all populations. (iii) Matrix 3 – dataset prepared for multivariate analyses, including all 111 individuals as OTU and reduced set of characters from matrix 1. Multicollinearity was assessed by variance inflation factor (VIF) for quantitative traits using vifstep (th = 5) command from the library usdm (Naimi 2017). Specifically, four quantitative variables or ratios (w2L, plH, lInT, lLSpV) were excluded from matrix 1 because of multicollinearity. The potential problem of multicollinearity in categorical characters was accessed by Cramer's V (Legendre & Legendre 2012), but no variable had Cramer's V greater than 0.5 in paired analyses. Therefore, all qualitative variables were used.

Principal coordinate analysis (PCoA), using Gower's dissimilarity coefficient for mixed data (calculated by the StatMatch library; D'Orazio 2015), was used to obtain information on the phenetic relationships between all the individuals studied (matrix 3). Before analyses, some quantitative characters were log-transformed to improve normality. The significance of the correlation of quantitative characters with the first two PCoA factors and the goodness-of-fit of the levels of the class variables in the ordination diagram were tested using the Monte Carlo permutation test (MC test) with 999 permutations. Subsequently, matrix 3 was subjected to constrained principal coordinate analysis (db-RDA; Legendre & Anderson 1999) to test the null hypothesis of no morphological differences between taxonomic groups using a MC test with 999 permutations. PCoA and db-RDA were run using vegan (Oksanen et al. 2020) and Canoco 5, respectively.

To compare taxonomic groups, matrices 1 and 2 were analysed using univariate statistics. One-way ANOVA and Bonferroni multiple comparison tests were used for quantitative characters and their ratios, except for several traits for which the Kruskall-Wallis test and Dunn's multiple comparison test were applied. In the analyses of qualitative variables, log-linear models (LogLM) were used. When the overall LogLM was significant, a separate LogLM was done for each pair of taxa. A small value (0.25; i.e. delta value) was added to each cell count when zeros were present in the table. Calculations were done using NCSS 9 (NCSS, LLC, Kaysville, USA).

Results

AFLP fingerprinting

A total of 601 AFLP fragments were scored for 84 individuals from which high-quality, reproducible AFLP fingerprints were obtained; 57 fragments were unique and were excluded from further analyses. The average replicate error rate was 5.3% and the average number of loci per individual was 120.

The STRUCTURE analysis proposed K = 2 as the most appropriate number of genetic groups. The first genetic group corresponds to the taxonomic groups *A. fuscum* and *A. marginatum*, and the second to *A. paniculatum* and *A. podolicum*. Thus, this division

separated the populations on the western and the eastern side of the Eastern Carpathians (Fig. 1A, B). At K = 3, three genetic groups (clusters) corresponded to the three taxonomic groups: *A. fuscum* (~ genetic cluster 1), *A. marginatum* (~ genetic cluster 2) and the group of *A. paniculatum* and *A. podolicum* (~ genetic cluster 3; Fig. 1A, B). Solutions with higher K (4–24) differed from those with K = 2–3 by separating individual populations (not shown). Several individuals, mostly from the *A. marginatum* group (population no. 15), were found to have a higher admixture of *A. fuscum* (Fig. 1A, B).

The first and second PCoA dimensions explained 11.1% and 6.2% of the variability and confirmed the results of the STRUCTURE analysis. Three well-separated genetic clusters were distinguished in the ordination space that correspond to three taxonomic groups (Fig. 1C): *A. fuscum*, *A. marginatum* and the group of *A. paniculatum* and *A. podolicum*. The Neighbor-Net network produced comparable results, showing a clear split between the eastern populations (*A. paniculatum*, *A. podolicum*) and the western populations (*A. fuscum*, *A. marginatum*). Within the western populations, a split was subsequently found between *A. fuscum* and *A. marginatum* (Fig. 1D). Population 13, the type locality of *A. marginatum* (see below), falls within genetic cluster 2.

The maximum level of genetic diversity (D) was recorded in two populations of *A. fuscum* (no. 1: 0.20, no. 4: 0.20), and in a population of *A. paniculatum* (no. 23: 0.20), the lowest D was recorded in a population of *A. paniculatum* (no. 21: 0.05). In general, the taxonomic groups did not differ from each other in D (pANOVA, F = 1.93, P = 0.157, Table 1). The frequency-down-weighted marker values (DW) ranged from 5.30 in a population of *A. marginatum* (no. 11) to 10.25 in a population of *A. fuscum* (no. 21). *Allium marginatum* had a significantly lower DW than other groups (pANOVA, F = 5.56, P < 0.007, Table 1). Except for a significant positive correlation between DW and longitude (r = 0.42, P = 0.046) across the entire complex and a significant negative correlation between D and longitude (r = -0.82, P = 0.004) in *A. marginatum* (genetic cluster 2), no significant relationship between D or DW and latitude or longitude was found across the entire complex and within taxonomic groups (all P > 0.07).

Analysis of molecular variance showed that most of the variance occurs within populations of individual taxonomic groups (69.9%). Only 16.3% of the variance was attributed to differences between taxonomic groups. The remaining 13.8% was attributed to variance among populations within taxonomic groups. The second AMOVA was performed separately for each individual taxonomic group. Among-population variance was higher for the eastern than for the western taxonomic groups (Supplementary Table S3).

The Mantel test revealed a significant correlation between geographical distance and genetic differentiation (Slatkin's F_{ST}) for the entire complex (r = 0.38, P = 0.004). However, when Mantel tests were applied to each of the taxonomic groups or genetic clusters 1–3 separately, a significant correlation between geographical distance and genetic differentiation (with P ≤ 0.05) was detected only in *A. marginatum* (= genetic cluster 2, r = 0.52, P < 0.001).

Ploidy levels, genome size and GC content

Flow cytometric analyses of 248 plants from 39 populations (Supplementary Table S4) revealed the occurrence of two ploidy levels: diploids (2n = 16), dominant in all taxonomic groups (98.4% of total) and rare triploids (2n = 24; 1.6%), detected in one popula-
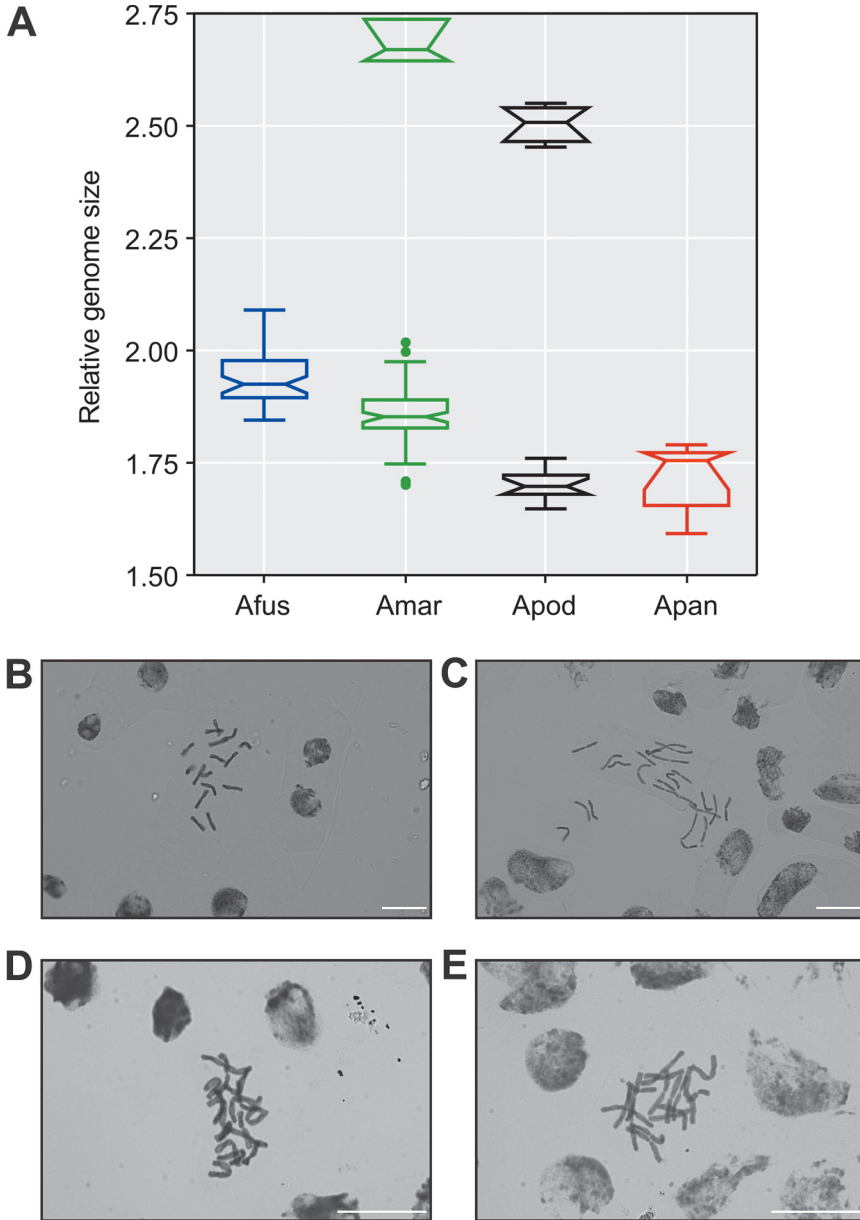
Fig. 2. (A) Box-and-whisker plot with a multiple comparison notch of relative genome size (RGS) of the taxonomic groups studied (Afus, *Allium fuscum*; Amar, *A. marginatum*; Apod, *A. podolicum*; Apan, *A. paniculatum*) and separately for each ploidy level (diploids and triploids in lower and upper part of the plot, respectively). If the notches of two boxes do not overlap, it may be assumed that the medians are significantly different at the 95% level of significance. (B–E) Mitotic metaphase chromosomes of the taxonomic groups studied: *A. marginatum* (B; 2n = 16; population no. 12, Romania, Visea), *A. podolicum* (C; 2n = 24; population no. 18, Ukraine, Vikno), *A. fuscum* (D; 2n = 16; population no. 25, Romania, Orsova), and *A. paniculatum* (E; 2n = 16; population no. 39, Ukraine, Kovalyn). Bar = 20 μm. Photographs by Alena Fišerová, Michaela Jandová and Lucie Kobrlová.

tion of *A. podolicum* and two of *A. marginatum* (Fig. 2A). These data were confirmed by chromosome counts for several plants from each group studied, with triploids being established only within *A. podolicum* (Fig. 2B–E). Except for *A. podolicum* and *A. paniculatum*, mean RGS of diploids differed significantly between taxonomic groups (ANOVA, $F = 94.9$, $P < 0.001$; Fig. 2A; population no. 38 was excluded from testing because of high CV). Both *A. podolicum* (mean±SD; 1.70±0.03) and *A. paniculatum* (1.72±0.07) have a low and similar RGS, while *A. marginatum* has an intermediate (1.86±0.05) and *A. fuscum* has the highest RGS (1.94±0.06). The mean RGS of triploids (*A. podolicum*: 2.50±0.04; *A. marginatum*: 2.69±0.05) was approximately 1.5 times that of diploids (*A. podolicum*: 1.47, *A. marginatum*: 1.45), but when comparing both cytotypes within cytotype-mixed populations, the ratio triploid/diploid RGS approached 1.50 (*A. podolicum*: 1.49, *A. marginatum*: 1.46 and 1.48).

In addition, several plants from different populations were selected for each taxonomic group and the AGS and GC content were determined (Supplementary Table S5). The AGS of the taxonomic groups studied were slightly different and followed the pattern observed for RGS. The GC content was similar both within and between taxonomic groups and varied between 36.1 and 37.9%.

Morphometric analyses

The PCoA of the morphological data (matrix 3) showed a nearly complete segregation of individuals into three clusters partially corresponding to the taxonomic grouping: *A. fuscum*, *A. marginatum* and a cluster containing *A. paniculatum* and *A. podolicum* (Fig. 3A). The first factor was positively correlated with several quantitative characters measured on leaves (i.e. w1L, w_l1L, w_l2L) and ovaries (lO, wO) and was also related to the distribution of categories of several qualitative characters that describe the shape of transverse section of leaf (shL), the colour of the petals (colP, T) and the ovary surface (papO; Fig. 3B). The second factor was negatively associated with the size of the plant (plHwS), the number of leaves (nrL) and flowers (nrF), and the ratio of length to width of the ovary (pIHwS), and related to the distribution of categories of the ovary shape (shO; Fig. 3B). Some individuals of *A. marginatum* overlapped with the *A. fuscum* cluster due to the presence of colours of petals typical for the other group. Triploids of *A. podolicum* and those of the *A. marginatum* were mainly located inside the clusters of their diploid relatives in ordination space (Fig. 3A).

Constrained principal coordinate analysis confirmed the results of PCoA that there is clear morphological differentiation between taxonomic groups except for *A. podolicum* and *A. paniculatum* (the first canonical axis: $F = 9.30$, $P = 0.002$, all canonical axes: $F = 16.51$, $P = 0.002$; Supplementary Fig. S1). The following characters were most strongly correlated with the first two canonical axes and thus important in the morphological differentiation between taxonomic groups: perigon background colour (colP), shape of transverse section of leaf (shL), ovary surface (papO), ovary width (wO), ovary length (lO), width of leaf (w1L) and ratio width/length of leaf (w_l1L, w_l2L).

Descriptive statistics of all characters measured and scored in the taxonomic groups studied are listed in Supplementary Tables S6 and S7, while selected diagnostically significant characters for taxa determination are listed in Tables 2 and 3 and shortly discussed below. Figures 4–7 show representative photographs of selected characters of the four taxonomic groups.
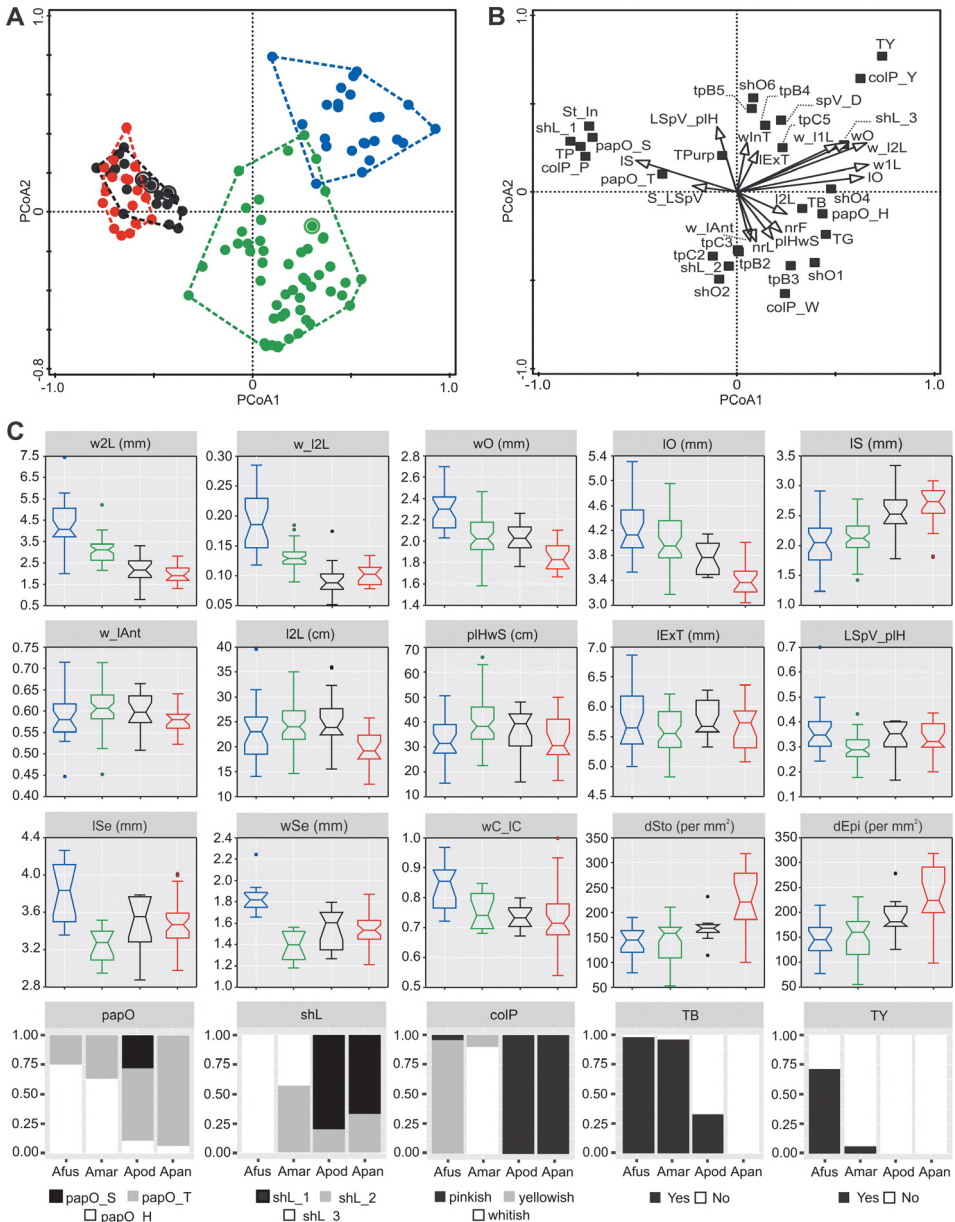
Fig. 3. Results of multivariate and univariate analyses of morphological characters of the *Allium paniculatum* complex. (A) Principal coordinate analysis (PCoA) of matrix 3. Ordination diagram of individual plants as OTUs. (B) PCoA ordination diagram of quantitative (vectors) and qualitative characters (centroids of each category). Only quantitative characters with significant correlations ($P \leq 0.05$) with at least one of the first two dimensions, and qualitative characters differing in the positions of centroids of levels ($P \leq 0.05$) are shown in the diagram. (C) Notched box plots or stacked bar plots of selected morphological characters and their ratios (matrices 1, 2) for the taxonomic groups grown in a common garden. If the notches of two boxes do not overlap, it may be assumed that the medians are significantly different at the 95% level of significance. Afus (blue), *A. fuscum*; Amar (green), *A. marginatum*; Apod (black), *A. podolicum*; Apan (red), *A. paniculatum*. Triploids are marked by an additional circle around the point. Abbreviations of characters are explained in Tables 2 and 3, and Supplementary Table S2.
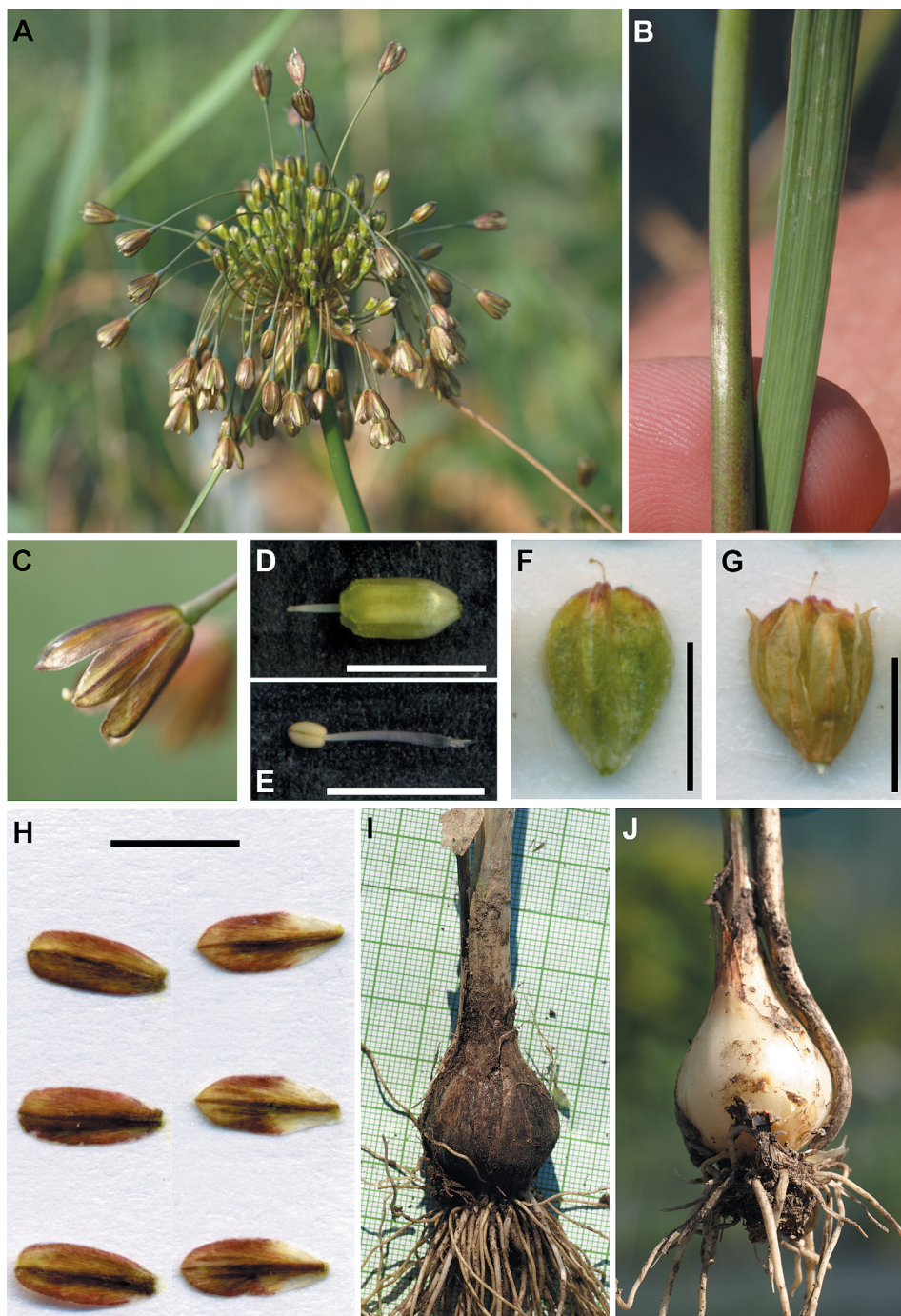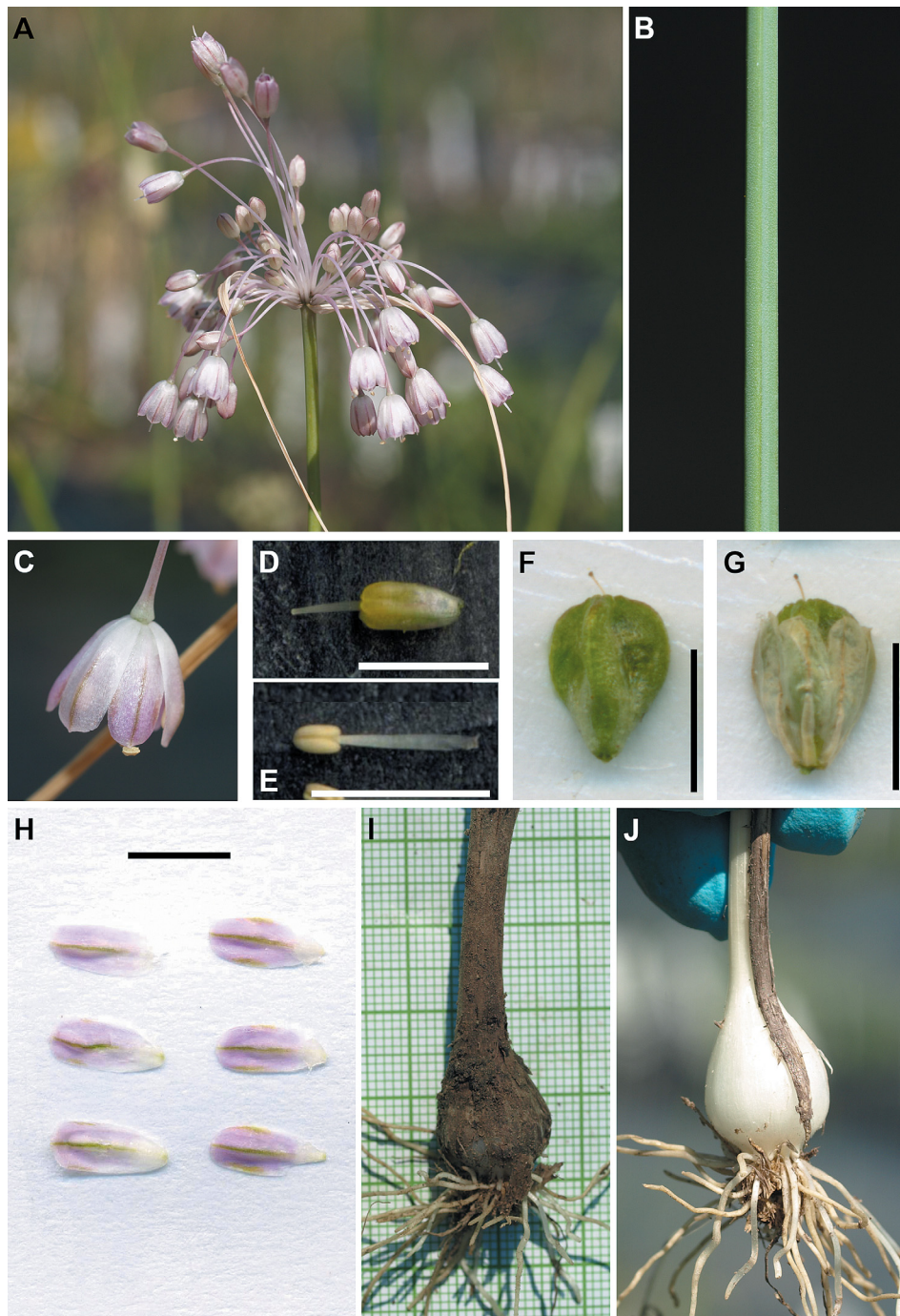
Fig. 4. *Allium fuscum*. (A) Inflorescence with spathes. (B) Leaf. (C) Perigon. (D) Ovary with style. (E) Stamen with filament. (F) Capsule. (G) Capsule within perigon. (H) Outer (left) and inner (right) petals. (I) Bulb within the outer tunic. (J) Bulb within the inner tunic. Scale bar = 0.5 cm. Photographs by Kateřina Vojtěchová and Martin Duchoslav.

Fig. 5. *Allium marginatum*. (A-B) Inflorescence with spathes: variability in perigon colour. (C-D) Perigon. (E) Ovary with style. (F) Stamen with filament. (G) Capsule. (H) Capsule within perigon. (I) Outer (left) and inner petals. (J) Leaf. (K) Bulb within the outer tunic. (L) Bulb within the inner tunic. Scale bar = 0.5 cm. Photographs by Kateřina Vojtěchová and Martin Duchoslav.

The traits measured on the leaves related to size and shape clearly differentiated groups, with *A. paniculatum* and *A. podolicum* having the narrowest leaves with the lowest width/length leaf ratio, *A. marginatum* being intermediate, and *A. fuscum* having the widest leaves with the highest width/length leaf ratio. Unlike the other groups, *A. fuscum* had significantly denser papillae on margin of leaf (Table 2).

Fig. 6. *Allium podolicum*. (A) Inflorescence with spathes. (B) Leaf. (C) Perigon. (D) Ovary with style. (E) Stamen with filament. (F) Capsule. (G) Capsule within perigon. (H) Outer (left) and inner (right) petals. (I) Bulb within the outer tunic. (J) Bulb within the inner tunic. Scale bar = 0.5 cm. Photographs by Kateřina Vojtěchová and Martin Duchoslav.
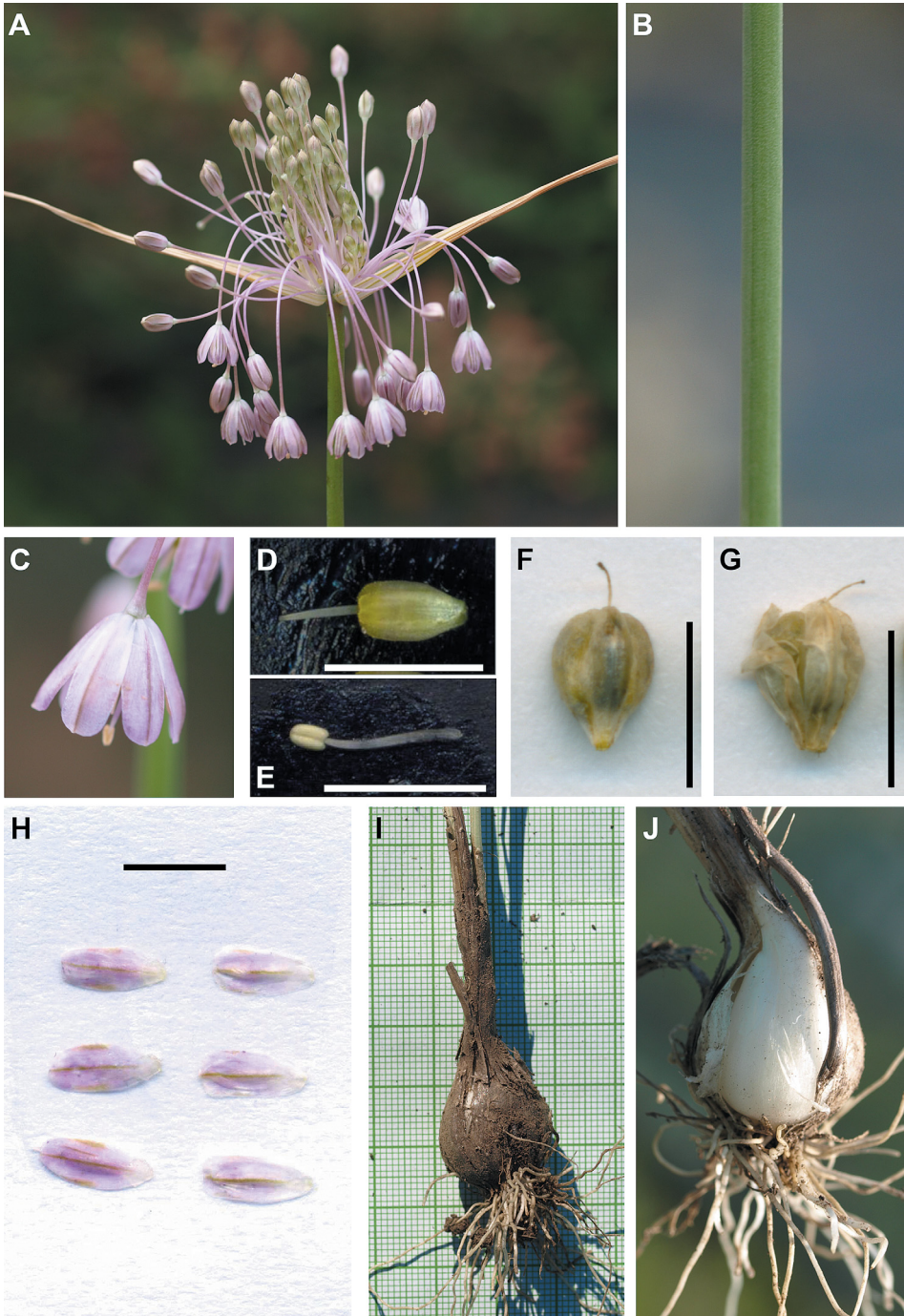
Fig. 7. *Allium paniculatum*. (A) Inflorescence with spathes. (B) Leaf. (C) Perigon. (D) Ovary with style. (E) Stamen with filament. (F) Capsule. (G) Capsule within perigon. (H) Outer (left) and inner (right) petals. (I) Bulb within the outer tunic. (J) Bulb within the inner tunic. Scale bar = 0.5 cm. Photographs by Kateřina Vojtěchová and Martin Duchoslav.

Table 2. Descriptive statistics of selected quantitative characters and their ratios (mean±standard deviation; minimum, 10% and 90% quantile, and maximum) useful for the discrimination of the taxonomic groups, including common di- and rare triploids recorded in *Allium podolicum* and *A. marginatum*. Linear models (LM) with taxonomic category as fixed effect factor, with accounting for different variance in each group, were used for comparison of means of each character among groups. Descriptive statistics based on the original (untransformed) values are presented in the table. Bonferroni correction was also applied to raw P values and those remaining significant are indicated in bold. LM were also recalculated with all triploids excluded from taxonomic groups and if any changes in significance of the tests with and without including triploids were detected, they are reported. Bonferroni multiple comparison test was used after a significant result of LM; different letters in a row indicate significant differences between groups at P ≤ 0.05. Abbreviations of each character are added before the name of the respective character (first column). [#] When only diploids of *A. podolicum* and *A. paniculatum* were compared, no significant differences were found in paired comparison (P > 0.05). Reported only for the case of significant LM tests.

Character	A. fuscum	A. marginatum	A. podolicum	A. paniculatum	F	P
Vegetative morphological characters						
w1L: second uppermost leaf width (mm)	3.5±1.0[a] (1.7–) 1.8–5.0 (–5.7)	2.7±0.8[b] (1.4–) 2.1–3.4 (–7.0)	1.9±0.4[c] (1.1–) 1.4–2.6 (–2.7)	1.8±0.4[c] (1.3–) 1.4–2.3 (–2.8)	27.50	**< 0.001**
w2L: second uppermost leaf width (mm)	4.3±1.2[a] (2.0–) 2.4–5.7 (–7.5)	3.1±0.6[b] (2.2–) 2.4–3.9 (–5.2)	2.2±0.6[c] (0.8–) 1.5–3.2 (–3.3)	2.0±0.4[c] (1.3–) 1.5–2.5 (–2.8)	45.10	**< 0.001**
papB: density of papillae per 2 mm of leaf margin at the base of the second uppermost leaf	9.1±6.9[a] (0.0–) 0.0–19.0 (–25.0)	3.2±5.0[b] (0.0–) 0.0–11.8 (–18.0)	4.2±4.8[b] (0.0–) 0.0–12.0 (–12.0)	2.6±4.1[b] (0.0–) 0.0–10.2 (–12.0)	5.22	0.004
papC: density of papillae per 2 mm of leaf margin in the middle of the second uppermost leaf	13.4±6.1[a] (0.0–) 4.0–22.5 (–26.0)	7.2±6.4[b] (0.0–) 0.0–15.0 (–26.0)	3.8±4.5[c] (0.0–) 0.0–9.3 (–12.0)	3.0±4.7[c] (0.0–) 0.0–10.4 (–14.0)	15.54	**< 0.001**
Generative morphological characters						
wExT: external tepal width (mm)	2.4±0.2[a] (2.0–) 2.1–2.7 (–2.8)	2.2±0.2[b] (1.8–) 2.0–2.4 (–2.6)	2.4±0.2[a] (2.0–) 2.2–2.7 (–2.7)	2.5±0.2[a] (2.2–) 2.3–2.8 (–3.0)	12.48	**< 0.001**
wInT: internal tepal width (mm)	2.5±0.2[a] (2.0–) 2.2–2.7 (–2.7)	2.3±0.2[b] (1.7–) 2.0–2.4 (–2.6)	2.4±0.3[ab] (1.8–) 1.9–2.8 (–2.8)	2.4±0.2[a] (2.1–) 2.2–2.6 (–2.7)	7.26	**< 0.001**
IS: style length (mm)	2.0±0.4[a] (1.2–) 1.5–2.6 (–2.9)	2.1±0.3[a] (1.4–) 1.8–2.5 (–2.8)	2.5±0.4[b] (1.8–) 1.9–3.0 (–3.1)	2.6±0.4[b] (1.8–) 1.8–3.0 (–3.1)	15.03	**< 0.001**
IO: ovary length (mm)	4.2±0.5[a] (3.5–) 3.7–5.0 (–5.3)	4.1±0.4[a] (3.2–) 3.6–4.6 (–5.0)	3.8±0.3[b] (3.4–) 3.5–4.1 (–4.1)	3.4±0.2[c] (3.0–) 3.1–3.7 (–4.0)	31.11	**< 0.001**

Character	A. fuscum	A. marginatum	A. podolicum	A. paniculatum	F	P
wO: ovary width (mm)	2.3±0.2^a (2.0–)2.0–2.6 (–2.7)	2.0±0.2^b (1.6–) 1.8–2.2 (–2.5)	2.0±0.1^{ab} (1.8–) 1.8–2.2 (–2.3)	1.9±0.1^c (1.7–) 1.7–2.1 (–2.1)	24.26	**< 0.001**
lC: capsule length (mm)	5.1±0.4^a (4.5–) 4.6–5.6 (–5.6)	4.8±0.4^a (3.5–) 4.3–5.3 (–5.6)	4.8±0.6$^{c\#}$ (3.8–) 3.9–5.8 (–5.9)	4.3±0.4$^{b\#}$ (3.4–) 3.6–4.9 (–5.0)	8.97	**< 0.001**
wC: capsule width (mm)	4.3±0.4^a (3.6–) 3.7–4.9 (–5.1)	3.5±0.3^b (3.0–) 3.1–3.8 (–4.2)	3.5±0.4^b (3.0) 3.0–4.2 (–4.2)	3.2±0.4^b (2.5–) 2.6–3.7 (–3.8)	25.22	**< 0.001**
lSe: seed length (mm)	3.8±0.3^a (3.4–) 3.4–4.2 (–4.3)	3.5±0.2^b (3.0–) 3.2–3.9 (–4.0)	3.5±0.3^b (2.9–) 2.9–3.8 (–3.8)	3.3±0.2^b (3.0–) 3.0–3.5 (–3.5)	9.75	**< 0.001**
wSe: seed width (mm)	1.8±0.2^a (1.7–) 1.7–2.1 (–2.2)	1.5±0.1^b (1.2–) 1.3–1.7 (–1.9)	1.6±0.2$^{bc\#}$ (1.3–) 1.3–1.8 (–1.8)	1.4±0.1$^{c\#}$ (1.2–) 1.2–1.6 (–1.6)	20.94	**< 0.001**
Micromorphological and anatomical characters of leaf surface and stomata (epidermal characters)						
dSto: density of stomata (number per mm^2)	141.5±29.5^a (79.6–) 96.7–180.7 (–189.5)	141.9±40.6^a (52.9–) 79.3–190.2 (–210.8)	169.6±27.7^a (113.9–) 120.8–221.5 (–232.1)	225.5±60.1^b (99.5–) 116.4–306.4 (–317.9)	9.66	**< 0.001**
dEpi: density of epidermal cells (number per mm^2)	146.4±33.5^a (77.6–) 94.6–192.3 (–214.2)	148.5±44.0^a (55.6–) 82.1–202.9 (–231.4)	189.6±38.2^b (125.6–) 134.4–266.2 (–278.1)	233.9±63.3^c (98.1–) 125.4–315.0 (–317.9)	10.20	**< 0.001**
Ratios						
w_l1L (w1L/l1L)	0.16±0.05^a (0.08–) 0.12–0.24 (–0.29)	0.12±0.04^b (0.07–) 0.09–0.15 (–0.34)	0.08±0.03^c (0.05–) 0.05–0.13 (–0.18)	0.09±0.02^c (0.07–) 0.07–0.15 (–0.15)	18.97	**< 0.001**
w_l2L (w2L/l2L)	0.19±0.05^a (0.12–) 0.13–0.27 (–0.29)	0.13±0.02^b (0.09–) 0.10–0.16 (–0.18)	0.09±0.02^c (0.05–) 0.05–0.13 (–0.17)	0.10±0.02^c (0.08–) 0.08–0.13 (–0.13)	33.55	**< 0.001**
lC_wC (lC/wC)	0.84±0.08^a (0.72–) 0.72–0.96 (–0.97)	0.73±0.09^b (0.54–) 0.65–0.84 (–1.00)	0.73±0.04^b (0.67–) 0.67–0.79 (–0.80)	0.75±0.06^b (0.68–) 0.68–0.84 (–0.85)	6.99	**0.001**
wSe_lSe (wSe/lSe)	0.49±0.04^a (0.43–) 0.44–0.54 (–0.54)	0.44±0.04^b (0.30–) 0.39–0.49 (–0.51)	0.44±0.02^b (0.39–) 0.40–0.47 (–0.47)	0.43±0.04^b (0.35–) 0.36–0.47 (–0.48)	5.77	0.004

Table 3. Descriptive statistics of the selected qualitative characters (percentage of each category for each categorical variable studied within each group) useful for the discrimation of the taxonomic groups, including common di– and rare triploids recorded in *Allium podolicum* and *A. marginatum*. Categorical characters were analysed using log–linear models (logLM). Bonferroni correction was also applied to raw P values and those remaining significant are indicated in bold. logLMs were also recalculated with all triploids excluded from taxonomic groups but any changes in significance of the tests with and without including triploids were not detected. If the main logLM test was significant, paired logLM tests were applied for each pair of taxonomic groups, using Bonferroni correction of P; different letters in a row indicate significant differences in proportions between groups. Abbreviations of each character/category are added before the name of the respective character/category (first column).

| Character	*A. fuscum*	*A. marginatum*	*A. podolicum*	*A. paniculatum*	χ^2	P
Vegetative characters						
shL: shape of transverse section of leaf	a	b	c	c	116.75	**< 0.001**
shL_1: semicylindrical	0.0	0.0	77.8	66.7		
shL_2: compressed	0.0	56.9	22.2	33.3		
shL_3: flat	100.0	43.1	0.0	0.00		
tpC: leaf margin of the central part of the second uppermost leaf	a	b	bc	c	39.94	**< 0.001**
tpC1: smooth margin, a narrow lining without any papillae	0.0	9.8	38.9	44.5		
tpC2: vesicular papillae homogeneously present along entire margin	4.2	17.6	11.1	22.2		
tpC3: minority verrucose, majority vesicular papillae along entire margin	8.3	19.6	16.7	0.0		
tpC4: minority vesicular, majority verrucose papillae along entire margin	12.5	21.6	11.1	0.0		
tpC5: verrucose papillae homogeneously present along entire margin	75.0	31.4	22.2	33.3		
Generative characters						
papO: ovary surface	a	a	c	b	48.66	**< 0.001**
papO_S: smooth ovary	0.0	0.0	27.8	0.0		
papO_T: upper third of ovary papillose	25.0	37.3	61.1	94.4		
papO_H: upper half of ovary papillose	75.0	62.7	11.1	5.6		
P: length of capsule vs perigon length	a	b	b	b	22.78	**< 0.001**
P_ex: capsule longer than the perigon	92.3	0.0	0.0	0.0		
P_in: capsule shorter than the perigon	7.7	100.0	100.0	100.0		
colP: perigon (tepal) background colour	a	b	c	c	185.82	**< 0.001**
colP_W: white–whitish	0.0	90.2	0.0	0.0		
colP_P: pink–pinkish	4.2	0.0	100.0	100.0		
colP_Y: yellow–yellowish	95.8	9.8	0.0	0.0		
T: colour pattern of internal and external tepals	a	b	c	c	33.03	**< 0.001**
T1: same (identical)	8.3	50.9	83.3	77.8		
T2: different	91.7	49.1	16.7	22.2		
T: colour present in tepals						
TY: yellow	a	b	c	c	49.20	**< 0.001**
	70.8	5.9	0.0	0.0		
TG: green	a	a	b	b	39.85	**< 0.001**
	66.7	62.8	11.1	0.0		
TB: brown	a	a	c	b	88.54	**< 0.001**
	95.8	98.0	33.3	0.0		
TW: white	a	b	b	b	12.98	0.005
	79.2	100.0	100.0	100.0		
TP: pink	a	a	b	b	119.05	**< 0.001**
	0.0	3.9	100.0	100.0		
TPurp: purple	a	b	b	ab	13.02	0.005
	95.8	60.8	66.7	83.3		

The length of the tepals was rather similar between the groups, while the width of the tepal was significantly narrower in *A. marginatum* than other groups. *Allium paniculatum* and *A. podolicum* had smaller ovaries and longer styles than the other groups. The shape of the ovary was predominantly ellipsoid-cylindrical in all groups, except for *A. fuscum*, where both ellipsoid-cylindrical and cylindrical ovaries were recorded with similar frequencies. Both *A. podolicum* and *A. paniculatum* had a papillose surface only in the upper third of the ovary, whereas most plants in the other groups had a papillose surface in the upper half of the ovary. The characters measured on capsules and seeds clearly differentiated *A. fuscum* from the other taxonomic groups, having larger capsules and seeds with higher width/length ratio (Fig. 3C, Tables 2, 3).

The taxonomic groups also differed in the colour of the petals (Figs 3C, 4–7). Both *A. podolicum* and *A. paniculatum* always had pink (or pinkish) tepals, rarely tinged with white, green, brown and purple, with a darker (usually purple) midrib. In contrast, *A. fuscum* had dirty yellow tepals, tinged with brown-green or purplish-brown. *Allium marginatum* had rather variably coloured tepals, with a white or whitish background, tinged predominantly with purple, but also green, brownish, or pink, but in most cases without the presence of yellow. In some populations, all flowering plants had identically coloured tepals, while in others continuous variability occurred ranging from dirty whitish background and green-brownish midrib and margins to white backgrounds with purple to dark pink midrib and margins. The internal and external tepals of both *A. podolicum* and *A. paniculatum* were similarly coloured whereas in *A. marginatum* and especially *A. fuscum* they were differently coloured (lighter vs darker tepals; Figs 4–7, Table 3).

## Leaf transverse shape, epidermal micromorphological characters and anatomy

Different shaped transverse sections of leaves were detected in the taxonomic groups: mostly semicylindrical in *A. podolicum* and *A. paniculatum*, compressed in *A. marginatum* and always flat in *A. fuscum* (Fig. 8). In contrast, the leaf surface was found to be quite uniform (Fig. 9). In general, the leaves were unifacial, without hairs and with a thick cuticle. The cuticle bore a longitudinal striation over cells and a row of micropapillae. Above the stomatal apparatus, which was sunk in the cuticle layer, a cuticular ridge (the entrance to the front stomatal cavity) was present (Supplementary Material S1). The highest epidermal cell density was found in *A. paniculatum*, it was intermediate in *A. podolicum* and lowest in *A. fuscum* and *A. marginatum* (Table 2). When comparing all groups, a significantly higher density of stomata was found in *A. paniculatum*. However, the stomatal index did not differ between the groups (Table 2, Supplementary Table S6). Micropapillae were usually arranged in groups, forming teeth or variously shaped formations on the leaf margins or the midvein. However, no type of micropapillae formation was taxon-specific. Various types of papillae on the leaf margin were observed in most of the taxonomic groups, except for *A. fuscum* with mostly verrucose papillae (Fig. 9, Table 3).

The anatomical structure of leaf blades was similar in the taxonomic groups (Fig. 8); a detailed description is provided in Supplementary Material S1.
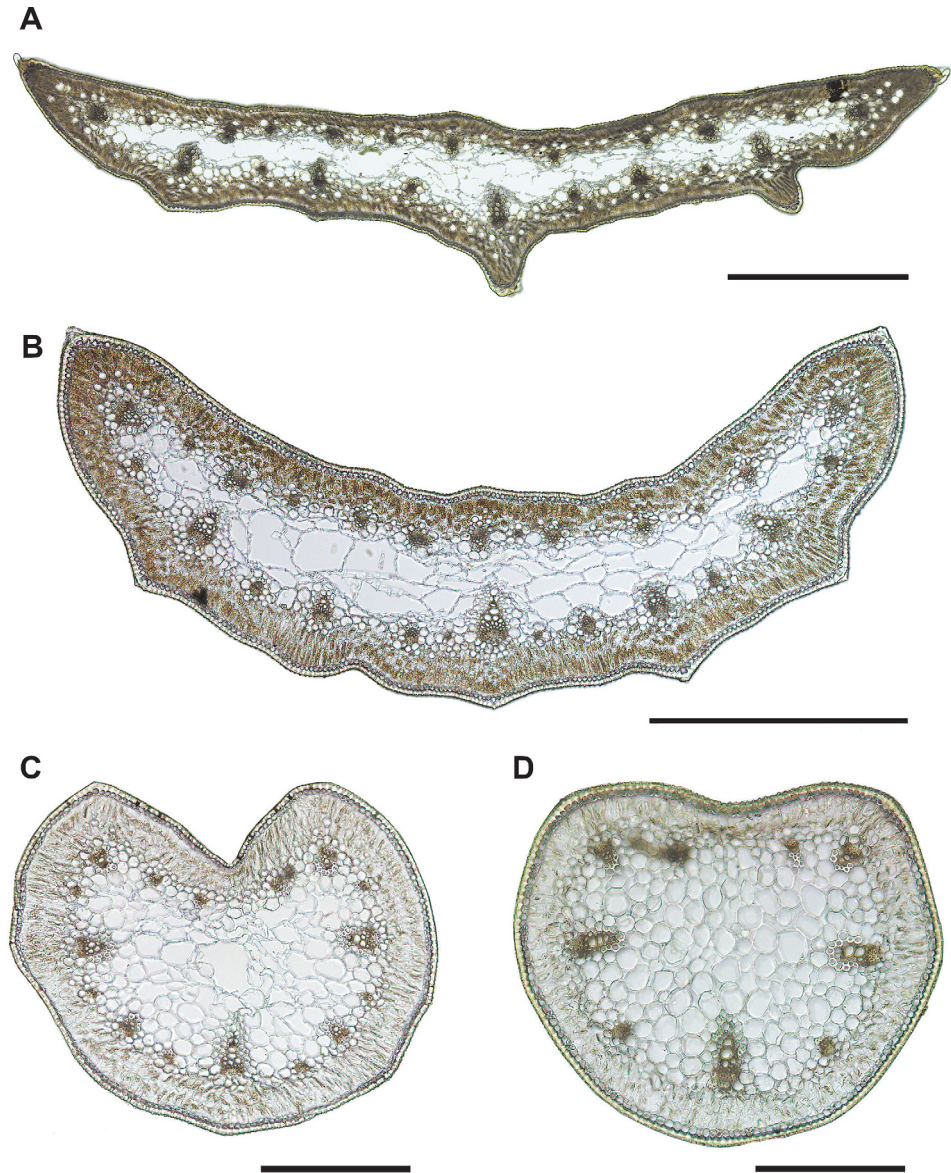
Fig. 8. Light micrographs of transverse sections of the leaves of the taxa studied. (A) *Allium fuscum.* (B) *A. marginatum*. (C) *A. podolicum*. (D) *A. paniculatum*. The adaxial surface is oriented upwards. Scale bar = 1 mm (A, B), 0.5 mm (C, D). Photographs by Kateřina Vojtěchová.
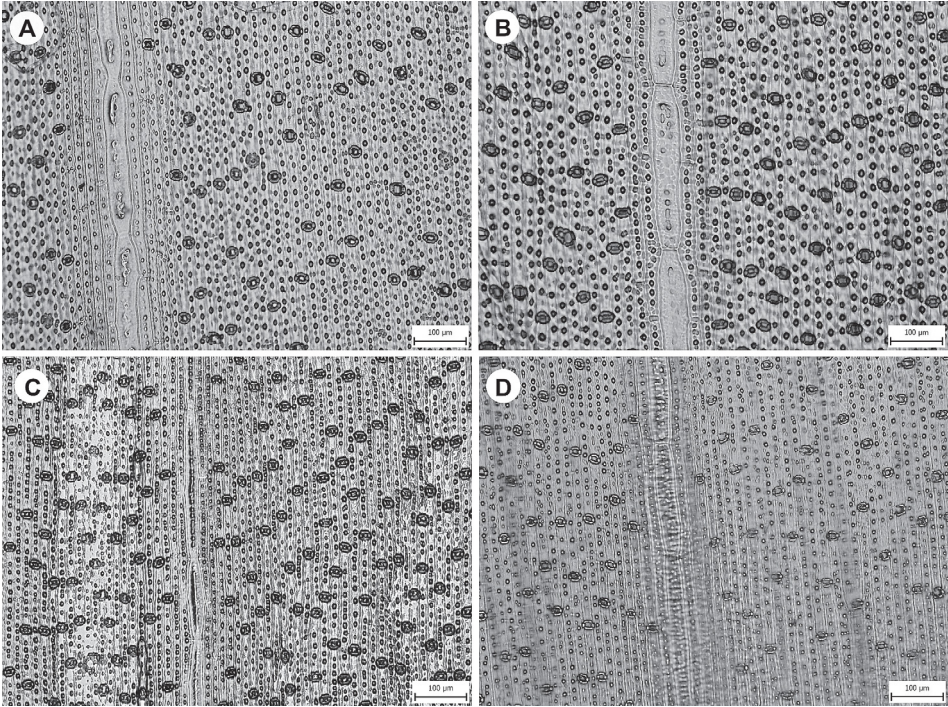
Fig. 9. Dermograms of the adaxial leaf surface. (A) *Allium fuscum*. (B) *A. marginatum*. (C) *A. podolicum*. (D) *A. paniculatum*. Scale bar = 100 μm. Photographs by Kateřina Vojtěchová.

Discussion

Recent clarification of the taxonomic identity of true *A. paniculatum*, with its distribution area restricted to southern Ukraine and southern Russia (Salmeri et al. 2016), questioned the taxonomic identity of populations referred to as *A. paniculatum* in other parts of the range of this species complex. This is also the case in central and the western part of eastern Europe, from where four other species of this complex (i.e. *A. fuscum*, *A. fussii*, *A. marginatum* and *A. podolicum*) were originally described, but their taxonomic value has long been a matter of controversy. Here, the use of an integrated approach revealed the existence of three genetic clusters with distinct genome sizes and morphology corresponding to the three morphological species in the material sampled. On the basis of this data, *A. marginatum* and *A. fuscum*, are recognized as two species occurring in the Pannonian and Transylvanian basins and the Iron Gate area, respectively. On the other hand, the two eastern-European taxa, i.e. *A. paniculatum* and *A. podolicum*, are similar in terms of the AFLP, genome size and morphology. On this basis, these species are designated conspecific, with *A. podolicum* a heterotypic synonym of *A. paniculatum*.

Genetic structure and relative genome size variation (RGS) elucidate the evolutionary history of the distinguished taxa

The Bayesian clustering of AFLP data suggested that the most likely number of genetic groups is two (eastern and western genetic groups), but perfect congruence of STRUC-TURE, PCoA and Neighbor-Net analyses supported the recognition of three genetic clusters. Considering the clustering position of populations from or close to the type localities of the species studied, these genetic clusters correspond with three taxonomic groups: genetic cluster 1, representing *A. fuscum*, genetic cluster 2, *A. marginatum* and the most genetically distant genetic cluster 3, *A. paniculatum* + *A. podolicum* (Fig. 1B–D). In accordance with the genetic structuring, these three clusters are also significantly differentiated by their RGS (Fig. 2A).

   The clear genetic differentiation between genetic groups to the west (clusters 1, 2) and east (cluster 3) of the Eastern Carpathians appears to be the result of geographic barriers that prevent gene flow between these two lineages (Fig. 1A). This is illustrated by the fact that despite the similarity of plant composition in these zones (i.e. the co-occurrence of many species with continental, Pontic-Pannonian, and eastern sub-Mediterranean distributions; Chytrý et al. 2022), the extrazonal (forest) steppes in the Pannonian and Transylvanian Basins are now isolated from the zonal steppe and forest steppes in eastern Europe (Wesche et al. 2016). However, several phylogeographic studies indicate long-term (i.e. pre-Holocene) in situ persistence of isolated populations of several steppe species in the Pannonian and Transylvanian Basins (Magyari et al. 2010, Stewart et al. 2010, Varga 2010, Feurdean et al. 2015, Willner et al. 2019, 2021), which fostered the origin of distinct evolutionary units (Cieślak 2014, Kajtoch et al. 2016, Kirschner et al. 2020). Although the genetic pattern and the current distribution of the populations of the *A. paniculatum* complex studied imply a Pontic-Pannonian distribution pattern, which could have resulted from the above-mentioned scenario, this complex cannot be regarded as an example of a typical steppic taxon, because the evolutionary centre and maximum species diversity of the complex is situated in the eastern Mediterranean region (Stearn 1981, Salmeri et al. 2016). Therefore, it is possible that these two currently genetically distinct lineages could be separate branches of the original lineage, which in the past spread from the southern Balkan Peninsula to the northeast and northwest, respectively, and adapted to different environmental conditions in allopatry (see Kajtoch et al. 2016 for a similar scenario).

   The genetic structure of clusters 1 and 2, representing the western genetic group (Fig. 1B), is characterized by similar levels of within-population genetic diversity and the DW index, but also by less genetically differentiated populations when compared with the eastern group. The south-north decrease in the DW index (but not within-population diversity) observed between populations of the western genetic group could be explained by postglacial migration of its members to their current distribution areas from more southern areas (e.g. the southern margin of the Carpathian Basin), corresponding to the north–south 'contraction–expansion' pattern regularly observed in temperate species (Hewitt 1999, 2000, Petit et al. 2003). However, the observed pattern is entirely based on the comparison of two genetic clusters differing also in geographic distribution, genome size, morphology and ecology, and thus it is likely they represent different evolutionary histories. Therefore, it cannot be ruled out that members of the western genetic group

have persisted in partial isolation in refugial sites within the Pannonian and Transylvanian Basins and the Iron Gate region even during the last glacial period and the entire Holocene. Specifically, populations of *A. marginatum* inhabit dry grasslands and open-canopy dry oak forests (Zahariadi 1966, Soó 1973, Čeřovský et al. 1999, Dengler et al. 2012 sub *A. paniculatum* subsp. *fuscum*) and therefore tolerate a wide range of environmental conditions. Survival at or around the current sites is also likely for *A. fuscum*, since its populations inhabit rocky outcrops and tolerate being shaded by trees (Brullo et al. 1996) and thus may also have persisted in their present areas at least during the entire Holocene. Willner et al. (2021) show that glacial refugia of some rocky-steppe species of sub-Mediterranean origin also existed in the centre and north-western periphery of the Pannonian Basin, though it is likely they were restricted to microrefugia within the landscape matrix of cold steppe during the Last Glacial Maximum (LGM; Divíšek et al. 2022).

Strongly weighted splits and high bootstrap support, higher among-population genetic differentiation than seen in the western group, the highest DW value recorded and nonsignificant isolation-by-distance pattern of the eastern genetic group might reflect past and rapid, pre-Holocene spread followed by long-term persistence, isolation and limited gene flow due to habitat fragmentation and degradation (Sudnik-Wójcikowska et al. 2011, Dembicz et al. 2016). Similarly, recent palaeodistribution modelling supports long-term suitable habitat conditions for species of dry grasslands in most parts of the Pontic area from the LGM to the present (Divíšek et al. 2022). A weak genetic differentiation between western (*A. podolicum*) and southern Ukrainian populations (*A. paniculatum*) was detectable only in Neighbor-Net (Fig. 1D), which might be related to the fact that the western Podolian region is considered as a potential steppe refugium (Roleček et al. 2019, Willner et al. 2021), where isolated populations might have persisted for long periods. Despite this, molecular fingerprinting suggests that the populations of *A. podolicum* studied could be considered conspecific with those of *A. paniculatum*, which is also supported by nonsignificant differences in RGS of diploids of *A. podolicum* and *A. paniculatum* (Fig. 2A).

Variations in ploidy level, absolute genome size (AGS) and GC content:
dominant diploids and rare triploids of autopolyploid origin

Single (2n = 2x) or various ploidy levels (2n = 2x–6x) have been reported for plants assigned to the studied *Allium* taxa in the past (Rice et al. 2015), but most of these reports can be considered as erroneous and belonging to other species of the *A.* sect. *Codonoprasum* due to misleading interpretations of the species studied (Salmeri et al. 2016). Using both flow cytometry and chromosome counts, only two ploidy levels, i.e. diploid (2n = 16) and triploid (2n = 24), were recorded for the taxa studied.

Allium fuscum is exclusively diploid, which is consistent with Brullo et al. (1996). A similar situation also holds for *A. paniculatum*. Regarding the geographic origin of the samples, only the previous reports of di- and tetraploids (2n = 32) from Russia and Ukraine (Vakhtina & Kudrjashova 1985) can probably be assigned to *A. paniculatum*. The findings of diploids are congruent with those of Salmeri et al. (2016), who studied populations in the area of the locus classicus in Ukraine.

In both *A. podolicum* and *A. marginatum*, dominant diploids and rare triploids were found. The plant no. 674 with diploid chromosome count (2n = 16; Levan 1937), cultivated by Levan (1937) and used in his experimental crosses, probably represents *A. podolicum*, which agrees with the data presented. The diploid count originally reported as *A. paniculatum* (Murín et al. 1999) from one locality in southern Slovakia belongs to *A. marginatum* as this locality is close to population no. 8 of *A. marginatum* (see also Eliáš et al. 2013). The detected triploid plants most likely arose via the fusion of reduced and unreduced gametes of diploids within both taxa studied. This autopolyploid scenario is supported by (i) the RGS being 1.5 times the RGS of putative diploids, as reported for some other diploid-autotriploid complexes (e.g. Eilam et al. 2010, Čertner et al. 2017, Kobrlová et al. 2022), (ii) a strict co-occurrence of triploids with dominant diploids, (iii) intermingled position of triploids with putative diploids in PCoA and Neighbor-Net of AFLP data and (iv) the absence of local co-occurrence of diploids with autotetraploids or related tetraploid species (K. Vojtěchová et al., personal observation). The occurrence of autotriploids in different populations of *A. marginatum* also indicate their independent origins, now considered as a common phenomenon in plants (Soltis & Soltis 1999, Sharbel & Mitchell-Olds 2001, Ekrt et al. 2021). However, the absence of pure-triploid populations indicates that, despite the likely recurrent formation of triploids within diploid populations, a frequency-dependent mating disadvantage (Levin 1975) and the absence of asexual reproduction, which would allow the escape from the reproductive interference with diploids, probably prevent the coexistence of cytotypes in the long term (Kolář et al. 2017, Duchoslav et al. 2020, Čertner et al. 2022). Nevertheless, the record of a tetraploid count in conspecific *A. paniculatum* (Vakhtina & Kudrjashova 1985) does not exclude the possibility of autotetraploid establishment through a triploid bridge (Husband 2004).

The AGS and GC content data presented are the first estimates for all the taxa investigated (cf. Leitch et al. 2019). Previously, Jones & Rees (1968) report the AGS of "*A. fuscum*" using Feulgen photometry. However, this value (2C = 18.4 pg vs mean 2C = 31.5 pg in Supplementary Table S5) probably refers to another species since the plant material was collected in Turkey (Ved Brat 1965, Jones & Rees 1968), which is not within the distribution of *A. fuscum* (Brullo et al. 1996) and it has a different number of chromosomes (2n = 14, Jones & Rees 1968).

In general, the variation in GC content within plant genera is rather small and usually insufficient for species delimitation (Meister & Barow 2007), but may be significant in some evolutionary young groups of plants (Šmarda & Bureš 2012). The GC content estimates presented are similar both within and between the species investigated (range 36.1–37.9%), are at the lower limit for the genus *Allium* (Meister & Barow 2007, Šmarda et al. 2019) and lower than published GC contents for the other four species of the *A.* sect. *Codonoprasum* (range 40.3–41.2%; Meister & Barow 2007, Šmarda et al. 2019). This could be partly due to different methodology (e.g. use of different fluorochromes for FCM; Doležel et al. 1992) and/or the negative correlation between the GC content and the monoploid GS for a group of species with large GS (sensu Leitch et al. 1998, Šmarda et al. 2014, 2019), where all these species belong.

*Leaf anatomy and epidermal micromorphological characters: adaptation
to xeric environment, but weak differences between taxonomic groups*

Leaf anatomy and epidermal characters were considered to be diagnostically important traits for distinguishing *Allium* species in the past (Traub 1968, Zahariadi 1975, Krahulec 1980, Fritsch 1988, 1992, Brullo et al. 1996, Gregory 1996, Yousaf et al. 2008, Choi & Oh 2011, Lin & Tan 2015, Jandová et al. 2017) although they are only occasionally used in determination keys (Zahariadi 1975, Krahulec 1977, Yousaf et al. 2008, Özhatay et al. 2018). The results presented show that all the taxa studied are similar in terms of leaf anatomy and epidermal characters: thick epidermis with sunken stomata, cuticular ridge around the entrance to the front cavity, presence of micropapillae and several layers of palisade parenchyma are clearly adaptations to xeric environments (Shields 1950). Therefore, these traits alone have limited value in distinguishing closely related species of *Allium* sharing a similar ecology, as is previously reported by Krahulec (1977, 1980) and Gregory (1996). However, narrow leaves with higher densities of (smaller) epidermal cells and stomata observed in the eastern taxa *A. podolicum* and especially *A. paniculatum*, compared with *A. fuscum* and *A. marginatum*, correlate well with patterns in the genome size of these taxa and might be interpreted as an adaptation (Mashayekhi & Columbus 2014, Doyle & Coate 2019) to the slightly more extreme (drier) conditions at sites in the more continental eastern part of the area studied. The nucleotypic effect of increased ploidy on the size of the stomata (Beaulieu et al. 2008) has been observed in *A. podolicum*, with triploids having 15–20% wider stomata than diploids.

*Morphological variation correlates with genetic grouping: taxonomic consequences
and distribution of distinguished taxa*

The *A. paniculatum* complex contains a wide range of morphotypes, with the variability being particularly high as regards habitus, vegetative characters (e.g. shape of transverse section of leaves, the length and the shape of the spathes) and generative traits (shape, size and colour of ovary, anther and tepals; Wilde-Duyfes 1973, Zahariadi 1975, Brullo et al. 2008, Salmeri et al. 2016). Multivariate analyses of the morphological data of the samples studied confirmed the diagnostic importance of some of the characters mentioned above and identified three morphological groups (Fig. 3) corresponding to the three genetic clusters revealed by AFLP (Fig. 1).

The first group, corresponding to genetic cluster 1, clearly differed from the others, being characterized by robust plants with broad flat leaves, greenish-yellowish tepals tinged with brown or purple and large capsules and seeds. This combination of characters fits well with the original description (Waldstein & Kitaibel 1808) as well as with the more recent study of plants of *A. fuscum* from its locus classicus (Brullo et al. 1996) and confirms the opinion of Brullo et al. (1996) that *A. fuscum* is a distinct species. However, it is necessary to consider all of the morphological traits to correctly identify this species. Up to now, many populations of the *A. paniculatum* complex have been attributed to *A. fuscum*, usually based on brownish tepals (e.g. Reichenbach 1828, Stearn 1980, Kollmann 1984), which includes also some populations of *A. marginatum* (e.g. Zahariadi 1966, see also below) and probably many populations of the *A. paniculatum* complex from other parts of the Balkan Peninsula (K. Vojtěchová et al., unpublished results).

South-eastern Serbia was added to the distribution of *A. fuscum* (south-eastern Romania, north-western Bulgaria; Brullo et al. 1996; Fig. 1A). Recent reports of *A. fuscum* from other regions on the Balkan Peninsula (e.g. Goranova & Vassilev 2006, Anačkov 2009, Cheshmedzhiev 2011, Assyov & Petrova 2012, Vladimirov et al. 2013, Nikolov 2021) and in the Pontic region (e.g. Ghendov 2015) require thorough revision and should be considered as doubtful, due to the confusion and inconsistency of taxonomic concepts in national floras (see Introduction). Moreover, specimens of *A. fussii*, described from the Eastern Carpathians (Munţii Haşmaş Mts; Kerner 1878) growing on calcareous outcrops in upper montane to subalpine belts in the Eastern and Southern Carpathians (Ciocârlan 2000) and now treated as an intraspecific taxon within *A. fuscum* in Romanian Floras (Zahariadi 1966, Ciocârlan 2000), were not studied. *Allium fusii* should differ from *A. fuscum* in several characters, i.e. semicylindrical and narrow leaves, pink perigon with tepals 7–8 mm long and the capsule shorter than the perigon (Zahariadi 1966, Brullo et al. 1996, Ciocârlan 2000). However, Mráz (2005) notes that plants of *A. fusii* sampled by him from the same mountain range do not fully match the diagnostic characters given in Brullo et al. (1996). Unfortunately, attempts to find this taxon at the locus classicus were unsuccessful. Overall, this taxon needs thorough revision.

The specific morphology resulting in a nearly separate group in the PCoA (Fig. 3A), corresponding to genetic cluster 2, unequivocally indicates that this group comprises another separate species, for which the most appropriate name is *A. marginatum*. Diagnostic characters of the leaves (compressed leaf cross shape with the width of leaf between 2.5 and 3.5 mm), flowers and fruits (narrow tepals, regular presence of whitish and almost complete absence of yellow as a background colour of tepals, perianth exerting the capsule) correspond well with the original description of *A. marginatum* (Janka 1884), especially the contrasting colour of petals, having whitish background and purple or brownish midrib and petal margins. This character is also considered diagnostic for this taxon (sub *A. fuscum* var. *fuscum* f. *marginatum*) by Zahariadi (1966) in Flora of Romania. However, the colour of the petals, both within and between populations of *A. marginatum*, varied with different plants having midrib and margins of petals and sometimes also surface between them suffused with purplish, purple-brownish, or brownish (Fig. 5), which is also previously reported by Zahariadi (1966). Consequently, the presence of plants of *A. marginatum* with petals suffused with purple-brownish, similar to those of *A. fuscum*, resulted in a slight overlap of both taxonomic groups in the PCoA of morphological characters (Fig. 3A). It cannot be ruled out that the variation in petal colour was one of the causes of the taxonomic uncertainty about some populations of *A. marginatum* in the past (e.g. Zahariadi 1966) and it is likely that a future study of more extensive material may lead to finer taxonomic subdivisions within this species. It cannot also be ruled out that rare hybridization may be occurring between *A. marginatum* and *A. fuscum*, as indicated by the elevated admixture values in population 15 of *A. marginatum* (Fig. 1B), where a mixture of plants with flowers with dirty yellow perianth and purplish-brownish margin and midvein and "typical" *A. marginatum* plants were recorded.

The original description of *A. marginatum* (Janka 1884) does not mention any specimens, only the specific locality (Sucutard near Cluj, Transylvanian Basin, Romania), which might be considered a type locality. Searching for the specimens of *A. marginatum* collected by Janka yielded only one herbarium sheet with three plant specimens originating from the locality mentioned above, which is deposited in the herbarium CJ. Careful

study of these dried plants revealed that they are small, with a compact inflorescence the petals of which are bleached. To eliminate any doubts about the taxonomic identity of these plants, this locality was visited (Fig. 10A, E, F) and scattered plants were found (Supplementary Figure S2) that match the description of Janka (1884). The plants from this population analysed using AFLP clustered together with the individuals of the other populations sampled in the Pannonian and Transylvanian basins and together formed a separate genetic cluster (Fig. 1C, D), which undoubtedly confirmed our opinion that the populations studied could be assigned to *A. marginatum*. Since there is no holotype for the name *A. marginatum*, a lectotype is designated here (see Taxonomic treatment below). The current distribution of *A. marginatum* (Fig. 1A) includes ecologically suitable sites within the Pannonian Basin, stretching from northern Serbia (Titelski breg; Anačkov 2009) through Hungary (Bartha et al. 2022) to southern Slovakia (Somogyi 1999, Eliáš et al. 2013) and the Transylvanian Basin (Zahariadi 1966). The records of *A. paniculatum* within this region should be treated as *A. marginatum*. However, it cannot be ruled out that the range of this species is larger and extends southward to North Macedonia (Nikolov 2021 sub *A. fuscum*?) and Croatia (sub *A. paniculatum* subsp. *fuscum* and subsp. *paniculatum*, cf. Nikolić 2022).

Based on the morphological results presented, *A. podolicum* is very similar or even identical to *A. paniculatum*, especially when only diploids are considered (Fig. 3A), which corresponds to genetic cluster 3 (Fig. 1). Original descriptions of both species (Linnaeus 1759, 1762, Ascherson & Graebner 1905, Raciborski & Szafer 1919) do not provide a set of diagnostic characters for distinguishing between them. Descriptions of the species in the Ukrainian and Russian Floras (Bordzilovsky 1950, Omelchuk-Myakushko 1979, Dobrochaeva et al. 1999) are inconsistent, but generally emphasize tepal length, the position of stamens vs tepals and different colour of the midrib of tepals as diagnostically important morphological characters for distinguishing *A. podolicum* from *A. paniculatum*. With the exception of the occasional presence/absence of purple in addition to the dominant pink of the tepals in *A. paniculatum*/*A. podolicum*, the above characters did not differ between these taxa when considering the data presented. However, morphological data for both species (excluding triploid *A. podolicum* from the comparison) agree well with Salmeri et al. (2016), who studied *A. paniculatum* populations at its locus classicus in southern Ukraine.

The name *A. podolicum* was first used by the Polish botanist B. Błocki for plants he sampled at Probabin near Horodenka, Podolia region, Ukraine, and distributed as exsiccates to various European herbaria. However, Błocki never published this name. Ascherson & Graebner (1905) used the epithet 'podolicum' for this collection and validly published this taxon, treating it as a variety of *A. paniculatum* (*A. paniculatum* var. *podolicum* Asch. et Graebn.). Thus, this name is a basionym. Raciborski (in Raciborski & Szafer 1919) reports a new combination of species rank (*A. podolicum* Błocki ex Racib.) based on the same collection of Błocki's specimens. Soviet authors (Omelchuk-Myakushko 1979, Czerepanov 1981, 1995) consider the name *A. podolicum* (Asch. et Graebn.) Błocki ex Racib., but here the following notation is preferred: *A. podolicum* (Błocki ex Asch. et Graebn.) Racib. No type specimen is designated for *A. podolicum* in the protologue. Although there were several attempts to select a lectotype (e.g. Krytzka et al. 2000), the name was never properly typified. For the name *A. podolicum*, therefore, a lectotype from the type collection of Błocki's specimens was chosen (see Taxonomic treatment below).
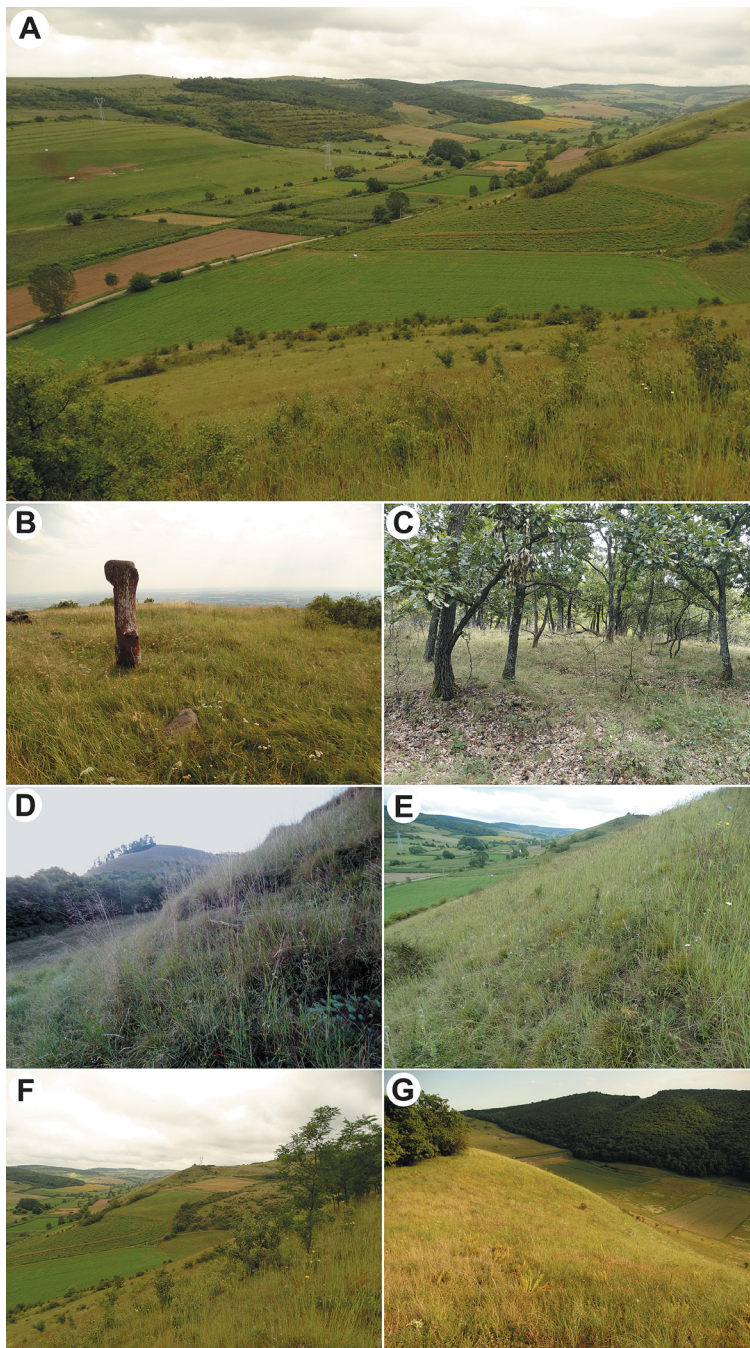
Fig. 10. Habitats at localities where *Allium marginatum* was present. (A) Landscape near Sucutard in Transylvanian basin, with steppe grasslands on slopes, separated by arable fields (population no. 13, type locality). (B) Sub-Pannonian steppe grasslands at the top of the Cseplye-této hill near Abasár (Hungary) (no. 11). (C) Xerothermic *Quercus pubescens* Willd. forest, andesite bedrock, Mátra Mts, Cserepes near Szurdokpüspöki (Hungary) (no. 37). (D) Pannonian steppe grasslands on slopes of steep hills near Boarta (Romania) (no. 14). (E-F) Pannonian steppe grasslands on slopes near Sucutard (Romania), with sparse population of *A. marginatum* ([no. 13, type locality). (G) Sub-Pannonian steppe grasslands on loess soils near Visea (Romania) (no. 12). Photographs by Roman Kalous, except for C by András Schmotzer.

However, based on the findings presented, the name *A. podolicum* should be treated as a heterotypic synonym of *A. paniculatum*. The semicylindrical narrow leaves, uniformly pink perigon (rarely tinged with white, green, brown and purple, usually with darker midrib) and small ovary, smooth or papillose only at the top are diagnostic characters that allow one to distinguish *A. paniculatum* from the other taxa studied.

The current distribution of *A. paniculatum* (incl. *A. podolicum*) includes steppe and forest steppe regions in eastern Europe (Fig. 1A), stretching from the Podolia region in western Ukraine (Bordzilovsky 1950, Salmeri et al. 2016), Moldova (Ghendov 2015) and north-eastern Romania (Tulcea and Galaţi regions; Zahariadi 1966) to the southern and lower Volga regions in southern Russia (Vvedensky 1935, Omelchuk-Myakushko 1979, Seregin 2007, Salmeri et al. 2016).

To complete the overall picture, it should be noted that another species closely related to *A. paniculatum*, *A. praescissum* Rchb., also occurs in the area studied, but has a distribution that ranges from east of the Dnieper river to western Siberia (Friesen 1988, Seregin 2007, Sinitsina 2019). Although the two species have very similar general habitus, leaf shape and colour of perigon, they morphologically clearly differ mainly in the shape of the inflorescence and the flowers, and also ecologically, with *A. praescissum* typically occurring on saline soils (Omelchuk-Myakushko 1979, Dobrochaeva et al. 1999, Seregin 2007).

Northern and central parts of the Balkan Peninsula as targets for future research

This study confirmed the validity of some of the previously described species in the *A. paniculatum* complex at the northern edge of its range, suggesting that quaternary climatic oscillations might have resulted in the isolation of populations of species in different refugia, resulting in genetic divergence and eventually speciation (Kadereit & Abbott 2021). This scenario is considered to be the most parsimonious explanation of the high species diversity and endemism in many species' complexes in the Mediterranean region (Nieto Feliner 2014). It cannot be ruled out that a similar scenario may apply to *A.* sect. *Codonoprasum*, although the main diversification within this section is suggested to date back to the Miocene-Pliocene during and after the Messinian salinity crisis (Bogdanović et al. 2009, Salmeri et al. 2016). Future comparative biosystematic studies should focus on the representatives of the *A. paniculatum* complex from the northern and central parts of the Balkan Peninsula, from where there are doubtful records of *A. paniculatum* and *A. fuscum* and some other species with ambiguous taxonomic concepts (e.g. *A. tenuiflorum* Ten., *A. coppoleri* Tin., *A. longispathum* Redouté) (Anačkov 2009, Cheshmedzhiev 2011, Nikolov 2021, Nikolić 2022). It is also necessary to resolve the taxonomic value, morphological variation and phylogenetic relationships of several species of this complex described as new to science from this region, about which there is still extremely little information (*A. macedonicum* Zahar., Zahariadi 1975; *A. serbicum* Vis. et Pančić, Clementi et al. 2015).

Taxonomic treatment

Provided here is a taxonomic treatment, description and distribution of *A. marginatum*, mostly based on data collected in this study. Taxonomic treatment and description of *A. fuscum* follow Brullo et al. (1996), which is supplemented and refined based on

additional samples included in the morphometric study. The description and distribution of *A. paniculatum* (Salmeri et al. 2016) was revised after the new circumscription of the species.

Allium fuscum Waldst. et Kit., Descr. Icon. Pl. Hung. 3: 267 (1807)

≡ *Codonoprasum fuscum* (Waldst. et Kit.) Rchb., Fl. Germ. Excurs.: 115 (1830)
≡ *Allium paniculatum* subsp. *fuscum* (Waldst. et Kit.) Arcang., Comp. Fl. Ital., ed. 2: 136 (1894)

Lectotype (iconotype; designated by Brullo et al. 1996: 466): [Romania], "Habitat in rupibus calcareis Banatus, frequens ad thermas Herculis" in Waldstein & Kitaibel, Pl. Rar. Hungar. 3, t. 241, 1808. – Epitype (designated by Brullo et al. 2008: 247): "Romania, rupi ombreggiate di Băile Herculane", 29. 7. 1993, Brullo & Scelsi s. n. (sheet 59.048 in CAT).

Description: Bulb ovoid or ovoid-elliptical, (5–) 10–15 (–20) mm, daughter bulbs not developed; outer coats pale brown or blackish-brown, coriaceous, inner coats whitish, membranous. Stem robust, (15–) 21–45 (–51) cm long, glabrous, erect, covered by leaf sheaths up to two thirds of its length. Leaves (3–) 4–6 (–7), (12–) 14–31 (–40) cm long and (1.7–) 2.4–5.7 (–7.5) mm wide, flat, slightly fistulose, with dense verrucose micropapillae on leaf margins. Inflorescence laxly hemispheric, with (15–) 29–142 (–162) flowers, without bulbils; pedicels unequal, (1.4–) 1.6–3.0 (–3.7) cm long. Spathe bivalve, persistent, valves unequal, ending in a long appendage, mostly divaricate or reflexed at anthesis, longer than the umbel, the bigger (10–) 11–26 (–27) cm long, the smaller (5–) 6–15 (–18) cm long. Perigon campanulate; tepals (5.0–) 5.1–6.6 (–6.9) mm long and (2.0–) 2.1–2.7 (–2.8) mm wide, oblong-elliptical, rounded at apex, green-yellowish, tinged with brown-green, purplish-brown, midrib brown-green. Stamens with anthers slightly exerted from perigon, simple; filaments whitish or pinkish, linear-subulate, (2.5–) 2.7–4.0 (–4.4) mm long, below connate into an annulus; anthers (1.0–) 1.0–1.4 (–1.7) mm long and (0.6–) 0.7–0.8 (–0.9) mm wide, whitish, elliptical, rounded at apex. Ovary mostly ellipsoid-cylindrical, papillose in the upper 1/2, (3.5–) 3.7–5.0 (–5.3) mm long and (2.0–) 2.0–2.6 (–2.7) mm wide. Style white, (1.2–) 1.5–2.6 (–2.9) mm long. Capsule subglobose to obovoid, (4.5–) 4.6–5.6 (–6.2) mm long and (3.6–) 3.7–4.9 (–6.0) mm wide, longer than the perigon. Seeds black, (3.4–) 3.4–4.2 (–4.3) mm long and (1.7–) 1.7–2.1 (–2.2) mm wide (Fig. 4). 2n = 16.

Phenology: Flowers from late June to August.

Habitats: Usually at least partly shaded limestone outcrops and fissures, sometimes also below rocks on limestone debris, rarely along forest pathways.

Distribution: Banat region in south-western Romania, north-western Bulgaria and south-eastern Serbia (Fig. 1A). Altitudinal range: 70–570 m a.s.l.

Conservation: This species is protected by law in Serbia (Službeni glasnik RS 2010–2011). IUCN Red List (IUCN 2022) puts this species in the category Least Concern (LC). However, this is not correct due to the wrong taxonomic interpretation of this species within the Red List. Despite the fact that populations of *A. fuscum* occur in habitats subject to little human pressure, its populations are small and distribution is rather limited. Therefore, following the IUCN Red List criteria, it is proposed to update its status to Near Threatened (NT).

Allium marginatum Janka, Term. Füz. 8: 29 (1884)

≡ *Allium longispathum* subsp. *marginatum* (Janka) Nyman, Consp. Fl. Eur., Suppl. 2: 309 (1890)
≡ *Allium paniculatum* subsp. *marginatum* (Janka) Soó, Feddes Repert. 83: 136 (1972)
≡ *Allium fuscum* var. *fuscum* f. *marginatum* (Janka) Asch. & Graebn., Syn. Mitteleur. Fl. 3: 140 (1905)

Lectotype (designated here): [Romania], "In herbidis collini elatioribus prope pag. Sz. Gothárd [Sucutard] Transilvaniae centralis haud rarum", 22. Augusti 1880, Janka (right plant on the sheet 03949 in CJ; Supplementary Figure S3)

Description: Bulb ovoid or ovoid-elliptical, (5–) 10–30 (–35) mm, daughter bulbs not developed; outer coats brown, coriaceous, slightly fibrous, inner coats whitish, membranous. Stem robust, (22–) 26–54 (–66) cm long, glabrous, erect, covered by leaf sheaths up to two thirds of its length. Leaves (4–) 4–6 (–7), (10–) 18–29 (–43) cm long and (1.4–) 2.1–3.9 (–7.0) mm wide, compressed or less frequently flat, fistulous in middle and upper part, with teeth or variously shaped formations of micropapillae on margins and midvein of the abaxial surface. Inflorescence laxly hemispherical, (30–) 47–134 (–164) flowers, without bulbils; pedicels unequal, (1.7–) 2.0–3.3 (–3.7) cm long. Spathe bivalve, persistent, valves unequal, opposite, erect or reflexed at anthesis, ending in a long appendage, the bigger (9–) 11–22 (–26) cm long, the smaller (5–) 6–13 (–17) cm long. Perigon campanulate; tepals (4.8–) 5.2–6.1 (–6.3) mm long and (1.7–) 2.0–2.4 (–2.6) mm wide, oblong-elliptical, rounded at apex or rarely apiculate, white to dirty whitish, with midrib and margins and sometimes also in between suffused with purplish, purplish-brown or brownish-green. Stamens with anthers slightly exerted from the perigon, simple; filaments whitish or rarely pinkish, (2.5–) 3.0–4.0 (–4.4) mm long, below connate into an annulus; anthers whitish to pale yellow, oblong-ovate, rounded at apex, (1.0–) 1.1–1.3 (–1.6) long and (0.6–) 0.7–0.8 (–0.9) mm wide. Ovary ellipsoid-cylindrical, slightly narrowed at apex, papillose in the upper half, (3.2–) 3.6–4.6 (–5.0) mm long and (1.6–) 1.8–2.2 (–2.5) mm wide, style white, (1.4–) 1.8–2.5 (–2.8) mm long. Capsule obovoid, (3.5–) 4.3–5.3 (–5.6) mm long and (3.0–) 3.1–3.8 (–4.2) mm wide, shorter than the perigon. Seeds black, (3.0–) 3.2–3.9 (–4.0) mm long and (1.2–) 1.3–1.7 (–1.9) mm wide (Fig. 5). 2n = 16, 24.

Phenology: Flowers from July to August.

Habitats: Dry and semi-dry closed grasslands (Sub-Pannonian and Pannonian steppe grasslands, Ponto-Sarmatic steppes), dry deciduous, open-canopy forests (Pannonian-Balkanic turkey oak-sessile oak forests, Pannonian forests with *Quercus pubescens*) and thermophilous forest fringes (Fig. 10). Rarely also in secondary *Robinia pseudoacacia* forests planted in former dry grasslands and dry deciduous forests.

Distribution: Southern Slovakia, Hungary, north-western Romania (Transylvanian Basin), northern Serbia (Fig. 1A). Altitudinal range: 120–850 m a.s.l.

Conservation: This species is protected by Law and included in the Red List in the Slovak Republic (critically endangered, sub *A. paniculatum* subsp. *paniculatum*; Eliáš et al. 2015), Hungary (nearly threatened, sub *A. paniculatum* subsp. *marginatum* (Janka) Soó; Király 2007) and Serbia (sub *A. paniculatum* subsp. *marginatum*; Službeni glasnik RS 2010–2011). The IUCN Red List (IUCN 2022) lists the species (sub *A. paniculatum*) in category LC, but the revised taxonomic concept of this species presented here requires an update of the conservation status. Because *A. marginatum* inhabits dry grasslands threatened by abandonment of traditional management, its populations are usually small and isolated and the distribution of this species is rather narrow, this species is here classified as Near Threatened (NT).

Allium paniculatum L., Syst. Nat. ed. 10, 2: 978 (1759)

≡ *Cepa paniculata* (L.) Moench, Meth.: 243 (1794)
≡ *Porrum paniculatum* (L.) Moench, Meth. Suppl.: 264 (1802)
≡ *Codonoprasum paniculatum* (L.) Rchb., Fl. Germ. Excurs.: 115 (1830)
≡ *Kalabotis paniculatum* (L.) Raf., Fl. Tellur. 2: 19 (1836)
≡ *Raphione paniculatum* (L.) Salisb., Gen. Pl.: 89 (1866)

Lectotype (designated by Wilde-Duyfjes 1973: 75): "Allium juncifolium, floribus purpura-scentibus Gerb. 14 Tanais. Habitat in campis feris Tauronicensibus versus Koshinska" (right specimen on the sheet 419.21 in LINN)
= *Allium podolicum* (Błocki ex Asch. & Graebn.) Racib., Fl. Polska 1: 124 (1919). – **Lectotype (designated here)**: [Ukraine], "Probabin pr. Horodenka, Galiciae orient-aus-tralis, in collibus et ad saxa gypsacen – frequens", Błocki 1891 (right plant on the sheet 64837 in WU!; isolectotype BRNM!)

Description: Bulb ovoid, (5–) 10–15 (–18) mm, daughter bulbs not developed; outer coats pale brown, slightly fibrous, inner coats whitish, hyaline, membranous. Stem robust, (16–) 23–47 (–50) cm long, glabrous, erect, covered by leaf sheaths up to two thirds of its length. Leaves (3–) 4–6, (9–) 13–32 (–36) cm long and (0.8–) 2.1–2.6 (–3.3) mm wide, semicylindrical or rarely compressed, fistulous, with smooth margins or with teeth or variously shaped formations of micropapillae on margins and midvein of the abaxial surface. Inflorescence laxly hemispherical, (11–) 19–142 (–156) flowers, without bulbils; pedicels unequal, (1.5–) 1.6–4.3 (–5.1) cm long. Spathe bivalve, persistent, valves unequal, opposite, erect, less frequently divaricate or reflexed at anthesis, ending in a long append-age, the bigger (3–) 11–25 (–27) cm long, the smaller (2–) 5–17 (–19) cm long. Perigon campanulate; tepals (5.1–) 5.3–6.2 (–6.4) mm long and (1.8–) 2.0–2.7 (–3.0) mm wide, oblong, rounded at apex, pale pinkish to pink, sometimes suffused with purplish-pink, midrib purplish or greenish. Stamens with anthers slightly exerted or sometimes included, simple; filaments whitish or pinkish, linear-subulate, (2.7–) 2.9–4.3 (–4.7) mm long, below connate into an annulus; anthers (1.0–) 1.1–1.3 (–1.4) mm long and (0.6–) 0.6–0.8 (–0.8) mm wide, whitish to pale yellow, oblong, rounded at apex. Ovary mostly ellipsoid-cylindrical, smooth or papillose in the upper 1/3, (2.8–) 3.1–4.0 (–4.1) mm long and (1.4–) 1.7–2.2 (–2.3) mm wide. Style white, (1.8–) 1.9–3.0 (–3.1) mm long. Capsule obovoid, (3.4–) 3.6–5.8 (–5.9) mm long and (2.5–) 2.7–4.1 (–4.2) mm wide, shorter than the perigon. Seeds black, (2.9–) 3.0–3.5 (–3.8) mm long and (1.2–) 1.3–1.7 (–1.8) mm wide (Fig. 7). 2n = 16, 24.

Phenology: Flowers from middle June to early August.

Habitats: Dry and semi-dry grasslands (Sub-pannonic steppic grasslands, Ponto-Sarmatic steppes), rocky outcrops with pioneer vegetation.

Distribution: Ukraine, Moldova, north-eastern Romania, southern Russia (Fig. 1A). Altitudinal range: 0–350 m a. s. l.

Conservation: This species is included in the Red List for Ukraine (LC, least concern, sub *Allium paniculatum* and *A. podolicum*; Onyshchenko et al. 2022) and Moldova (CE, critically endangered, sub *Allium podolicum*; Munteanu 2015). IUCN Red List (IUCN 2022) lists this species in category LC (Least Concern). However, this is not correct due to wrong taxonomic interpretation of this species in the Red List. Because many popula-tions of *A. paniculatum* occur in island-like isolated localities within strongly degraded agricultural landscape and its distribution is limited it is proposed that its status be updated to Near Threatened (NT).

Identification key to the accepted species of *A. paniculatum* complex within the region studied

1a Leaves semi-cylindrical, narrow, usually less than 2.5 mm wide; tepals pale pinkish to pink, sometimes tinged with purplish-pink, white or brown, usually with purplish or rarely greenish midrib; ovary smooth or slightly papillose in the upper 1/3 .... ***A. paniculatum***

1b Leaves flat or compressed, usually more than 2.5 mm wide; tepals whitish or green-yellowish, suffused with purple or brown, with purplish or green-brownish midrib; ovary papillose in the upper 1/2 .... **2**

2a Leaves always flat, usually more than 3.0 mm wide, leaf margin densely papillose with prevalence of verrucose papillae; tepals green-yellowish, tinged with brown, purplish-brown, with brown-green midrib; capsule exerted from the perigon; seed usually more than 1.7 mm wide .... ***A. fuscum***

2b Leaves compressed (or rarely flat), usually less than 3.5 mm wide, leaf margin not densely papillose, with irregular distribution of papillae; tepals white to dirty whitish, tinged with purple or brown, with purplish, purplish-brown or brownish-green midrib; capsule shorter than the perigon; seed usually less than 1.7 mm wide .... ***A. marginatum***

Supplementary materials

Fig. S1. – Results of the redundancy analysis (RDA), testing the morphological differences among four taxonomic groups (predictors), using the same dataset (matrix 3) as in the PCA.

Fig. S2. – Plants of *Allium marginatum* Janka from the type locality (locality no. 13, Sucutard near Cluj, Romania).

Fig. S3. – Lectotype of *Allium marginatum* Janka, deposited in the herbarium CJ.

Table S1. – Survey of populations analysed in this study.

Table S2. – Survey of quantitative and qualitative morphological characters and ratios, and their abbreviations.

Table S3. – Analysis of molecular variance (three-level AMOVA analysis) of the total dataset (84 individuals) and separate AMOVAs (two-level) for each taxonomic group (genetic cluster).

Table S4. – Results of flow-cytometry and karyological analyses of the populations studied.

Table S5. – Absolute genome size (AGS), DNA base content (AT, CG, in %) of selected plants of the taxonomic groups studied.

Table S6. – Descriptive statistics of all quantitative characters and their ratios (mean±standard deviation; minimum, 10% and 90% quantile, and maximum) for the taxonomic groups, including common di- and rare triploids recorded in *A. podolicum* and *A. marginatum*.

Table S7. – Descriptive statistics of all qualitative characters studied (percentage of each category for each studied categorical variable within each group) for the taxonomic groups, including common di- and rare triploids recorded in *A. podolicum* and *A. marginatum*.

Supplementary Material S1. – Anatomical structure of the leaf blades of the taxonomic groups studied and the transverse cross section of the leaf of *Allium marginatum*.

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Rozklíčování taxonomické struktury komplexu česneku latnatého (*Allium paniculatum*) ve střední a východní Evropě pomocí molekulárních, cytogenetických a morfologických přístupů

Allium sekce *Codonoprasum* zahrnuje skupinu evolučně mladých taxonů s nejasnými taxonomickými hranicemi. Nejkomplikovanějším taxonem této sekce je druh *A. paniculatum*, který byl donedávna považován za nejrozšířenější a morfologicky nejvariabilnější v rámci celé sekce, s rozsáhlým areálem od Středomoří po střední Evropu a pontickou oblast. Nedávná studie prokázala, že "pravé" *A. paniculatum* je samostatný druh se specifickou morfologií, vyskytující se pouze na jižní Ukrajině a v jižním Rusku. Objasnění jeho taxonomické identity však zpochybnilo taxonomickou identitu populací označovaných jako *A. paniculatum* v jiných částech Evropy, včetně populací ve střední Evropě a v západní části východní Evropy (Slovensko, Maďarsko, Rumunsko, Ukrajina), odkud byly popsány další druhy tohoto komplexu (*A. fuscum*, *A. marginatum* a *A. podolicum*), později často uváděny pouze jako synonymum *A. paniculatum*. Tento článek se zabývá taxonomickou identitou populací výše zmíněných taxonů na území střední a východní Evropy pomocí různých biosystematických metod (morfometrika, anatomie listů, epidermální mikromorfometrika, karyologie, velikost genomu a obsah GC bází, AFLP). Pomocí tohoto kombinovaného přístupu byly ve studovaném materiálu zjištěny tři vzájemně separované populační skupiny, taxonomicky odpovídající *A. fuscum*, *A. marginatum* a skupině *A. paniculatum* + *A. podolicum*. Zjištěná genetická struktura naznačuje, že populace alespoň některých z rozlišovaných taxonů mohly přežívat ve svých současných areálech nebo v sousedních oblastech během posledního glaciálu a holocénu. *Allium fuscum* je druh, který převážně osídluje zastíněné skalní výchozy v širokém okolí oblasti Železných vrat (jihozápadní Rumunsko, severozápadní Bulharsko a severovýchodní Srbsko). *Allium marginatum* představuje druh blízce příbuzný druhu *A. fuscum*, který osídluje suché trávníky a xerotermní doubravy v Panonské a Transylvánské pánvi. Dva východoevropské taxony, *A. podolicum* a *A. paniculatum*, velmi podobné morfologií, velikostí genomu a genetickými markery, jsou považovány za konspecifické a pojmenovány *A. paniculatum*. Tento druh obývá zonální stepní a lesostepní oblasti východní Evropy od západní Ukrajiny po jižní Rusko. V závěru je uvedeno taxonomické a nomenklatorické zpracování a klíč k určování akceptovaných druhů komplexu *A. paniculatum* ve studovaném regionu.