



## *Allium urusakiorum* (Amaryllidaceae), a new member of the Balkan clade of the section *Oreiprason* from European Turkey

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### Abstract

The taxonomy of the *Allium saxatile* group (sect. *Oreiprason*) has been studied recently upon morphological and molecular data. New specimens collected from the European part of Turkey near the Istranca Mountains and identified as '*A. saxatile*' proved to be a new species confirmed by sequences of internal transcribed spacer (ITS) and two plastid DNA regions (*rpl32-trnL* and *trnL-trnF*) and supported by morphological characters. *Allium urusakiorum*, a new species from the *A. saxatile* group, is described here through living and herbarium specimens. It is the only species of the sect. *Oreiprason* in the country and seems to be an endemic species of the Turkish flora. Characteristics of the species include morphological description, identification key, molecular dataset, and karyotype (2n = 16).

**Key words:** *Allium* subgen. *Polyprason*, taxonomy

### Introduction

Seregin *et al.* (2015) recently published a large molecular and morphological revision of the *Allium saxatile* Marshall von Bieberstein (1798: 114) species complex. *Allium saxatile* is the type species of *A. sect. Oreiprason* Hermann (1939: 57) from the subgenus *Polyprason* Radić (1990: 250, 253) as specified by Friesen *et al.* (2006). In order to finally revise the whole section, Seregin & Friesen (2015) performed additional molecular samplings of many poorly known Central Asian species; the results of this study led to their complete transfer to *A. sect. Falcatifolia* N.Friesen in Friesen *et al.* (2006: 390). Accessions of *A. tianschanicum* Ruprecht (1869: 33), another Central Asian species formerly assigned to *A. sect. Oreiprason*, formed a separate clade out of the *A. saxatile* group (Seregin & Friesen 2015). Thereby, recent studies show that *A. sect. Oreiprason* in its current circumscription should include 15 species, i.e. only the *A. saxatile* species complex revised earlier (Seregin *et al.* 2015).

Özhatay *et al.* (2012) reported '*A. saxatile*' from Turkey-in-Europe. This record was supported with high-quality photographs showing white-flowered plants with long filaments of apparently unknown identity. As Seregin *et al.* (2015) did not study Turkish material, we present in this paper the results of molecular, morphological and karyological analysis of the Turkish plants which appear to be a species new to science.

### Materials and methods

**Taxon sampling for molecular analysis:**—The Turkish plants were sampled from two close localities (isolates ## Gl-115, Gl-116). To infer their phylogenetic relationships within the European clade of *A. sect. Oreiprason*, we employed 28 samples for 12 related taxa published earlier (Seregin *et al.* 2015): *A. austrodanubiense* N.Friesen & Seregin in Seregin *et al.* (2015: 89), *A. horvatii* Lovrić (1972: 569), *A. marschallianum* Vvedensky (1935: 184), *A. psebaicum* Mikheev (2004: 96), *A. rubriflorum* (Adamović 1908: 200) Anačkov, N.Friesen & Seregin in Seregin *et al.* (2015: 90),

*A. savranicum* (Nyman 1882: 741) Oxner (1935: 301), *A. saxatile* var. *saxatile*, *A. saxatile* var. *globosum* (M.Bieb. ex Redouté 1807: tab. 179) Seregin & Sennikov in Sennikov & Seregin (2015: 1299), *A. schistosum* N.Friesen & Seregin in Seregin *et al.* (2015: 93), *A. tarkhankuticum* Seregin (2012: 11), as well as *A. cretaceum* N.Friesen & Seregin in Seregin *et al.* (2015: 85) and *A. montanostepposum* N.Friesen & Seregin in Seregin *et al.* (2015: 85). The authorship of *A. savranicum* was corrected after Sennikov & Seregin (2014). 30 ITS and 30 plastid DNA sequences were included in the phylogenetic analysis. GenBank accession numbers and voucher information of the original sequences are listed alphabetically in Appendix 1. Total genomic DNA was sampled using the “InnuPREPP Plant DNA Kit” (Analytic Jena AG) according to the instructions of the manufacturer and was used directly in PCR amplifications.

**DNA sequencing:**—We sequenced the internal transcribed spacer (ITS) from ribosomal nuclear DNA of all samples. The nrDNA ITS region (ITS1, 5.8S, and ITS2) was amplified using primers ITS-A and ITS-B (Blattner 1999). The PCR condition was the same as described in Friesen *et al.* (2006). Additionally, we sequenced the plastid *trnL*–*trnF* and *rpl32*–*trnL* regions using primers described by Shaw *et al.* (2007) for *rpl32*–*trnL* and Taberlet *et al.* (1991) for *trnL*–*trnF*. PCR products were sent to SeqLab (Göttingen, www.seqlab.de). Forward and reverse (if necessary) sequences from each individual were manually edited in CHROMAS Lite 2.1.1 (Technesylum Pty Ltd) and combined in single consensus sequences. The sequences of all samples were aligned with CLUSTAL X (Thompson *et al.* 1997) with subsequent manual correction of alignments in MEGA6 (Tamura *et al.* 2013). The simple indel coding method of Simmons & Ochoterena (2000) was used in aligned sequences.

**Phylogenetic analysis:**—*Allium montanostepposum* and *A. cretaceum* from the Siberian clade of *A. sect. Oreiprason* have been chosen as outgroup based on the analysis by Seregin *et al.* (2015). Parsimony analysis was performed with PAUP\* 4.0b10 (Swofford 2002) using heuristic searches with TBR and 100 random addition sequence replicates. Bootstrap support (BS) (Felsenstein 1985) was estimated with 1000 bootstrap replicates, each with 100 random addition sequence searches. Bayesian analysis was implemented with MrBayes 3.1.23 (Ronquist & Huelsenbeck 2003). Sequence evolution models were evaluated using Akaike information criterion with the aid of Modeltest 3.7 (Posada & Crandall 1998). Two independent runs were initiated for 10 million generations using Markov chains, sampling every 100 trees. A quarter (25%) of the initial trees was discarded as burn-in and excluded from the analysis. The remaining trees were combined into a single dataset, and a majority-rule consensus tree was obtained. Bayesian posterior probabilities were calculated for that tree in MrBayes3.1.23.

**Karyotype analysis:**—Specimens were collected from the natural habitat and potted for the karyological studies. Root tips were pre-treated with 0.05% 1-bromonaphthalene solution at 4°C for 24 h and then fixed in fresh Carnoy’s solution overnight. Root tips were hydrolysed for 10–12 min in 1 n HCl at 60°C, washed and stained in Feulgen solution for 1–2 h. Stained meristems were squashed in a drop of 2% aceto-orcein and permanent preparations made by the liquid CO<sub>2</sub> method. The image analysis systems KAMERAM© and Canon A640 camera were used for metaphase handling and chromosome measurements. They were calculated with a formula of the relative variation in chromosome length (CV<sub>CL</sub>), mean centromeric asymmetry (M<sub>CA</sub>) following Zuo & Yuan (2011) and Peruzzi & Eroğlu (2013). The classification of chromosomes as having centromeres in metacentric (m), submetacentric (sm), subtelocentric (st), and telocentric (t) was used, according to Levan *et al.* (1964). Chromosome data of the specimens are summarized in Table 1. For each chromosome, the relative length (percentage of total autosomal length) and centromeric index (length of short arm as a percentage of the whole chromosome length) were calculated.

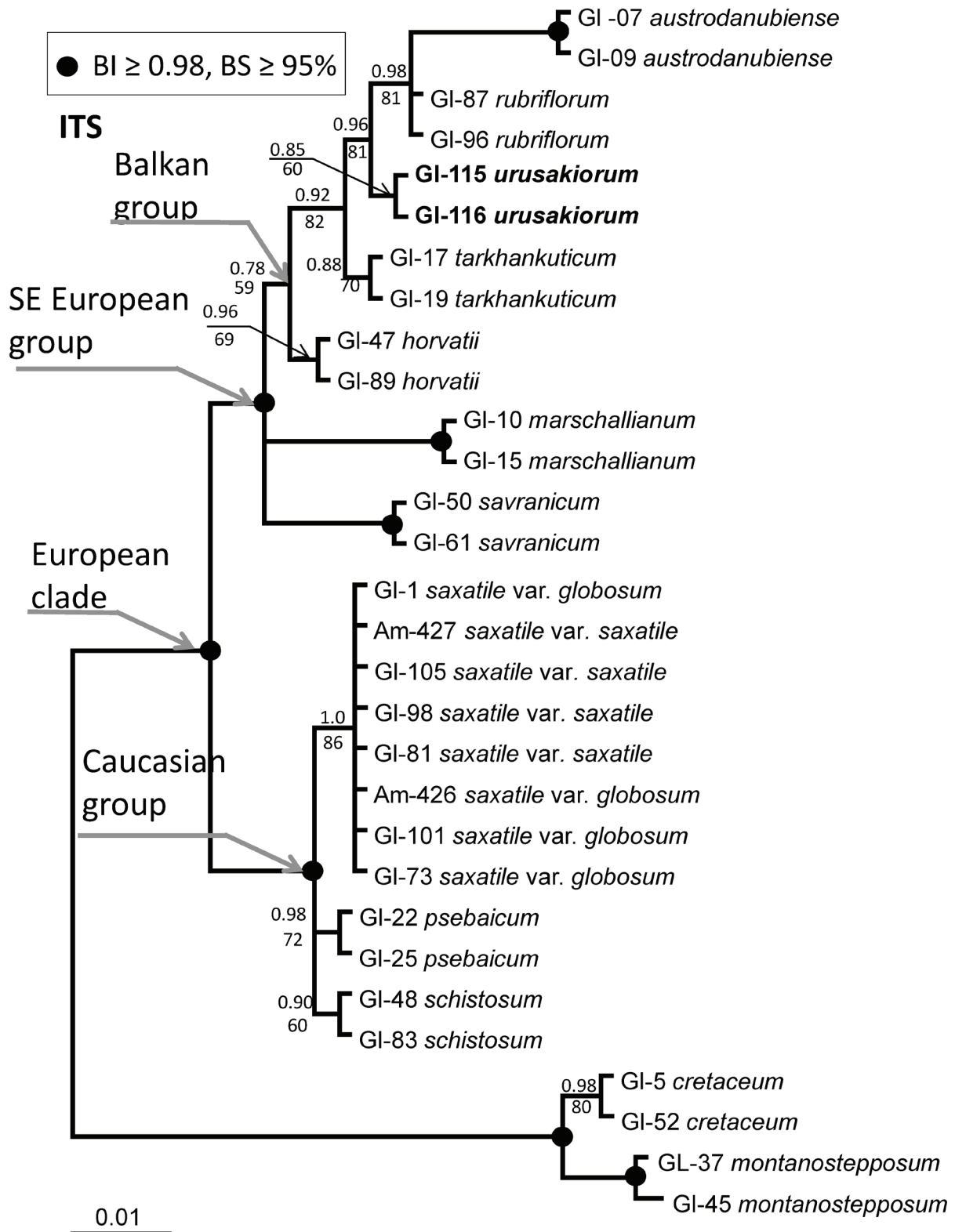
**TABLE 1.** Morphometric data on the haploid chromosomes of *Allium urusakiorum* (MC = metacentric chromosome; SMC = submetacentric chromosome).

Chromosome pair	Centromeric position	Total absolute length (µm)	Short arm (S) (µm)	Long arm (L) (µm)	R = L/S
1	MC1	8.53	3.75 ± 0.08	4.78 ± 0.08	1.27
2	MC2	7.86	3.74 ± 0.02	4.12 ± 0.11	1.10
3	MC3	7.67	3.27 ± 0.39	4.40 ± 0.38	1.35
4	MC4	7.25	2.99 ± 0.12	4.26 ± 0.04	1.42
5	MC5	6.33	2.95 ± 0.02	3.38 ± 0.22	1.15
6	MC6	5.76	2.70 ± 0.10	3.06 ± 0.04	1.13
7	SMC1	6.90	2.18 ± 0.11	4.72 ± 0.01	2.17
8	SMC2	6.78	1.98 ± 0.13	4.80 ± 0.18	2.42

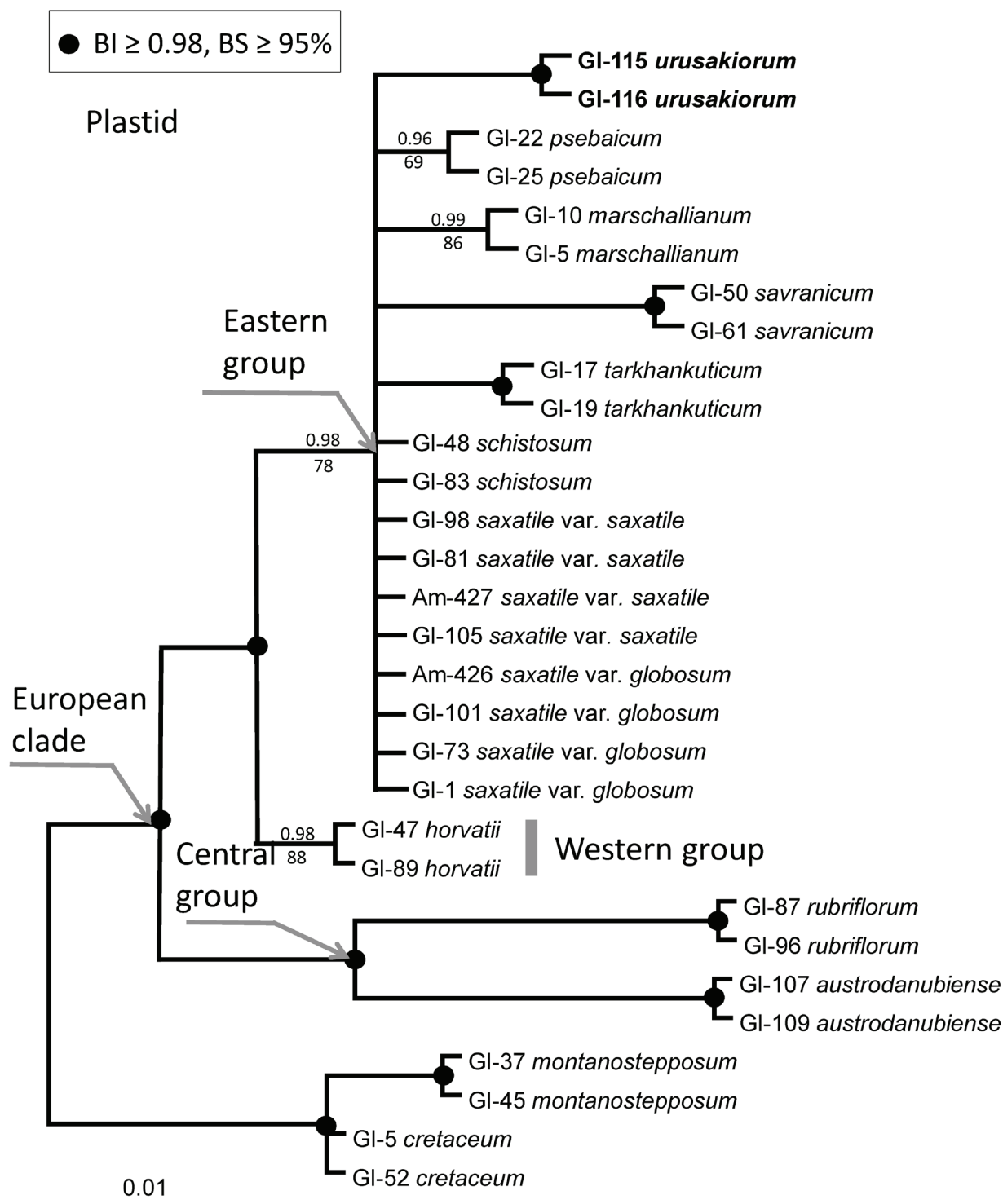
Total karyotype length: 114.16 ± 1.3 µm

## Results

Sequences of nrITS and plastid DNA fragments (*trnL-trnF* and *rpl32-trnL*) were analysed separately. The summary of statistics for the phylogenetic framework is presented in Table 2, and the Bayesian phylograms are shown in Figs. 1 and 2.



**FIGURE 1.** ITS Bayesian consensus tree of the European species of *Allium* sect. *Oreiprason*. Numbers by nodes represent bootstrap support (100 replicates) and Bayesian probabilities. Bayesian probabilities over 0.98 and bootstrap support over 95% are indicated with a black dot.



**FIGURE 2.** Plastid DNA Bayesian consensus tree of the European species of *Allium* sect. *Oreiprason* from *trnL*–*trnF* and *rpl32*–*trnL* (UAG). Numbers by nodes represent bootstrap support (100 replicates) and Bayesian probabilities. Bayesian probabilities over 0.98 and bootstrap support over 95% are indicated with a black dot.

**ITS sequence data:**—The alignments of combined ITS1 and ITS2 sequences (including the 5.8S gene) consist of 644 characters of which 81 variable characters are parsimony informative. The substitution model TVM+G was chosen by AIC in Modeltest 3.7 for the Bayesian analysis. Unweighted parsimony analysis of the 30 sequences resulted in four most parsimonious trees of 88 steps (CI = 0.9545; Fig. 1). All species of the European clade are divided into two sister groups—Caucasian (three species) and SE European (seven species, including the Turkish material). The Caucasian group with 100% BP and 1.00 PP consists of three monotypic lineages, i.e. *A. schistosum*, *A. psebaicum*, and

*A. saxatile*. The SE European clade with 100% BP and 1.00 PP also has three lineages. It is composed of two strongly monophyletic basal lineages: (1) the Crimean endemic *A. marschallianum* and (2) *A. savranicum* from riverine sands of Ukraine and SW Russia. The third lineage with lower support (59% BP, 0.78 PP) comprises four Balkan taxa (*A. horvatii*, *A. austrodanubiense*, *A. rubriflorum*, and the Turkish accessions Gl-115 and Gl-116), and closely allied Western Crimean endemic *A. tarkhankuticum*. Within the third lineage, accessions of the westernmost *A. horvatii* form a basal clade which is a sister to a well-supported clade (82% BP, 0.92 PP) in which the easternmost *A. tarkhankuticum* lineage (70% BP, 0.88 PP) is a sister to three endemics of the Balkan Peninsula (i.e. *A. rubriflorum*, *A. austrodanubiense* and the Turkish species). The latter endemic Balkan clade has 81% BP and 0.98 PP in the ITS phylogram.

**Plastid DNA sequence data:**—The alignment of combined *trnL–trnF* and *rpl32–trnL* (UAG) sequences from 30 accessions generate a matrix of 1,170 characters divided into two partitions (*trnL–trnF* spacer, 1–316; *rpl32–trnL*, 317–1170) of which 63 variable characters are parsimony informative (see Table 2). Parsimony and Bayesian analysis yielded the same topology but with lower BP than PP. For the Bayesian analysis, the substitution model TVM+I+G was chosen by AIC in Modeltest 3.7. Unweighted parsimony analysis of the 30 sequences resulted in 4,326 most parsimonious trees of seven steps (CI = 0.9286; Fig. 2). All Caucasian and Crimean taxa of the section *Oreiprason* as well as *A. savranicum* have a peculiarly long deletion of about 400 bp. Three Balkan taxa, i.e. *A. rubriflorum*, *A. austrodanubiense* and *A. horvatii*, and those from the Siberian ITS clade do not have this deletion. Turkish accessions Gl-115 and Gl-116 also show this long deletion in the *rpl32–trnL* spacer. Accessions of *A. austrodanubiense* and *A. rubriflorum* (both species with a long *rpl32–trnL* spacer) build a strong supported sister clade (100% BP, 1.00 PP) to all other taxa of the European clade. On the next step, *A. horvatii*, a species distributed west of *A. austrodanubiense* and *A. rubriflorum*, forms a sister lineage (88% BP, 0.98 PP) to the Eastern group comprising seven taxa distributed in the Crimea, Caucasus and adjacent steppe regions of Russia and the Ukraine, as well as the Turkish accessions. The latter Eastern group (78% BP, 0.98 PP) is unresolved, but all terminal plastid DNA lineages are the same as the terminal lineages from the ITS tree and, therefore, suitable for species delimitation. The Turkish accessions form a strong supported clade with 100% BP and 1.00 PP.

**Karyological data:**—Chromosome number is  $2n = 16$  (ISTE 93421, paratype). The karyotype formula is  $12m + 4sm$  (Figs. 3–5). Metaphase chromosome length ranges from 5.76 to 8.54  $\mu m$ ; total haploid chromosome length is  $57.09 \pm 0.94 \mu m$  (see Table 1). Mean centromeric asymmetry ( $M_{CA}$ ) is 17.46 and inter chromosomal asymmetry index ( $CV_{CL}$ ) is 11.63.

**TABLE 2.** Summary of phylogenetic analysis of the *Allium saxatile* group from Modeltest and MP analysis of separate and combined datasets (AIC = Akaike information criterion; CI = consistency index; RI = retention index).

	ITS	<i>trnL–trnF</i>	<i>rpl32–trnL</i>	Combined <i>trnL–trnF</i> + <i>rpl32–trnL</i>
No. of included accessions	30	30	30	30
No. of included characters	644	316	854	1170
No. of constant characters	562	303	805	1107
No. of variable characters	82	13	48	63
No. of parsimony informative sites	81	13	48	63
No. of trees	4	1	2	4326
No. of steps (tree length)	88	14	53	70
CI	0.9545	0.9286	0.9434	0.9286
RI	0.9862	0.9524	0.9727	0.9645
Model selected by AIC	TVM+G	K81uf+G	HKY+G	TVM+I+G

## Discussion

**Molecular data:**—Both nuclear and plastid DNA analysis show that the Turkish accessions of the section *Oreiprason* represent an independent taxon, described here as a new species. However, our analysis shows that there are conflicting topologies between the ITS and combined plastid DNA trees (Figs. 1, 2) concerning the position of the Turkish samples, as well as other species of the European clade of the section. The Turkish species, *A. urusakiorum*, is clearly nested within the Balkan clade in the ITS tree, but is grouped to the Eastern group in the combined plastid DNA tree, mainly due to the two-fold reduction of *rpl32–trnL* spacer in *A. marschallianum*, *A. psebaicum*, *A. savranicum*, *A. saxatile*, *A. schistosum* and *A. tarkhankuticum*. As a rule, the *rpl32–trnL* spacer of all sequenced *Allium* species is ca. 800 bp long or slightly longer (Guetat *et al.* 2010; Oyuntsetseg *et al.* 2012; Herden *et al.* 2012, 2016; Wheeler *et al.* 2013;





**FIGURE 3.** The metaphase chromosomes of *Allium urusakiorum* ( $2n = 16$ ). Scale bar 10  $\mu\text{m}$ .

Mashayekhi & Columbus 2014; Huang *et al.* 2014; Friesen & Fragman-Sapir 2014; Friesen *et al.* 2015), whereas *A. urusakiorum* and six abovementioned species from the Eastern plastid group have ca. 400 bp in this spacer. The positions of *A. urusakiorum* on both trees are comparable with those of *A. tarkhankuticum* known to occur in the Western Crimea. A possible explanation for the conflicting topology would be an ancient hybrid origin of the Turkish taxon and *A. tarkhankuticum* as well. Because *A. urusakiorum* has the same deletion in the *rpl32-trnL* spacer as other Crimean and Caucasian species (incl. *A. savranicum*) and is placed in the ITS tree between *A. tarkhankuticum* and two Balkan endemics (*A. rubriflorum* and *A. austrodanubiense*), we assume the ancestors of these three species to be probable progenitors of *A. urusakiorum*. We can probably rule out the direct hybridization between modern species because *A. urusakiorum* has already accumulated several independent mutations in both plastid and nuclear spacers. The same scenario is likely to be true for *A. tarkhankuticum*.

**Karyological data:**—The chromosome number of the taxa previously identified as ‘*A. saxatile*’. was reported by Levan (1935), Cheshmedzhiev (1973), Magulaev (1976), Vosa (1977), Zakirova & Vakhtina in Moore (1977), Miceli & Garbari (1980), Van Loon & Kieft in Löve (1980), Pogosian (1983, 1997), Labani & Elkington (1987), Kudrjashova (1988), Agapova *et al.* (1990), Draghia *et al.* (2013). Their results indicated that the chromosome number of the *A. saxatile* species complex is always  $2n = 16$ . Reports on the presence of tetraploidy in the closely related *A. globosum* (Jones & Rees 1968; Nanushyan & Polyakov 1989) is very likely due to misidentification. The chromosome morphology of ‘*A. saxatile*’ was first investigated on specimens cultivated in Lund, Sweden however, no data as to

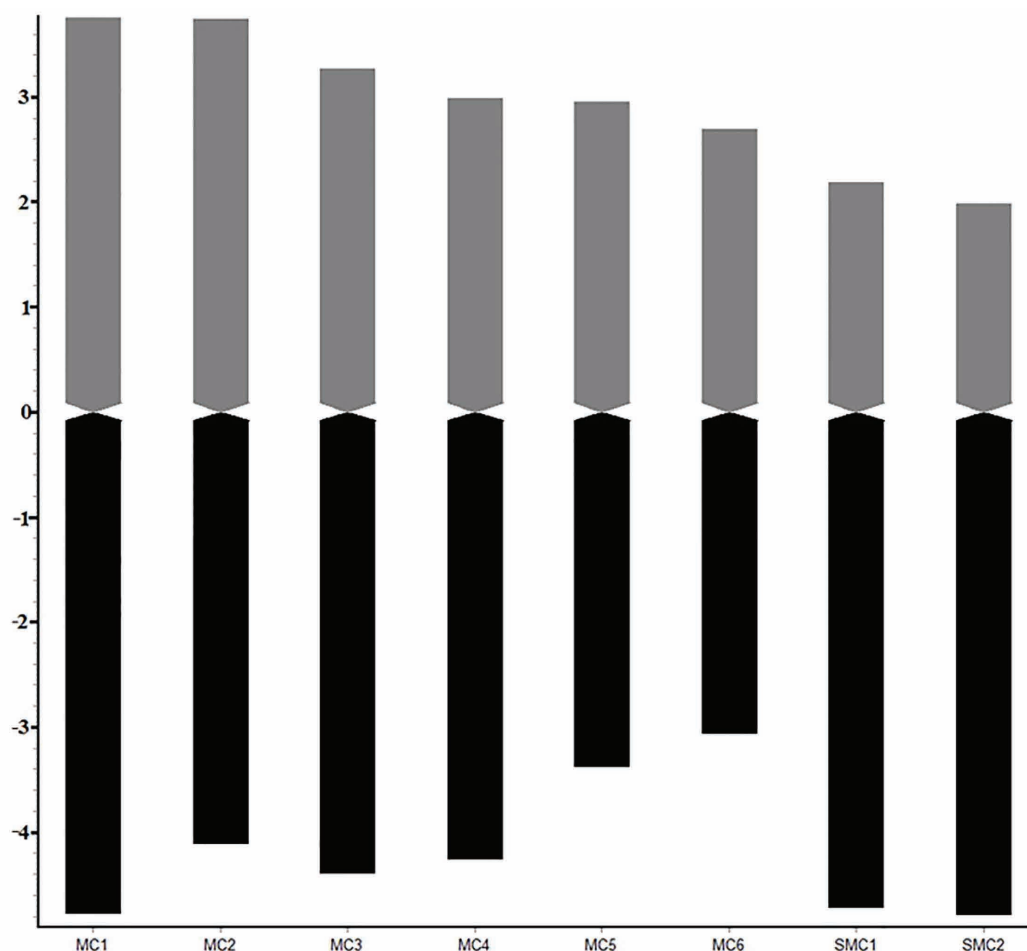


FIGURE 4. Idiogram of *Allium urusakiorum*.

their origin was supplied (Levan 1935). The haploid ( $n = 8$ ) karyotype formula in Levan's study was  $7m + 1st^{SAT}$  and the chromosome size  $5-7 \mu m$ . The chromosome morphology of the closely related *A. horvatii* was investigated on plants from the Eastern Adriatic Coast (Bedalov & Lovrić 1978). The haploid karyotype formula of *A. horvatii* was  $7m + 1ms^{SAT}$ . Finally, the chromosome morphology of the new species clearly differs from this, exhibiting six pairs of metacentric chromosomes and two pairs of submetacentric chromosomes. Peruzzi *et al.* (2016) have recently published a representative study of the karyotype asymmetry in the genus *Allium* and showed that, in the subgenus *Polyprason*,  $CV_{CL}$  shows values intermediate with respect to known range variation in *Allium*, while  $M_{CA}$  reaches relatively low values. Our data for *A. urusakiorum* ( $M_{CA} = 17.46$ ;  $CV_{CL} = 11.63$ ) from the section *Oreiprason* mostly confirm this picture. Another species from the section *Oreiprason* mentioned by Peruzzi *et al.* (2016), i.e. *A. marschallianum* sampled by Sarker *et al.* (1997) from the Crimea, also has similar values ( $M_{CA} = 16.3$ ;  $CV_{CL} = 10.7$ ). As for '*A. saxatile*' from Turkey, counts based on measurements of Özhatay *et al.* (2012) should be discarded as erroneous.

**Morphological data:**—*Allium urusakiorum* described below is a new species and the section *Oreiprason* is a new section for the Turkish flora. This *Allium* clearly differs from the closely related SE European taxa mainly with white short tepals, which are almost twice shorter than filaments. Other important diagnostic features are yellow anthers and the shape of the filaments, which are not widened at the base. A complete description, diagnosis, and identification key are given below.

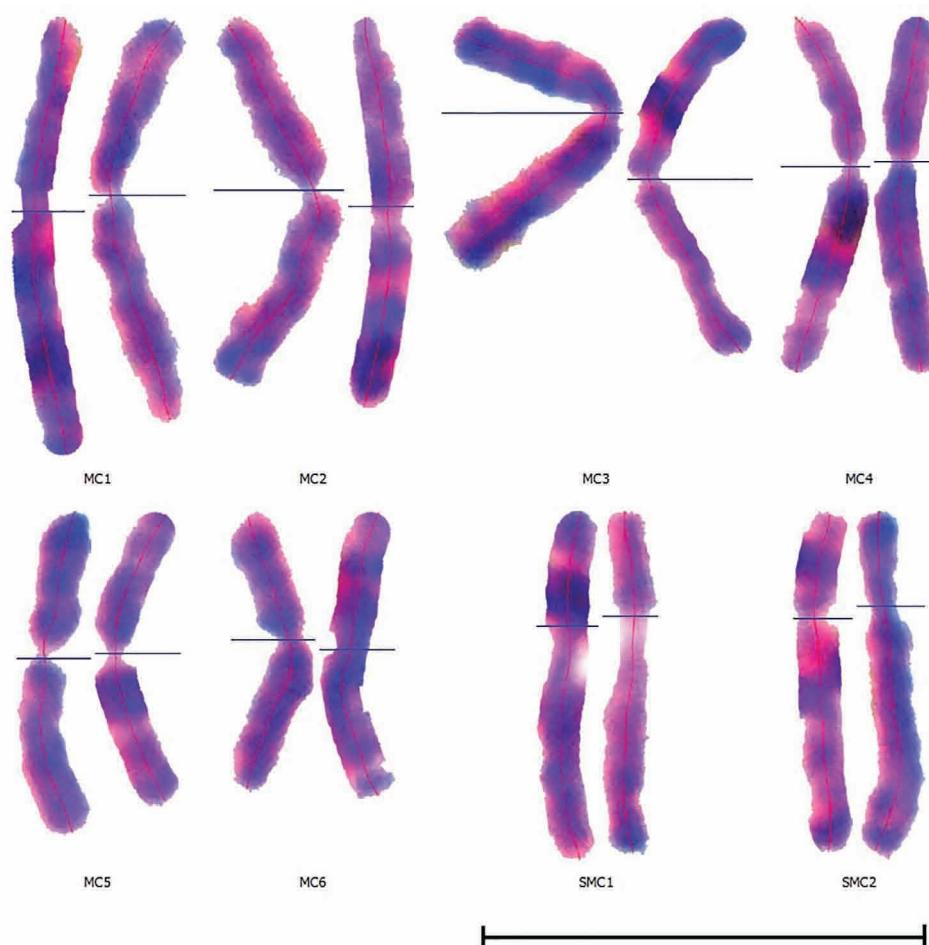


FIGURE 5. Karyotype of *Allium urusakiorum*. Scale bar 10  $\mu$ m.

### Description of the new species

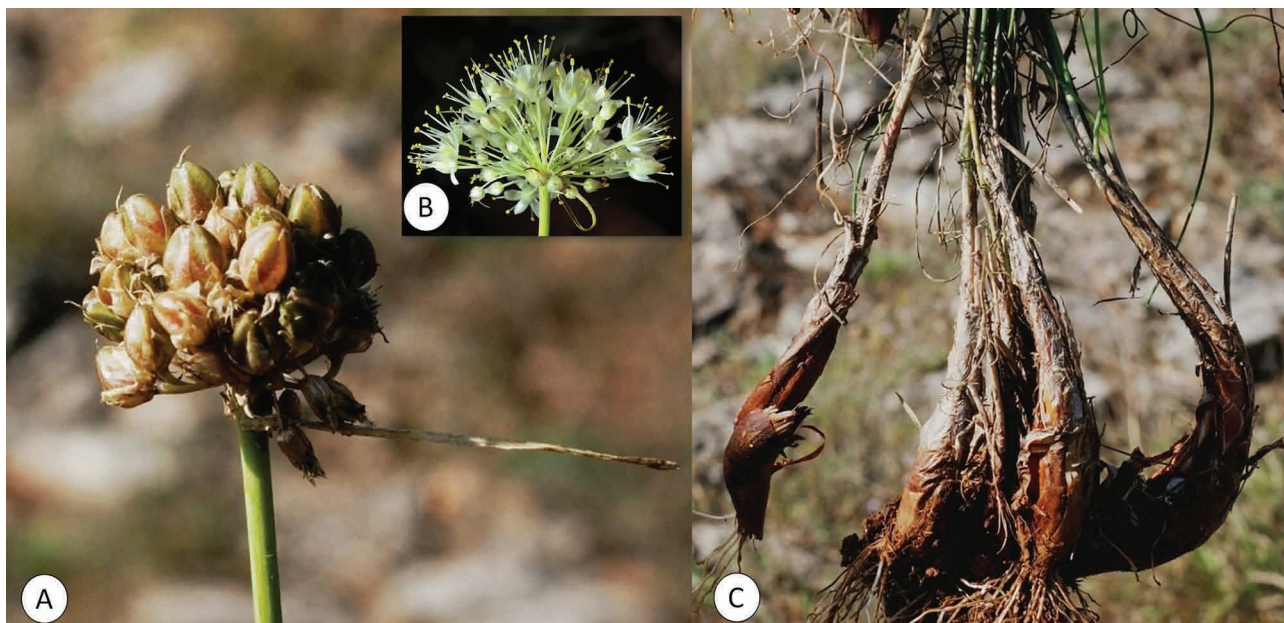
*Allium urusakiorum* Özhatay, Seregin & N.Friesen *sp. nov.* (Fig. 6)

From the closely related *A. rubriflorum* and *A. austrodanubiense* differs for the shorter tepals, 3.5–4.0 mm long (vs. inner tepals distinctly > 4.0 mm long) and white flowers (vs. purplish, rose, or yellowish). From the Caucasian *A. saxatile* differs for the yellow young anthers (vs. brick red or violet) and longer pedicels.

**Type:**—TURKEY: A1 (E): Kırklareli: Demirköy, Mahya Mt, Sarpdere village, 358 m, 3 October 2009, E. Akalın Urusak & Y. Yeşil (holotype, ISTE 92497!).

Stems usually clustered, attached to a short rhizome, 20–60 cm high, 1.5–2.0 mm in diameter, cylindrical. Bulb-like base of the stem (false bulb, or “bulb”) 0.5–1.5 cm in diameter, 2–3.5(–4.0) cm long, ovoid-cylindrical; outermost tunics brown to red-brown, coriaceous, somewhat splitting lengthwise into strips; inner tunics light brown, membranous. Leaves 3–7, filiform, 0.5–1.0 mm wide, 5–20 cm long, canaliculate, sheathing the lower 1/3 to 1/4 of the stem. Top leaves green in the beginning of anthesis, almost dry at the end of anthesis, longer than scape. Spathe bivalved, persistent; valves unequal, the longer 15–30 mm long with a long filiform beak, the shorter 5–8 mm long; beak 3–5 veined. Umbel hemispherical to subglobose in anthesis, 15–20 mm in diameter in anthesis, larger in fruiting, 15–35-flowered, subglobose or hemispherical; pedicels 5–15 mm long, subequal in anthesis, elongate in fruit, bracteolate. Flowers 1.5–2(–3) times shorter than pedicels. Perigone campanulate; tepals slightly unequal, 3.5–4.0 mm long, 1.5–2.0 mm wide, white with a light green vein, elliptic-ovate, acute inner ones slightly longer than outers. Stamens long exserted; filaments filiform, white, slightly widened at base, 1.5–2 times longer than tepals. Anthers 0.8–1.0 mm long,





**FIGURE 6.** *Allium urusakiorum*: fruiting umbel (A), flowering umbel (B), bulb-like bases of stems (C).

yellow; fully dehiscent anthers dark yellow, adnate to tepals; oblong, rounded at apex. Ovary subglobose-oblong, yellowish-green,  $0.8\text{--}1.0 \times 1.0\text{--}1.5$  mm. Style exerted, almost as long as stamens, white. Capsule globose-rhomboid,  $3.0\text{--}4.0 \times 3.5\text{--}4.5$  mm. Valves of capsule rhomboid-ovate. Seeds black, angled, 2 mm long.

**Etymology:**—The epithet “*urusakiorum*” originates from the surname of Emine Akalın Uruşak and her husband Uğur Uruşak, who collected many *Allium* specimens around Turkey during their excursions, including the first collection of the new species.

**Flowering time:**—August to September depending on weather conditions.

**Habitat:**—Dry limestone rocky places, 225–460 m above sea level (Fig. 7).



**FIGURE 7.** Habitat of *Allium urusakiorum*.





FIGURE 8. Distribution of *Allium urusakiorum*.

**Distribution:**—*Allium urusakiorum* is distributed in the NE of the European Turkey (Fig. 8), in Euro-Siberian phytogeographical region. It is known from two localities mentioned below in specimen labels. For neighbouring Bulgaria, Assyov *et al.* (2012) have reported '*A. saxatile*' for several regions. Revision by Seregin *et al.* (2015) have shown that these records should be interpreted as *A. austrodanubiense* and to some extent as *A. rubriflorum*. Considering that *A. urusakiorum* was collected in NW Turkey, we could assume that a report of '*A. saxatile*' by Assyov *et al.* (2012) from adjacent Strandzha Mts. in SE Bulgaria might also refer to this species. As far as we could trace, this record is based on '*A. globosum*' from Northern Strandzha by Hermann (1936). Unfortunately, Hermann did not provide any locality details and no voucher specimen was found in his collection deposited in GAT. Also, there is no supporting material from Strandzha in the major Bulgarian herbaria (SO, SOA, and SOM).

**Additional specimens examined (paratypes):**—TURKEY: A1 (E): Kırklareli: Demirköy, Dupnisa Cave, 458 m, 26 October 2009, E. Akalın Uruşak, Y. Yeşil & M. Koçyiğit (ISTE 93421!); *ibidem*, 260 m, 20 July 2010, N. Özhatay, E. Özhatay, E. Akalın Uruşak & M. Koçyiğit (ISTE 93422!); *ibidem*, 4 October 2015, M. Koçyiğit (ISTE 109445!).

## Conclusions

*Allium urusakiorum* is a species with a transitional position between the Balkan species (ITS data) and species from the Crimea and Caucasus (*trnL-trnF* and *rpl32-trnL* plastid DNA data). In this regard, its phylogenetic position is close to *A. tarkhankuticum*, an off-shoot of the Balkan group known from the western coast of the Crimea. *Allium urusakiorum*, which we assume to be a rare endemic to European Turkey, is the seventh member of *A. sect. Oreiprason* (and the first one on the Balkan Peninsula) with a highly reduced *rpl32-trnL* spacer—an exceptional case in the genus *Allium*. We have come a long way in our studies of *A. sect. Oreiprason*, especially in SE Europe, which has turned out to be an important and formerly neglected centre of diversification. Since recognition of the single aggregate species *A. saxatile* and the Krk Island endemic *A. horvatii* by Stearn (1980), the current list of the European species formerly known as '*A. saxatile*' includes eight taxa: *A. savranicum*, *A. marschallianum*, *A. tarkhankuticum*, *A. ×agarmyschicum*, *A. austrodanubiense*, *A. rubriflorum*, a completely recircumscribed *A. horvatii*, and finally *A. urusakiorum*.

## Identification key for species of the European clade of *Allium* sect. *Oreiprason*

1. Young anthers yellow ..... 2
- Young anthers brown, brick red or violet ..... 9
2. Filaments purplish; tepals uniformly rose; young stems spirally arching ..... *Allium psebaicum*
- Filaments white or yellowish; tepals white, yellowish or greenish, sometimes tinged rose in the upper part, with obscure or conspicuous veins, which might be green or purple; young stems erect ..... 3
3. Fully dehiscent (empty) anthers yellow ..... 4
- Fully dehiscent (empty) anthers light brown to violet ..... 7
4. Filaments not widened at base ..... 5
- Odd filaments (those attached to inner tepals) widened at base ..... 6
5. Tepals white or purple, (4.0–)4.5(–5.0) mm long; filaments up to 1.5 times longer than tepals ..... *Allium rubriflorum*
- Tepals white, 3.5–4.0 mm long; filaments 1.5–2(–3) times longer than tepals ..... *Allium urusakiorum*
6. Tepals turn rose after anthesis ..... *Allium marschallianum*
- Tepals ± whitish or tinged green after anthesis, but might be tinged rose prior to or in anthesis ..... *Allium horvatii*
7. Young anthers usually light brown, rarely yellow; more robust plants of open sands ..... *Allium savranicum*
- Young anthers always yellow; plants of stony habitats or steppes ..... 8
8. Filaments < 1.5 times longer than tepals, not widened at base ..... *Allium austrodanubiense*
- Filaments twice as long as tepals; odd filaments (those attached to inner tepals) widened at base ..... *Allium saxatile* var. *saxatile*
9. Filaments rose to purple ..... *Allium saxatile* var. *globosum*
- Filaments white ..... 10
10. Ovary green (sometimes tinged purple) in anthesis ..... 11
- Ovary purplish in anthesis ..... 12
11. Young anthers light brown; pedicels 2–3 times longer than tepals; tepals white, sometimes rose in upper part; robust plants of open sands ..... *Allium savranicum*
- Young anthers brick red (fully dehiscent anthers could be yellow); pedicels almost equal tepals; tepals white; dwarf alpine and subalpine plants of rocks and scree ..... *Allium schistosum*
12. Tepals oblong; filaments 1.5 times longer than tepals; filaments not widened at base ..... *Allium tarkhankuticum*
- Tepals lanceolate; filaments twice as long as tepals; odd filaments (those attached to inner tepals) slightly widened at base ..... *Allium saxatile* var. *saxatile*

## Acknowledgements

Contribution of M. Koçyiğit and N. Özhatay is financially supported by “Protection and Sustainable Development of Natural Resources and Biodiversity in the Yıldız Mountains, Turkey” (project no: EuropeAid/125289/D/SER/TR) and the Scientific Investigation Project Coordinator of Istanbul University (project no: 28749). Contribution of A.P. Seregin (molecular work and curation of distributional database) is supported by Russian Science Foundation–PHΦ (project # 14–50–00029). Lucille Schmieding (University of Osnabrück, Germany) made careful proofreading of the final English text.

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**APPENDIX 1.** Accessions of *Allium* sect. *Oreiprason* from which 30 ITS sequences and 30 plastid DNA (*trnL–trnF* and *rpl32–trnL*) sequences were obtained, with corresponding voucher information and GenBank reference numbers. The information for each taxon is listed as follows: COUNTRY, ISOLATE NAME (ITS, *trnL–trnF*, *rpl32–trnL*)-locality-herbarium (voucher information) and/or botanical garden accession number. Isolates ## GL-115 & GL-116 are sequenced for the first time; other sequences (HG794xxx) were published by Seregin *et al.* (2015).

- A. austrodanubiense*:**—BULGARIA, GL-107 (HG794212, HG794059, HG794129)—Black Sea Coast, Kaliakra Reserve—SOM 159122 (*Ant. Petrova*, 13 September 1999); GL-109 (HG794214, HG794061, HG794131)—Konjavsko Mt, Smudertsı—SO 96081 (*Gusev*, 1 August 1992).
- A. cretaceum*:**—RUSSIA, GL-5 (HG794150, HG794010, HG794080)—Volgograd Oblast, Ilovinsky distr., Kondrashi—MW (*Sukhorukov*, 23 August 1999); KAZAKHSTAN, GL-52 (HG794175, HG794033, HG794103)—Aktyubinsk Oblast, to NE from Uil, Akshatau—MW (*Tscherkassova*, 15 June 1965);
- A. horvatii*:**—ITALY, GL-47 (HG794171, HG794029, HG794099)—Trieste Prov., San Dorligo della Valle, Val Rosandra—Accession 09-08-0026-10 in Osnabrück Univ. BG; MONTENEGRO, GL-89 (HG794198, HG794050, HG794120)—Orjen Mt.—BUNS (*Anačkov & Zlatković*, 27 August 2006).
- A. marschallianum*:**—RUSSIA, GL-10 (HG794154, HG794014, HG794084)—Crimea, Sevastopol—MW (*Seregin T-233*, 6 September 2002); GL-15 (HG794158, HG794017, HG794087)—Crimea, Karadag—MW (*Kamenskikh* 8284, 28 September 2005).
- A. montanostepposum*:**—RUSSIA, GL-37 (HG794167, HG794025, HG794095)—Altai Republic, Ust-Koksinsky distr., 65 km to E from Ust-Koksa—OSBU 18724 (*Neuffer et al.*, 13 July 2008); GL-45 (HG794170, HG794028, HG794098)—Altai Krai, Krasnoschekinsky distr., Akimovka, Sopka Mursinka—Accession 10-20-0021-20 in Osnabrück Univ. BG.
- A. psebaicum*:**—RUSSIA, GL-22 (HG794163, HG794022, HG794092)—Krasnodar Krai, Tuapsinsky distr., Arkhipo-Osipovka—MW (*Zernov & Firsanov*, 1 August 1995); GL-25 (HG794164, HG794023, HG794093)—Krasnodar Krai, between Lake Abrau and Lake Limanchik—MW (*Zernov*, 21 September 1996).
- A. rubriflorum*:**—HUNGARY (cultivated), GL-96 (HG794205, HG794053, HG794123)—ex Hort. Bot. Univ. Budapest—GAT 7525 (*anonymous*, 12 July 1984) & Accession TAX631 in Gatersleben; SERBIA, GL-87 (HG794196, HG794048, HG794118)—East Serbia, Niš, Sicevo Gorge—BUNS (*Anačkov*, 17 August 2006).
- A. savranicum*:**—RUSSIA, GL-50 (HG794173, HG794031, HG794101)—Rostov Oblast, the Chir River, Khutor Demin—MW (*Pimenov* 18, 21 August 2006); GL-61 (HG794180, HG794037, HG794107)—Volgograd Oblast, Frolovsky distr., between Padok and Pilnya—MHA (*Shantser & Polonskaya*, 25 July 1993).

*A. saxatile* var. *saxatile*:—GEORGIA, AM-427 (HG794225, HG794068, HG794138)—Imeretia, between Tskaltubo and Tsageri—OSBU (*Lobin & Gröger*, 30 August 2012); GL-81 (HG794192, HG794045, HG794115)—Kazbegi distr., Daryalskoye Gorge—MHA (*Gogina 2139*, 15 August 1966); GL-98 (HG794206, HG794054, HG794124)—Dariali Gorge, 8 km to N from Kazbegi—GAT 7515 (*Pistrick & Akhalkatsi*, 20 July 2002) & Accession TAX6047 in Gatersleben; GL-105 (HG794210, HG794058, HG794128)—Mtiuleti, Kazbegi—OSBU (*Lobin*, 27 June 2005).

*A. saxatile* var. *globosum*:—GEORGIA, AM-426 (HG794224, HG794067, HG794137)—Racha-Lechkhumi, Lajanuri valley—OSBU (*Lobin & Gröger*, 30 August 2012); GL-101 (HG794209, HG794057, HG794127)—Lower Racha, Ambrolauri, Mukhli—GAT 7553 (*Pistrick & Akhalkatsi*, 14 July 2002) & Accession TAX6045 in Gatersleben; RUSSIA, GL-1 (HG794148, HG794008, HG794078)—Karachay-Cherkessia, Zelenchuksky distr., Kobu-Bashi—MW (*Zernov & Anurov 6471*, 8 August 2007); GL-73 (HG794189, HG794042, HG794112)—Stavropol Krai, Pyatigorsk, Mt Mashuk—MHA (*Dayeva 311*, 27 August 1966).

*A. schistosum*:—RUSSIA, GL-48 (HG794172, HG794030, HG794100)—Karachay-Cherkessia, Arkhyz—MW (*Zernov & Onipchenko 7011*, holotypus, 6 August 2008); GL-83 (HG794193, HG794046, HG794116)—Karachay-Cherkessia, Uchkulan—MHA (*Gogina 203*, 29 July 1968).

*A. tarkhankuticum*:—RUSSIA, GL-17 (HG794159, HG794018, HG794088)—Crimea, 3 km to WNW from Olenevka—MW (*Seregin & Seregina T-1110*, paratypus, 14 August 2008); GL-19 (HG794161, HG794020, HG794090)—Crimea, 4.5 km to NW from Olenevka—MW (*Seregin & Seregina T-1124*, paratypus, 14 August 2008).

*A. urusakiorum*:—TURKEY, GL-115 (LT593854, LT593856, LT593858)—Kırklareli, Dupnisa Cave—ISTE 93422 (*Özhatay et al.*, paratypus, 20 July 2012); GL-116 (LT593855, LT593857, LT593859)—Kırklareli, Dupnisa Cave—ISTE 92421 (*Akalın et al.*, paratypus, 26 October 2009).