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Allium toksanbaicum (Amaryllidaceae), a new species from Southeast Kazakhstan

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Abstract

Allium toksanbaicum from South East Kazakhstan is described as a new species. Molecular markers reveal a close relationship to *A. obliquum* and some other central Asian species of the section *Oreiprason*. We investigated the phylogenetic relationship of the new species based on sequences of two chloroplast spacers (*rpl*32-*trn*L and *trn*Q-*rps*16) and the nuclear ribosomal DNA internal transcribed spacer (ITS) region. The new species is diploid with a chromosome number of 2n = 2x = 16. A detailed morphological description, illustrations and karyotype features of the new species are given. With its falcate leaves, the new species is very similar to *A. carolinianum* from the section *Falcatifolia*, but in the shape of the inflorescence and flowers it is very different from it. From *A. obliquum* it differs for the purple colour of flowers and filaments, as well as the presence of teeth at the base of inner stamens.

Key words: *Allium* sect. *Oreiprason,* chromosomes, ITS, plastid DNA

Introduction

Allium Linneaus (1753: 294) is one of the largest monocot genera with about 1,000 species (Govaerts *et al.* 2020) naturally distributed throughout the northern hemisphere (Stearn 1978, 1992, Fritsch & Friesen 2002). The main centres of biodiversity are located in Southwestern and Central Asia and in the Mediterranean region, a smaller centre is found in western North America (Friesen *et al.* 2006, Nguyen *et al.* 2008, Li *et al.* 2010, Wheeler *et al.* 2013). The genus is characterized by bulbs (often in rhizomes) enclosed in membranous, fibrous or reticulate tunics, free or basally connate tepals and often a subgynobasic style (Friesen *et al.* 2006). The genus *Allium* is a member of Amaryllidaceae subfam. Allioideae, tribe Allieae (Chase *et al.* 2016).

Dzungar Alatau, a mountain system in Central Asia, stretches from west-south-west to east-north-east along the border of Kazakhstan and China, located between the Ili River (in the south) and Lake Alakol (in the north). The Dzungar Alatau consists of several parallel high mountain chains. The longest chain stretches in the north, and is accompanied by several lower and shorter chains on its northern side. South of the main ridge appear the Toksanbai, Bedzhintau and Tyshkantau ridges (north of the city of Zharkent), which are connected with the spurs of the Chinese Borokhoro ridge belonging to the eastern Tian Shan (Gvozdetsky & Mikhailov 1987). The small mountain ranges Altynemel and Koyandytau are located in the southwestern part of the Dzungar Alatau mountain system (Fig. 1). The Dzungar Alatau region is one of the richest floristic regions of Kazakhstan and included one genus and 76 species endemics to the country, which is 3.5% of the entire flora of the region (Goloskokov 1984). More endemic plant species in Kazakhstan are found only at the Syrdaya-Karatau ridge (88 species, Goloskokov 1969).

In the summer of 2019 we collected an interesting *Allium* species in the high mountains of the Toksanbai ridge that did not match any known species. Its falcate leaves are similar to *Allium carolinianum* Redouté (1804, t. 101), but the flowers and the bulb shape were different. A detailed examination of morphological and cytological features (e.g.

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bulbs, leaves, tepals, stamens, chromosome number, etc.) and the phylogenetic analysis based on molecular markers confirmed that it is a hitherto undescribed species. The present study aims to provide a comprehensive description of the new species named *A. toksanbaicum*, and to infer their taxonomic relationships.

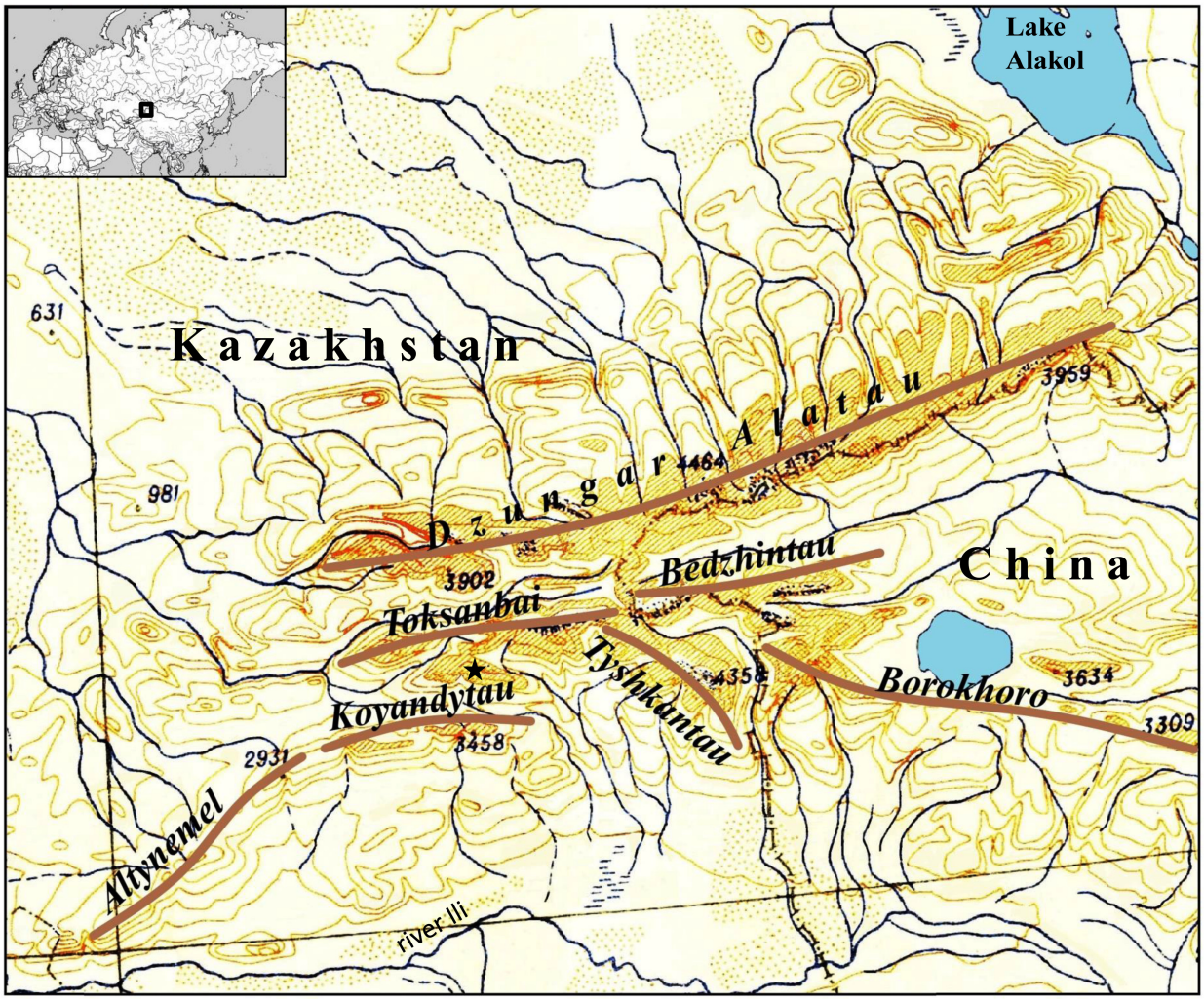

FIGURE 1. Dzungar Alatau mountains. The black star denotes the locus classicus of *Allium toksanbaicum.*

Material & Methods

Taxon sampling:—Bulbs and leaf samples for DNA isolation were collected in summer 2019 in Kazakhstan and grown at the Botanical Gardens in Almaty (Kazakhstan), Barnaul (Russia) and Osnabrueck (Germany). A BLAST analysis of the internal transcribed spacer (ITS) of nuclear rDNA and *rpl*32-*trn*L spacer (plastid DNA) sequences of the new species was made in order to find the closest related species to use for the following phylogenetic reconstructions using both nuclear and plastid sequences. The closest species was *Allium obliquum* Linnaeus (1753: 296) from *A.* sect. *Oreiprason* Hermann (1939: 57) and not, as previously expected based on morphological similarity, *A. caroliniaum* from *A.* sect. *Falcatifolia* N.Friesen in Friesen *et al.* (2006: 390). Other closely related species were those of the "Siberian clade" of the section *Oreiprason* (Seregin *et al.* 2015): *Allium petraeum* Karelin & Kiriloff (1842: 511)*, A. dshungaricum* Vvedensky (1971: 66)*, A. kirilovii* N.Friesen & Seregin in Seregin *et al.* (2015: 88)*, A. montanostepposum* N.Friesen & Seregin in Seregin *et al.* (2015: 85) *and A. cretaceum* N.Friesen & Seregin in Seregin *et al.* (2015: 85).

For the following phylogenetic analyses, we took all the available accessions of the above-mentioned species and one accession each of all the other known species from the section *Oreiprason.* A small selection of broadleaved species from the section *Falcatifolia* constituted by *A. carolinianum*, *A. platyspathum* Schrenk in Fischer & Meyer (1841: 7) and *A. hymenorrhizum* Ledeb. in Ledebour *et al.* (1830: 12) were also included. *Allium condensatum* Turczaninow (1856: 121) from *A.* sect. *Condensatum* N.Friesen in Friesen *et al.* (2006: 390) was selected as the outgroup, according to Friesen *et al*. (2006). Newly sequenced accessions are marked with Am, Gl, O or Tax number in the trees and their origin is shown in Table 1.

TABLE 1. Origin, source and GenBank accession numbers of *Allium* sequences used for phylogenetic analyses. Herbarium acronyms according to Index Herbariorum (Thiers 2020).

Voucher	Isolate Nr	Origin	Chromosomes	ITS	*trn*Q-*rps*16	*Rpl*32-*trn*L
		section *Oreiprason*				
		Allium toksanbaicum				
OSBU 27869	Am1090	Kazakhstan, South Dzungaria, Toksanbai Range	2n = 16	MW208958	MW201112	MW201081
		Allium obliquum				
GAT 0201	O-6	Russia, BG Ekaterinenburg	2n = 16	HG794228	MW201113	HG794141
OSBU 19369	O-36	Russia, Altai Krai, Tigiretzky range	2n = 16	MW208960	MW201114	HG794142
OSBU 23944	O-25	Russia, Altai Krai, Charyshsky rayon, Tulata		MW208959		
OSBU-2004-2096	O-37	Russia, Baschkiria	2n = 16	HG794230	MW201115	HG794143
GAT 3158	Tax3158	Russia, Altai, Teletzkoe lake, Estube	2n = 16	AJ412753		
OSBU 25595	Am874	Kazakhstan, Altai, Schemonaicha	2n = 16	MW208961	MW201116	MW201085
OSBU 25595	Am880	Russia, Orenburg region. Scheitantau	2n = 16	MW208962	MW201117	MW201084
OSBU 26005	Am898	Kazakhstan, Altynemel range, Usun-Bulak,		MW208963	MW201118	MW201086
OSBU 27817	Am1095	Kazakhstan, Altynemel range, Usun-Bulak	2n = 16	MW208964	MW201119	MW201083
OSBU 27991	Am1099	Kazakhstan, Tarbagatai, Alekseevka	2n = 16	MW208965	MW201120	MW201087
OSN 2018-0720	Am1125	Romania, Cheile Turzii, Distr. Cluj		MW208966	MW201121	MW201082
		Allium dshungaricum				
OSBU28024	Am783	Kazakhstan, Tarbagatai		MW208967	MW201122	MW201089
OSBU 26020	Am890	Kazakhstan, Saur, 2 km über den Dorf Scherkytsu	2n = 16	MW208968		
OSBU 27890	Am1094	Kazakhstan, Region Almaty, ca. 35 km N Koktal		MW208969		
OSBU 28056	Am1096	Kazakhstan, Tarbagatai, upper reaches of the Ayaguz river	2n = 16	MW208970	MW201123	MW201090
LE 5307	Gl-119	Kazakhstan, Ajagus		HG794222	MW201124	HG794136
		Allium kirilovii				
GAT3376	Tax 3376	China, Xingiang		AJ411865	MW201125	MW201088
MW 228460	Gl-166	Kazakhstan, Dzungar Alatau		MW208971		
		Allium petraeum				
MW Kljuikov 41	Gl-51	Kazakhstan, Alma Obl. Altynemel	2n = 16	HG794174	MW201126	HG794102
MHA Kuklina & Konovalova	Gl-70	Kazakhstan, Almata Obl. Toksanbai Range		HG794186	MW201127	HG794111
OSBU 24362	Am702	Kazakhstan, Pass Altynemel	2n = 16	MW208972		
OSBU 24365	Am704	Kazakhstan, Kolbai		MW208973	MW201128	MW201091
OSN-2015-0715	Am789	Kazakhstan, Uscharal		MW208974		
OSBU 24357	Am870	Kazakhstan, Kojandytau range		MW208975	MW201129	MW201094
OSBU 24339	Am871	Kazakhstan, Uscharal		MW208976	MW201130	MW201095
OSBU 24353	Am872	Kazakhstan, Koilyk		MW208977		
OSBU 26003	Am901	Kazakhstan, Altynemel range, Uzun-Bulak		MW208978		
OSBU 26011	Am904	Kazakhstan, Dzungar Alatau, Tekeli		MW208979	MW201131	MW201092
OSBU 27938	Am1092	Kazakhstan, Dzungar Alatau, Koksu	2n = 16	MW208980	MW201132	MW201093
		Allium cretaceum				
MHA Shreter	Gl-68	Kazakhstan, Kustanay region, Naurzumsky reserve		HG794184	MW201133	HG794109
OSN 2017-0807	Am879	Russia, Orenburg region	2n = 16	MW208981		
OSBU 26213	Am886	Russia, Samara region, Klimovka		MW208982	MW201134	MW201097
OSBU 25215	Am887	Russia, Uljanovsk region, Vyrypaevka		MW208983	MW201135	MW201096
OSBU 26214	Am946	Russia, Samara region, Mt Strelnaja		MW208984	MW201136	MW201098
		Allium montanostepposum				
OSBU 22278	Gl-37	Russia, Altai, Katun between r. Dety-Kochek and Turgunda	2n = 16	HG794223	MW201137	HG794095
OSN-2010-1148	Gl-43	Russia, Alrai Krai,. Antoschicha,	2n = 16	HG794170	MW201138	HG794097
OSN-2016-0971	Am877	Russia, Altai Krai, Alei		MW208985	MW201139	MW201099

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TABLE 1. (Continued)

Voucher	Isolate Nr	Origin	Chromosomes	ITS	*trn*Q-*rps*16	*Rpl*32-*trn*L
OSBU 26038	Am902	Kazakhstan, Kurchumsky range, Buchtarma		MW208986		
OSBU 28051	Am1097	Kazakhstan, Tarbagatai, ca. 35 km NW Urzhar,		MW208987	MW201140	MW201100
		Allium saxatile				
OSN-2009-1374	Gl 105	Georgia, Mtiuleti, Kazbegi		HG794210	MW201141	HG794128
		Allium globosum				
MHA Daeva	Gl 73	Stavropolsky Krai, Pyatigorsk, Mt Mashuk		HG794148	MW201142	HG794112
		Allium tarkhankuticum				
MW0605400	Gl 18	Crimea, 4,5 km to NW from Olenevka		HG794160	MW201143	HG794089
		Allium marschalianum				
MW	Gl 13	Crimea , Berg Argamysch		HG794154	MW201144	HG794086
		Allium agarmyschicum				
MW	Gl 114	Crimea , Berg Argamysch		HG794219	MW201145	HG794135
		Allium savranicum				
MW0289525	Gl 50	Russia, Rostov Oblast, Chir River		HG794173	MW201146	HG794101
		Allium psebaicum				
MW0655498	Gl 22	Russia, Krasnodar Krai, Arkhipo-Osipovka		HG794163	MW201147	HG794092
		Allium horvatii				
BUNS, Anaskov	Gl 89	Montenegro. Orjen Mt.,		HG794198	MW201148	HG794120
		Allium schistosum				
MW Zernov & Onipchenko 7011	Gl 48	Russia, Karachay-Cherkessia, Arkhyz, Sofiyskoye Sedlo pass		HG794177	MW201149	HG794100
		section *Falcatifolia*				
		Allium platyspathum* subsp. *platyspathum				
GAT 2905	Tax2905	Kazakhstan, Almaty, Bolschaja Almaatinka		AJ411878	MW201150	MW201101
OSBU 27856	Am1104	Kazakhstan, Toksanbai Range	2n = 16	MW208988	MW201151	MW201102
		Allium platyspathum* subsp. *amblyophylum				
OSBU 22672	Am489	Mongolia, Dzungarian Gobi, Baitag Bogdo,		MW208989		
OSBU 25990	Am903	Kazakhstan, Kungei Alatoo, Fluss Kurmerty		MW208990	MW201152	MW201103
OSBU 27744	Am1093	Kazakhstan, Ile Alatau		MW208991		
OSBU 27899	Am1103	Kazakhstan, Kajandytau, Tamshi valey	2n = 16	MW208992	MW201153	MW201104
		Allium carolinianum				
GAT 2570	Tax 2570	Tajikistan, Anzob Pass		AM418362	LR700292	LR700264
OSBU 22819	Am463	Mongolia, Dzungarian Gobi, Baytag Bogd	2n = 16	MW208993	MW201154	MW201106
OSBU 20971	Am464	Kyrgyzstan, W-Tienshan, Pass Kara Bura		MW208994		
OSBU 20967	Am465	Nepal, Lower Dolpo		MW208995		
GAT2541390	Am972	Kazakhstan, Pamiro Alai, Gjulcha valley		MW208996		
OSBU 27922	Am1089	Kazakhstan, Koyandytau, Tamschi		MW208997	MW201155	MW201105
OSN-2017-1166	Am1105	Kazakhstan, Altynemel range, Uzun-Bulak		MW208998	MW201156	MW201107
		Allium hymenorrhizum				
GAT3135	Tax 3135	Tadjikistan, Saravshan Range, Lake Iskanderkul		AJ411879	LR700291	LR700263
OSBU 26038	Am892	Kazakhstan, Tarbagatai, r. Karasu		MW208999	MW201157	MW201108
OSBU 27717	Am1100	Kazakhsan, Region Ile Alatau, ca. 30 km W of Almaty by Uschknyr		MW209000	MW201158	MW201109

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TABLE 1. (Continued)

Voucher	Isolate Nr	Origin	Chromosomes	ITS	*trn*Q-*rps*16	*Rpl*32-*trn*L
		section *Condensatum* Outgroup				
		Allium condensatum				
OSBU 20559	Am440	Mongolia, Dornod Aymaq, Sumber		MW209001	MW201159	MW201110
OSBU 20705	Am443	Mongolia, Dornod Aymaq, Hingan Mountains		MW209002		
OSBU 28164	Am1108	Russia, Primorsky Krai, Tretjakovski		MW209003	MW201160	MW201111

Sequences from NCBI Genbank (https://www.ncbi.nlm.nih.gov/nucleotide/) are marked with Genbank accession number on the trees. Ten accessions of the seven closest related species to *A. toksanbaicum*, based on the ITS rDNA and cpDNA analyses, were included in the fingerprint (SCoT) analysis.

Bulbs were planted in pots and growing roots were used for the karyotype analysis.

Karyotype analysis:—Root tips were excised from the bulbs kept overnight in distilled water on ice. They were then transferred to room temperature for 20 min and pre-treated for 3 h at room temperature in an aqueous solution of 0.1 % colchicine. Roots were then fixed in a freshly prepared mixture of 96 % ethanol and glacial acetic acid (3:1 v/v). Root tips were stained using hematoxylin according to the protocol reported by Smirnov (1968). Well-spread metaphase plates were electronically documented (digitally photographed), and finally the chromosomes of the best plates were measured and pairwise arranged using the KaryoType software (Altýnordu *et al.,* 2016). Because the idiograms automatically assembled by the software were not satisfactory, we manually ordered the chromosome pairs according to their size and the position of their centromere. The idiograms were designed using the bar graph function implemented in MS Excel®. The terminology of Levan *et al.* (1964) was applied. The karyotype was reconstructed using ten or at least five metaphase plates.

DNA extraction, amplification and sequencing:—Total genomic DNA was isolated from leaves in silica gel using the InnuPREPP Plant DNA Kit (Analytic Jena AG) according to the instructions of the manufacturer, and used directly in PCR amplification. The complete nuclear ribosomal ITS region (ITS1, 5.8S and ITS2) was amplified using the primers ITS-A (Blattner 1999) and ITS-4 (White *et al.* 1990). The PCR conditions for ITS followed Friesen *et al*. (2006). PCR conditions and primers for the chloroplast regions *trn*L-*rpl*32 and *trn*Q-*rps*16 were as described in Shaw *et al.* (2007). PCR products were sent to Microsynth SeqLab (Göttingen, www.microsynth.seqlab.de) for sequencing. The sequences from all the individuals were manually edited in Chromas Lite 2.1 (Technelysium Pty Ltd) and aligned with ClustalX (Thompson *et al.* 1997); the alignment was manually corrected using MEGA X (Kumar *et al.* 2018).

Phylogenetic analyses:—Both data sets (nrITS and the combined cpDNA markers) were analyzed separately by means of Fitch parsimony with the heuristic search option in PAUP version 4.0b10 (Swofford 2002) with MULTREES, TBR branch swapping and 100 replicates of random addition sequence. Gaps were treated as missing data. The consistency index (CI) of Kluge & Farris (1969) was calculated to estimate the amount of homoplasy in the character set. The most parsimonious trees returned by the analysis were summarized in one consensus tree using the strict consensus method. Bootstrap analyses (BS) using 1,000 pseudoreplicates was performed to assess the support of the clades (Felsenstein 1985). Bayesian phylogenetic analyses were also performed using MrBayes 3.1.23 (Ronquist & Huelsenbeck 2003). The sequence evolution model was chosen following the Akaike Information Criterion (AIC) obtained from jModelTest2 (Darriba *et al.* 2012). Two independent analyses with four Markov chains were run for 10 million generations, sampling trees every 100 generations. The first 25% of trees were discarded as burn-in. The remaining 150,000 trees were combined into a single data set, and a majority-rule consensus tree was obtained along with posterior probabilities (PP).

SCoT fingerprint analysis:—SCoT (Start Codon Targeted DNA) markers are obtained by polymerase chain reaction (PCR) using primers that are designed from a short conserved region flanking the ATG start codon, which is conserved for all genes, and includes an additional 15 or more randomly selected nucleotides (Collard & Mackill 2009). To assess genetic polymorphisms, samples of 11 accessions including *A. toksanbaicum* (Am1090), *A. obliquum* (Am1095, Am1099, Am1125), *A. dshungaricum* (Am1094, Am1096) *A. kirilovii* (Tax3376), *A. cretaceaum* (Am887), *A. montanostepposum* (Am877, Am1097) and *A. petraeum* (Am870) were tested with 25 SCoT primers, and as a result 7 primers showing polymorphism were selected for analysis (Table 2). The markers have a dominant type of inheritance, where polymorphism is determined by the presence or absence of a fragment. Each fragment was regarded as a single locus. The bands were scored independently as either present (1) and absent (0) and summarized in a matrix. Only clear bands from 500 to 3500 bp with good reproducibility were recorded. The proportion of polymorphic loci (P) corresponding to the level of genetic variability was calculated. A dendrogram was constructed based on the UPGMA genetic distance (Sokal & Michener 1958) using MEGA 7 (Kumar *et al.* 2016). For a better graphical representation

of the genetic network of DNA sequences, we used SplitsTree4 (Huson & Bryant 2006). We used NJ distance method with the neighborNet algorithm to calculate the network.

Morphological analysis:—Five individuals of *A. toksanbaicum* were used for morphological analyses. Comparisons with closely related species *A. obliquum*, *A. dshungaricum* and morphological similar *A. carolinianum* with many herbarium sheets deposited in OSBU, ALTB and AA herbariums are based on these data (See Table 4).

TABLE 2. Karyomorphometric parameters of (A) *A. toksanbaicum* (Am1090), (B) *A. obliquum* (Am1095) and (C) *A. obliquum* (1199). Mean values come from 10 good metaphase plates. Abbreviations: TAL = total absolute length; LA = long arm; SA = short arm; RL = relative length; Sat = satellite; CI = centromeric index; m = metacentric, st = subtelocentric (Levan *et al.* 1964); THL = total karyotype haploid length; KCI = karyotype centromeric index.

A) *Allium toksanbaicum*, Am1090

Pair n.	TAL (µm)	RL %	LA (µm)	SA (µm)	**Sat**	**CI %**	**Type**
I	8.00 ± 0.163	16.1	4.20 ± 0.151	3.80 ± 0.189		47.5	m
II	7.20 ± 0.192	14.5	3.90 ± 0.145	3.30 ± 0.21		45.8	m
III	6.50 ± 0.21	13.1	3.50 ± 0.121	3.00 ± 0.157		46.9	m
IV	6.30 ± 0.2	12.7	3.50 ± 0.09	2.80 ± 0.11		46.2	m
V	5.70 ± 0.13	11.5	3.10 ± 0.081	2.60 ± 0.06		46,4	m
VI	5.70 ± 0.43	11.5	4.40 ± 0.32	1.30 ± 0.17	0.5 ± 0.04	22.8	st sat
VII	5.30 ± 0.181	10,6	3.00 ± 0.133	2.30 ± 0.132		41.1	m
VIII	5.00 ± 0.08	10.0	2.80 ± 0.023	2.20 ± 0.19		44.0	m

THL = 49.7 ± 9.23 µm; KCI = 42.9%

B) *Allium obliquum*, Am1095

Pair n.	TAL (µm)	RL %	LA (µm)	SA (µm)	**Sat**	**CI %**	**Type**
I	10.00 ± 0.214	15.1	5.3 ± 0.08	4.7 ± 0.02		47.5	m
II	9.20 ± 0.168	13.8	5.1 ± 0.49	4.1 ± 0.002		45.8	m
III	9.1 ± 0.045	13.7	4.9 ± 0.35	4.2 ± 0.064		46.2	m
IV	8.6 ± 0.52	13.0	4.5 ± 0.092	4.1 ± 0.5		47.7	m
V	8.0 ± 0.081	12.1	4.2 ± 0.012	3.8 ± 0.967		47,5	m
VI	7.8 ± 0.145	11.8	4.1 ± 0.074	3.7 ± 0.035		47.4	m
VII	7.3 ± 0.218	11.0	4.0 ± 0.22	3.3 ± 0.018		45.2	m
VIII	6.3 ± 0.086	9.5	5.2 ± 0.049	1.1 ± 0.005	0.7 ± 0.0026	17.5	st sat

THL = 66.3 ± 13.07 µm; KCI = 43.7%

C) *Allium obliquum,* Am1099

Pair n.	TAL (µm)	RL %	LA (µm)	SA (µm) µm	**Sat**	**CI %**	**Type**
I	9.7 ± 0.98	15.1	5.1 ± 0. 49	4.6 ± 0.25		47.4	m
II	8.8 ± 0.56 ±0.	13.7	4.8 ± 0.35	4.0 ± 0.27		45.5	m
III	8.7 ± 0.21	13.6	4.7 ± 0.29	4.0 ± 0.42		45.9	m
IV	8.2 ± 0.16	12.8	4.6 ± 0.57	3.6 ± 0.23		43.9	m
V	7.8 ± 0.28	12.2	4.4 ± 0.39	3.4 ± 0.39		43.6	m
VI	7.4 ± 0.49	11.5	4.0 ± 0.21	3.4 ± 0.19		45.9	m
VII	7.1 ± 0.5	11.0	5.6 ± 0.41	1.5 ± 0.04	0.5 ± 0.017	21.1	st sat
VIII	6.4 ± 0.32	10.0	3.5 ± 0.18	2.9 ± 0.04		45.3	m

THL = 64.1 ± 14.16 µm; KCI = 42.7%

Results

Karyotype analysis:—The chromosome number 2n = 16 was found for all the accessions (Table 1). For *A. toksanbaicum* (Am1090) and two accessions of *A. obliquum* (Am1095 and Am1099) we built the karyotype idiogram. All the three studied accessions show a similar karyotype formula: seven metacentric chromosomes pairs (m) and one pair of satellited subtelocentric chromosomes (st) (Fig. 3; Table 2). There are small differences in the position of satellited chromosome pairs in the idiograms (chromosomes arranged according to their length): in the case of *Allium toksanbaicum* (Am1090) satellites are on the sixth pair, in *A. obliquum* from Altynemel range (Am1095) on the seventh pair, and in *A. obliquum* from Tarbagatai range (Am1095) on the eighth pair. Karyotype total haploid length in *A. toksanbaicum* is higher than in *A. obliquum* (48.0 ± 9.23 µm compared to 64.0 ± 14.16 and 66.3 ± 13.07 µm; Table 2).

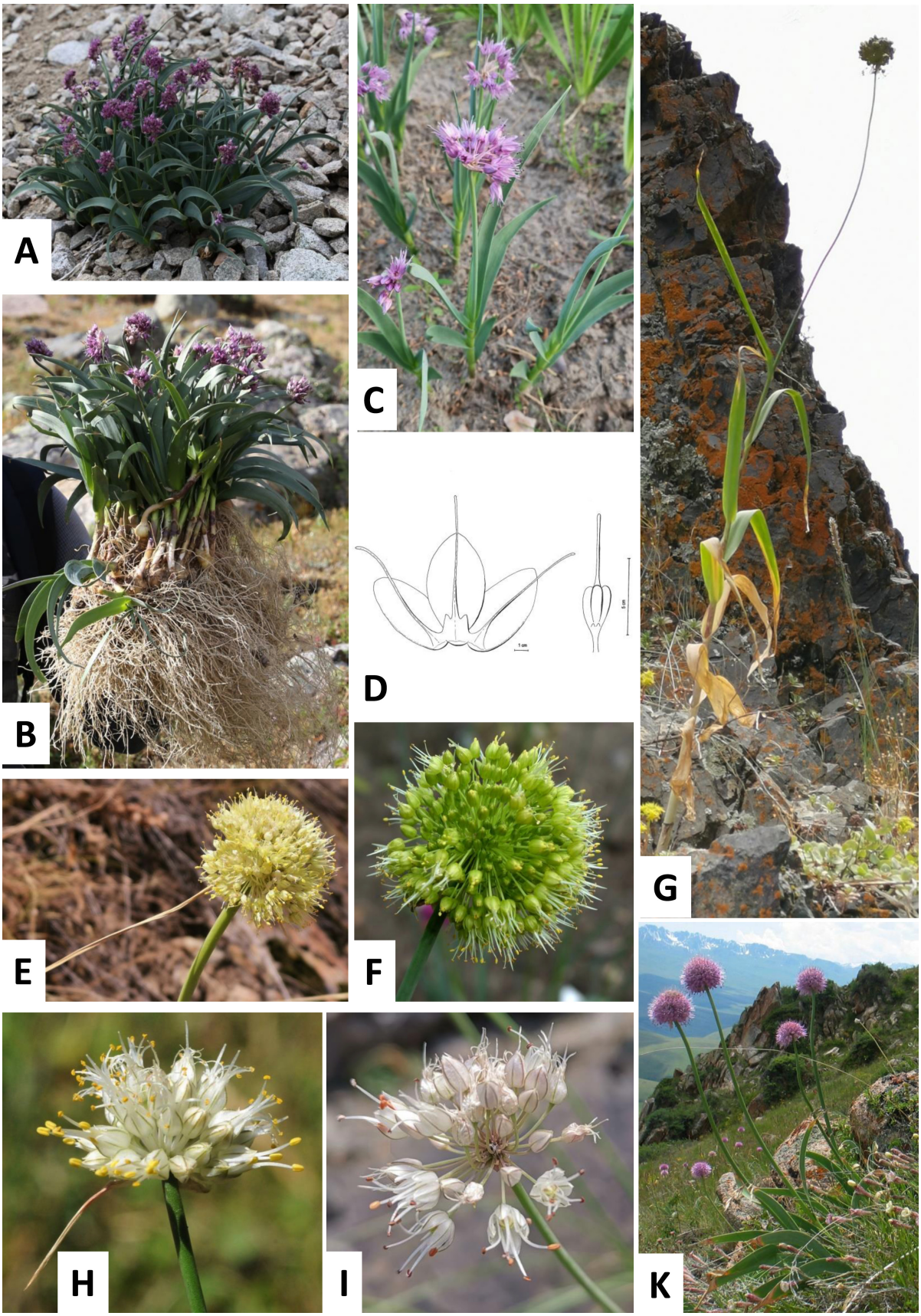

FIGURE 2. *Allium toksanbaicum* and related species. A–B, plant habit in Toksanbai range (photo by P. Vesselova); C, plant cultivated in South Siberian Botanical Garden, Barnaul (photo by A. Shmakov); D, perianth segment with stamens and ovary with style (drawn by S. Oevermann, Osnabrueck); E, *A. petraeum* in Dzhungar Alatau, Koksu (photo by N. Friesen); F–G, *A. obliquum* from Altai and Altynemel (specimens Am874 and Am1095) (photos by N. Friesen); H, *A. kirilovii* from Kazakhstan (photo by V. Epiktetov); I, *A. dshungaricum* in Dzungar Alatau (photo by N. Friesen); K, *A. carolinianum* from Kyrgyzstan (photo by N. Friesen).

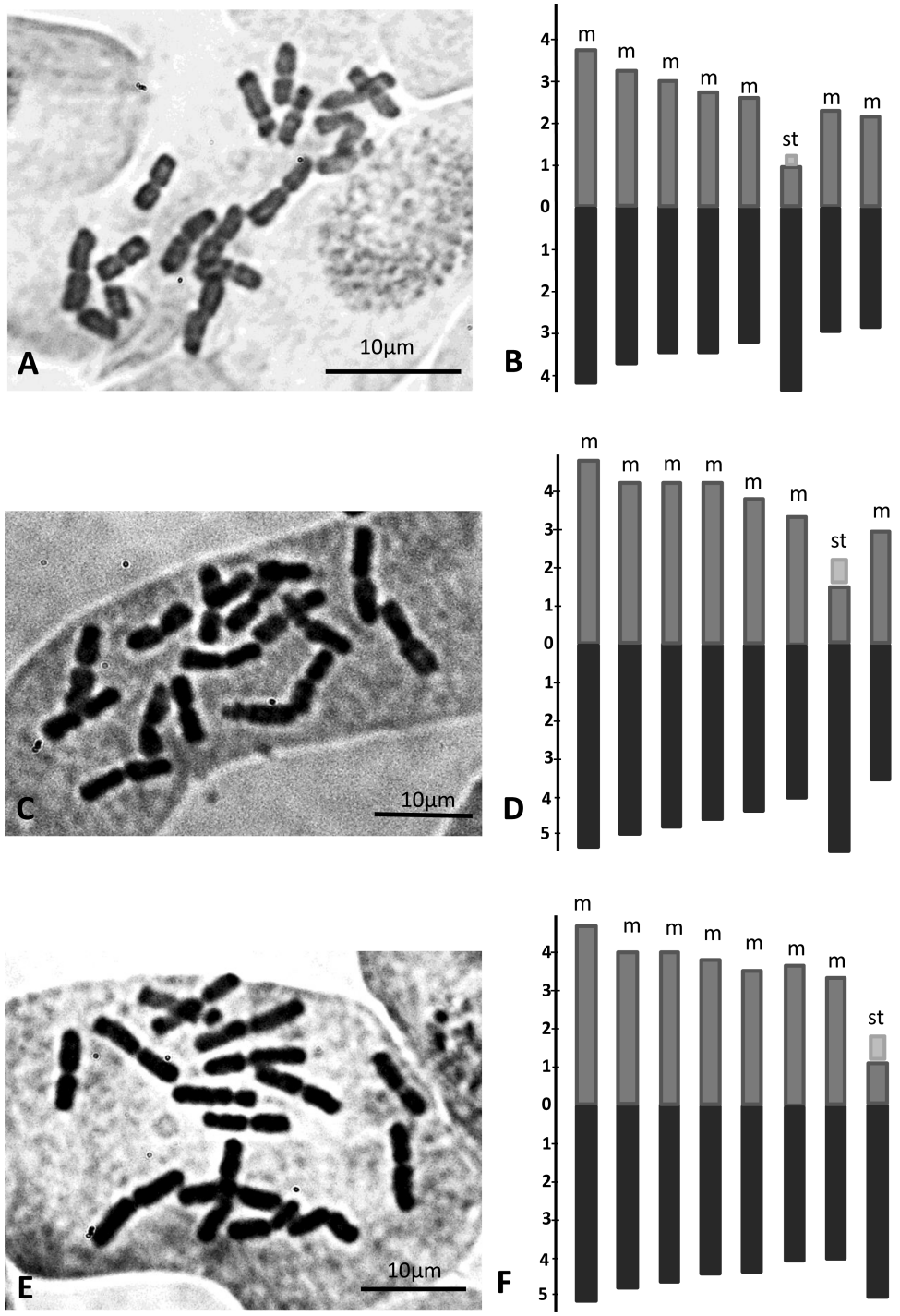

FIGURE 3. Metaphase plates and idiograms of *Allium toksanbaicum* (Am1090, A–B) and *A. obliquum* (Am1095, C–D; Am1099, E–F). For the origin of the accessions, see the Table 1.

ITS analysis:—The alignment of 94 nrITS sequences (including 43 new sequences obtained here; Table 1) is 626 bp long including 8 gaps, with 223 parsimony informative characters. Unweighted parsimony analysis of the 94 sequences returned 7,379 most parsimonious trees of 376 steps (CI = 0.8069). The substitution model TVM+G is the best fitting one. Parsimony and Bayesian analyses produce identical topologies. All ingroup samples included in the nrITS analysis are clearly divided according to their section (i.e., *Oreiprason* or *Falcatifolia*) (Fig. 4). Species of the section *Oreiprason* are divided in two sister clades: a strongly supported clade comprising only European representatives of the section and a less supported clade including all Asian taxa studied. The newly described *A. toksanbaicum* groups with two accessions of *A. obliquum* from Altynemel range (Am 898 and Am1095), which in turn

belong to a larger clade including *A. dshungaricum* and *A. kirilovii*. This latter clade is sister to all the samples of *Allium petraeum*. What is most surprising is the splitting of *A. obliquum* accessions into three geographically distant groups: the Dzungarian group, which is also phylogenetically distant to the other groups; the western group, spreading from Romania to South Ural; the south-eastern group, distributed in south Siberia, north Mongolia and east Kazakhstan. Samples of *A. obliquum* from the western and south-eastern groups form a clade sister to *A. montanostepposum* and *A. cretaceum* (Fig. 4), whereas the accessions of the Dzungarian group of *A. obliquum* (from south Dzungaria) cluster with *A. toksanbaicum*, as already reported above.

cpDNA analysis:—The alignment of the 53 combined plastid sequences is 1,794 bp long including 26 gaps, with 123 parsimony informative characters. Unweighted parsimony analysis of the 53 sequences returned 779 most parsimonious trees of 166 steps (CI = 0.8976). All ingroup samples included in the plastid analysis are clearly divided according to their section (i.e., *Oreiprason* and *Falcatifolia*) (Fig. 5), but the topology within the section *Oreiprason* is very different from that found in the ITS tree. Indeed, all accessions of *Allium* sect. *Oreiprason* are divided into two sister strongly supported clades (Fig. 5): one clade includes unresolved samples of *A. petraeum* and *A. kirilovii* and a weakly supported subclade (BS 61 %, PP 0.69) including all accessions of *A. obliquum, A. toksanbaicum* and *A. dshungaricum.* In this clade in turn two however only weakly supported subclades appear: European *A. obliquum* (Am1125, O6 and O37 with 0.95 PP / 65 BS) on the one side and *A. toksanbaicum* together with *A. obliquum* accessions from Tarbagatai and Dzungar Alatau (Am1099, Am1095) and *A. obliquum* from China (GenBank accession LT699701) with 0.65 PP / BS less than 50) on the other side; the other clade consists of a weakly supported subclade of European species and a strongly supported subclade with *A. cretaceum* and *A. montanostepposum*.

SCoT fingerprints analysis:—The SCoT dataset included the accession of *Allium toksanbaicum* and 10 accessions of *A. obliquum, A. dshungaricum, A. kirilovii* and *A. petraeum, A. cretaceum* and *A. montanostepposum* which are the closest ones to *A. toksanbaicum* according to ITS and cpDNA analyses. We detected 191 SCoT markers, only 9 of which were common for all taxa (Table 3). The UPGMA dendrogram shows three main clusters (Fig. 6a): the first cluster includes all *A. obliquum* accessions (strongly supported sub-cluster) and *A. toksanbaicum*, the second cluster includes the two accessions of *A. dshungaricum*, and the third cluster comprises all the other taxa studied, i.e. *A. kirilovii, A. petraeum*, *A. cretaceum* and *A. montanostepposum*. SplitsTree (Fig. 6b) shows more clearly the relationships within the examined species, with *A. toksanbaicum* and *A. obliquum* close to each other.

Morphological analysis:—*Allium toksanbaicum* differs from all closely related species in having up to 50 globose bulbs that are very compactly compressed on short-branched rhizomes (see Fig. 2B). Also, some flower characters like teeth in the inner filaments a rare in the section *Oreiprason* species. Comparison of the morphological characteristics between *Allium toksanbaicum* and phylogenetically most closely related species - according to the DNA analysis - *A. obliquum* and *A. dshungaricum* from section *Oreiprason* and the morphological very similar *A. carolinianum* from section *Falcatifolia* are shown in Table 4 and Figure 2. We have analyzed 51 herbarium sheets of *A. obliquum*. So far, we cannot find any significant qualitative morphological characters between plants from different isolated areas of the *A. obliquum*, only some quantitative such as height of the plants, which can be clearly attributed to the ecological peculiarities in different localities. In general, all three phylogenetically most closely related species *A. toksanbaicum*, *A. obliquum* and *A. dshungaricum* are morphologically very different (see Table 4 and Fig. 2).

TABLE 3. SCoT primers used and number of fragments obtained

Primer	Primer sequences 5' – 3'	Total number of fragments	Polymorphic fragments	Polymorphism (%)
SCoT 1	CAACA***ATG***GCTACCACCA	31	31	100%
SCoT 2	CAACA***ATG***GCTACCACCC	27	25	92.6%
SCoT 4	CAACA***ATG***GCTACCACCT	14	13	92.8%
SCoT 13	ACGAC***ATG***GCGACCATCG	39	36	92.3%
SCoT 14	ACGAC***ATG***GCGACCACGC	19	19	100%
SCoT 23	CACC***ATG***GCTACCACCAG	32	32	100%
SCoT 24	CACC***ATG***GCTACCACCAT	23	22	95.6%
		187	**178**	
Average level of polymorphism				95.2 %

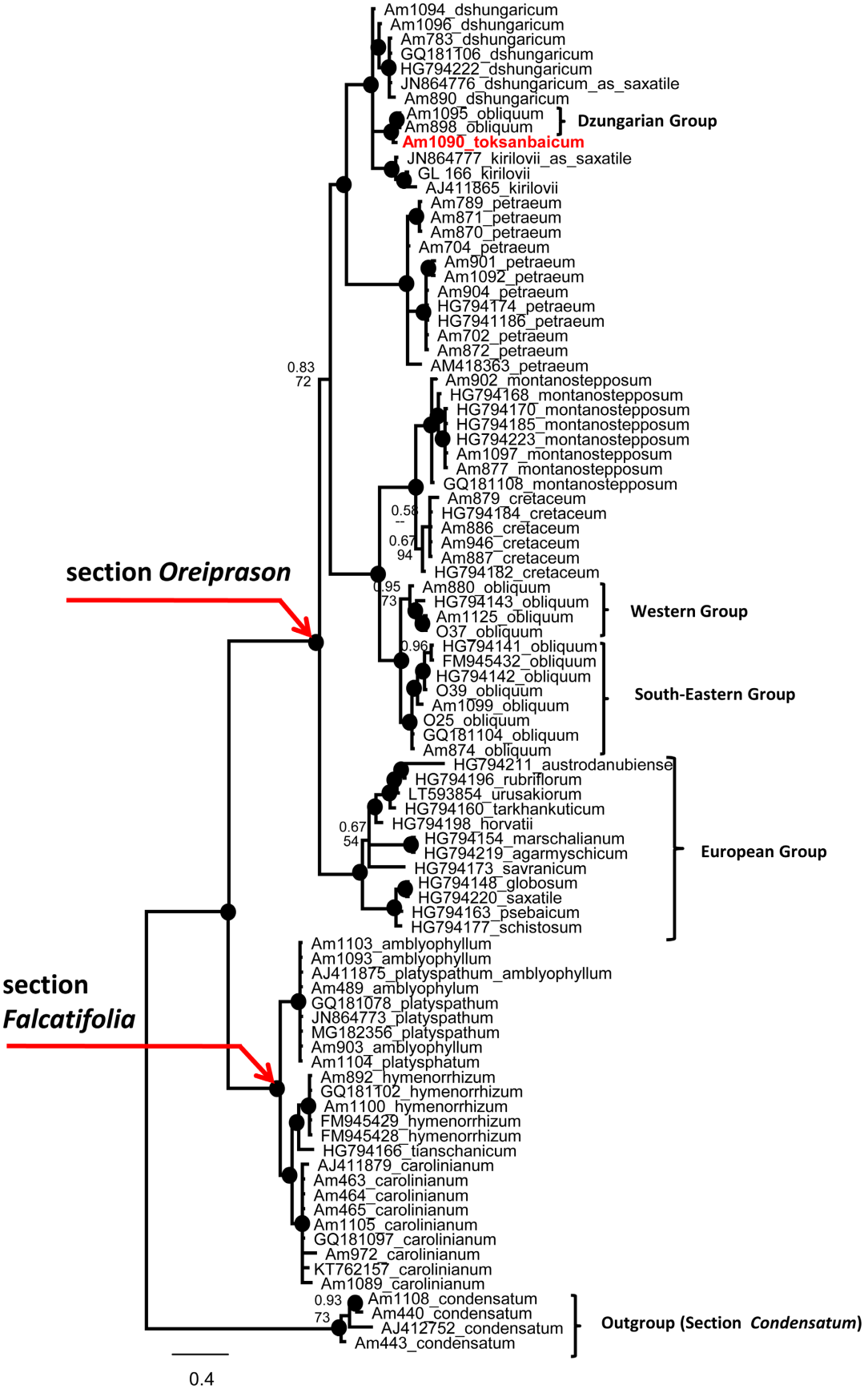

FIGURE 4. Phylogenetic tree of *Allium* sect. *Oreiprason* and *A.* sect. *Falcatifolia* based on ITS sequences. Numbers on nodes represent bootstrap support (1,000 replicates) and Bayesian probabilities. Joint presence of Bayesian probabilities over 0.98 and bootstrap support over 95% are indicated with a black dot. For the origin of samples without GenBank accession numbers, see the Table 1.

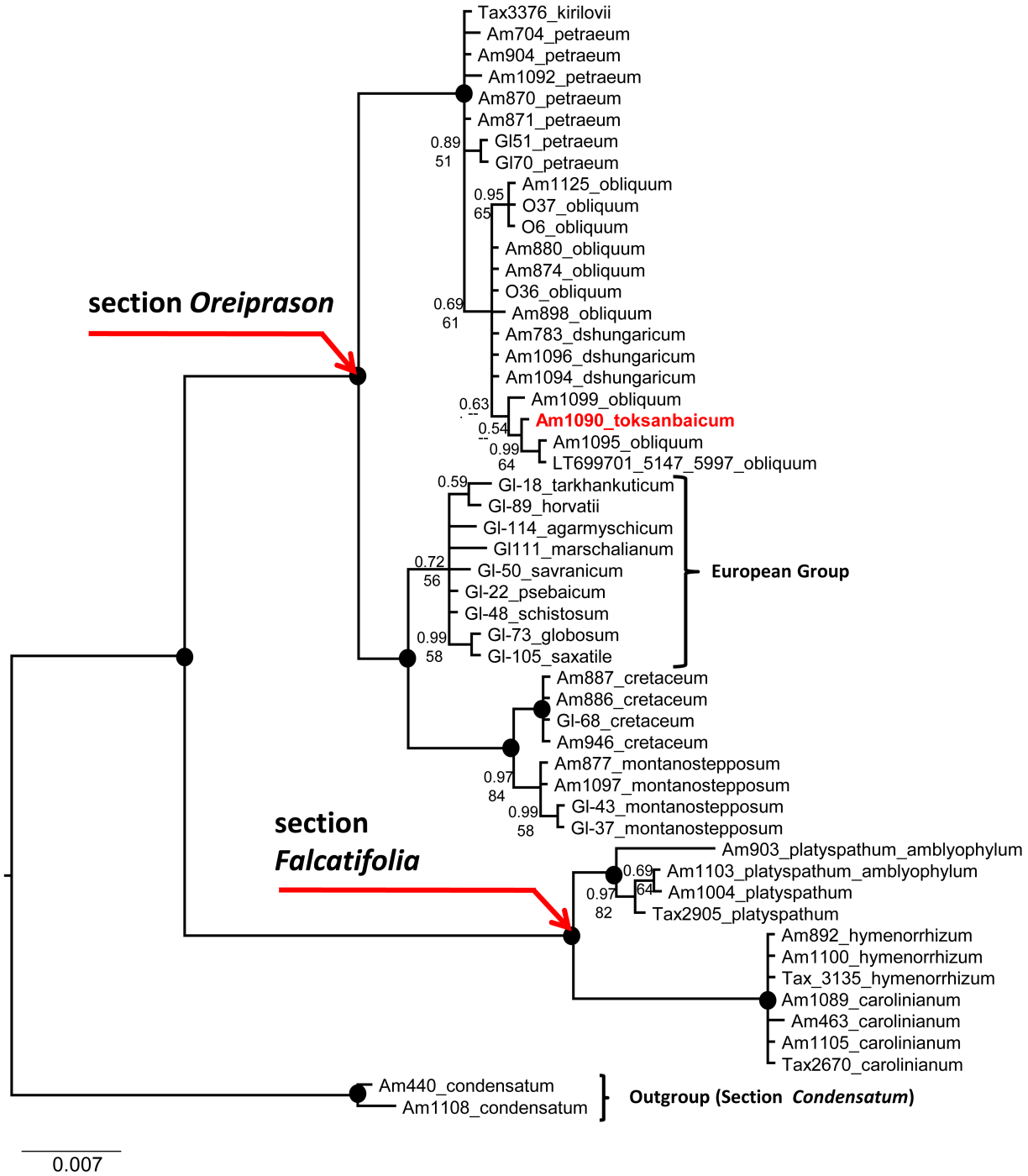

FIGURE 5. Phylogenetic tree of *Allium* sect. *Oreiprason* and *A.* sect. *Falcatifolia* based on combined plastid sequences. Numbers on nodes represent bootstrap support (1,000 replicates) and Bayesian probabilities. Joint presence of Bayesian probabilities over 0.98 and bootstrap support over 95% is indicated with a black dot.

TABLE 4. Morphological characters of *A. toksanbaicum* and related species.

Character	***Allium toksanbaicum*** (4 herbarium sheets)	***Allium obliquum*** (51 herbarium sheets: Romania, 2; South Ural, 5); Altai and West Sayan, 35; Tarbagatai, 5; Dzungar Alatau, 4)	***Allium dshungaricum*** (11 herbarium sheets)	***Allium carolinianum*** (39 herbarium sheets)
Form of bulbs	Globose, 15–25 mm in diam.	Oblong to ovate, 12–20(25) mm in diam.	Cylindrical to fusiform, 5–8(10) mm in diam.	Elongated to ovate, 10–15(20) mm in diam.

...continued on the next page

TABLE 4. (Continued)

Character	*Allium toksanbaicum* (4 herbarium sheets)	*Allium obliquum* (51 herbarium sheets: Romania, 2; South Ural, 5); Altai and West Sayan, 35; Tarbagatai, 5; Dzungar Alatau, 4)	*Allium dshungaricum* (11 herbarium sheets)	*Allium carolinianum* (39 herbarium sheets)
Number of bulbs	Clustered, with 30-50 bulbs together	Singular, rarely up to three	Two to six, sometimes in lose patches of 10–15	Clustered, with 5–8(10) bulbs together
Outer tunics of bulbs	Yellow coriaceous	Leathery, reddish brown	Brown, coriaceous	Leathery, reddish brown
Plant height	15–25 cm	70–100(130) cm	17–35 cm	25–45 cm
Portion of stem covered by leaf sheaths	1/3	2/3	1/2	1/4- 1/3
Leaf shape	Linear, flat, falcate, 9.5–11 mm wide in the lower part, only gradually narrowing to the tip	Linear, flat, oblique, (7)10-20(23) mm wide in the lower part, gradually narrowing to the tip	Filiform, semi-cylindrical, grooved 1.0–1.2(1.5) mm wide	Linear, flat, falcate, (7)10–13(17) mm wide in the lower part
Inflorescence shape	Hemispherical, lax	Globose, dense	Globose, lax	Globose, dense
Colour of flower	Purple	Yellow-Green	Light rose (with a purplish vein)	Red or purplish
Stamen to tepal length ratio	1/3 longer than tepals	2-3 longer than tepals	1.2–1.5 longer than tepals	0.4–0.7 shorter than tepals
Denticles in the inner filaments	Present	Absent	Absent	Absent
Anthers colour	Purple	Yellow	Rose	Purplish

Taxonomy

Allium toksanbaicum N.Friesen & Vesselova, *sp. nov.*

Type:—Kazakhstan, Almaty region, southern flank of the Toksanbai mountain range, N 44°33’14”, E 79°37’12.4”, 2880 m, on a scree slope. 22.07.2019. *P. Vesselova, K. Osmonaly, N. Friesen, B. Neuffer, H. Hurka* (Holotype OSBU 27869!, isotypes AA!, ALTB!).

It differs from the closely related *Allium obliquum* by purple perianth (not yellow), falcate leaves (not oblique), hemispherical and lax inflorescence, rounded bulbs (not oblong ovate) with coriaceous tunics. It differs from *A. carolinianum* by inner filaments with obtuse teeth at the base (not triangular without teeth), and rounded (not ovoid), bulbs with dirty yellow coriaceous (not brown, leathery) tunics.

Description:—Bulbs clustered, globose, 1.5–2.5 cm in diam., with dirty yellow coriaceous outer and white papery inner tunics. Stem 15–25 cm tall, cylindrical, glabrous, erect, covered by leaf sheaths for 1/5–1/3 of total length. Leaves shorter than scape (2)3–4(5), linear, flat, falcate; leaf blade 12–15 cm long, 10–11 mm wide in the lower part, gradually narrowing to the tip. Spathe persistent, shorter than pedicels, with 1 valve and 2 acute lobes. Inflorescence lax, many flowered, 40–45 mm across, hemispherical, pedicels sub-equal, 9–12 mm long. Perianth campanulate, segments purple, long elliptical, rounded at the apex, 6.5–8 mm long, midrib purple. Stamens for 1/3 longer than tepals (9–11 mm), inner filaments with obtuse teeth at the base; anthers dark purple, oblong, 1.5–2 mm long. Ovary ovoid, with concave nectaries covered by hood like projection at base, white, densely roughish above, style white-purple, exserted, 7 mm long. Seeds and fruits not seen. 2n = 16. Fig. 2 A–D.

Etymology:—The epithet refers to the Toksanbai range.

Distribution:—Kazakhstan, Toksanbai range, South Dzungaria Mountains (currently known from the type locality only).

Ecology:—On the scree slopes in the alpine belt.

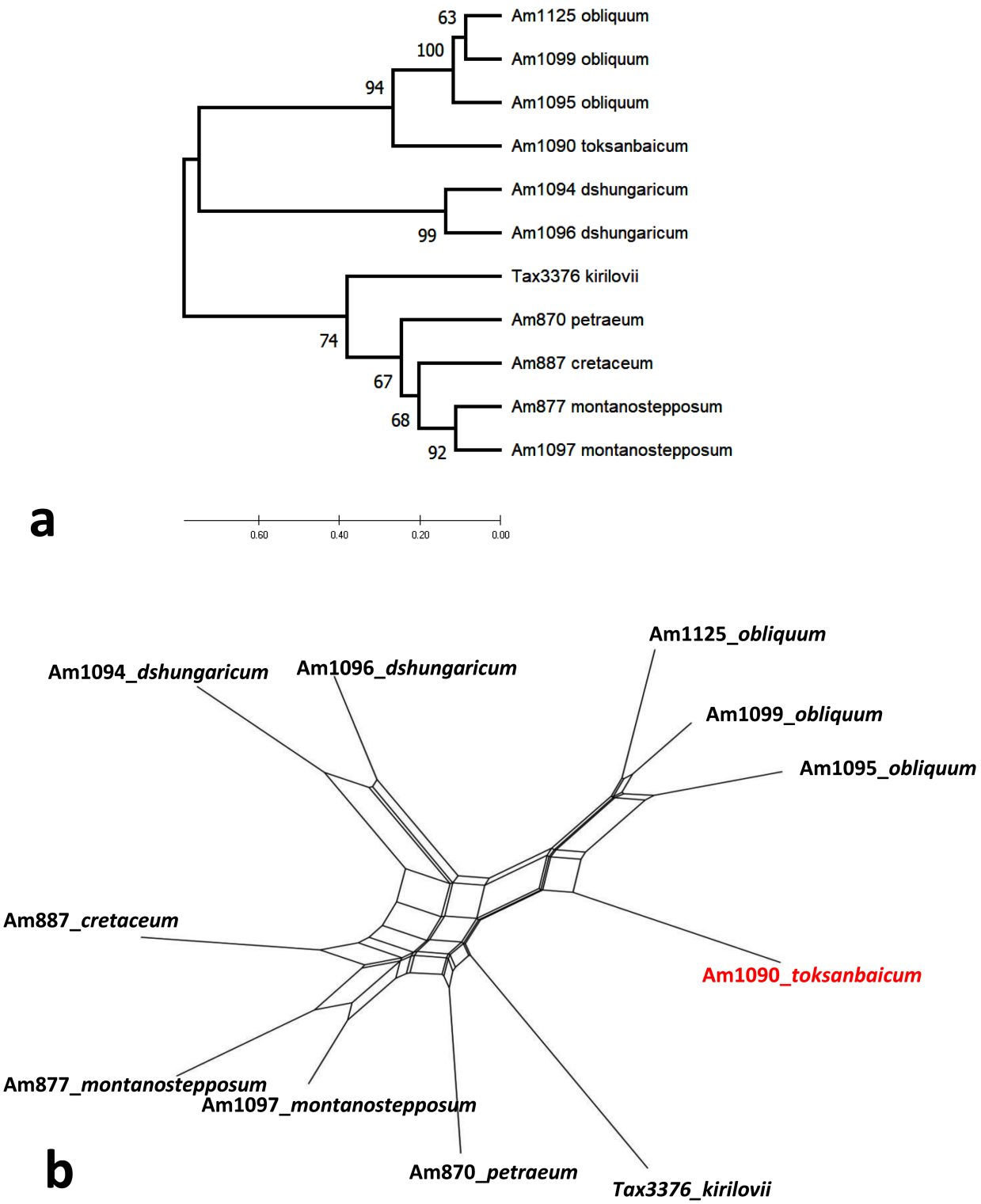

FIGURE 6. UPGMA (A) and SplitsTree (B) network of the *Allium toksanbaicum* and related taxa based on SCoT markers.

Discussion

All species of *Allium* sect. *Oreiprason* investigated so far are diploid with 2n = 16 (Seregin & Friesen 2015, Seregin *et al.* 2015 and references therein). Only one accession of *A. obliquum* from Uppsala Botanical Garden *s.n.* (vouchers in WS) was reported as 2n = 4x = 32 (Jacobsen & Ownbey 1977), whereas all the other published chromosome numbers for this species reports exclusively 2n = 16 (Dietrich 1967, Buttler 1969, Vosa, 1977, Friesen 1986, 1988, Ohri & Pistrick 2001). Our new counts of five *A. obliquum* accessions from different areas (Romania, South Ural,

Altai, Tarbagatai and Dzungaria) also revealed only 2n = 16 (Table 1). Karyotype of *A. toksanbaicum* is very similar to that of *A. obliquum* (Fig. 3). The difference in length of satellited chromosomes in the two karyotypes is very subtle, and may be explained by different chromosomes' condensation due to different colchicine treatments. The much shorter karyotype of *A. toksanbaicum* with respect to *A. obliquum* instead seems more significant (Table 3). For both *A. petraeum* and *A. dshungaricum* (Am790 and Am1092), our counts confirmed the previously published diploid chromosome numbers by Vachtina & Kudryaschova (1977, 1981, under *A. talassicum*), and Zakirova & Nafanailova (1990). Our results confirm that all species of the section *Oreiprason* are diploid with 2n = 16.

Both molecular markers used here clearly show that *Allium toksanbaicum* belongs to the section *Oreiprason* and is closely related to some *Allium* species from Dzungaria but not to the morphologically similar *A. carolinianum* (Fig. 2). The latter species clusters in a well-supported monophyletic clade along with other species of the section *Falcatifolia* (Figs. 4, 5). The splitting of *Allium obliquum* accessions into two groups in the nrITS tree is interesting and surprising (Fig. 4). *Allium obliquum* was described by plants originally collected in Siberia and cultivated at the Uppsala Botanical Garden. However, the exact location of origin is not indicated neither in the protologue nor on the label of the type specimen designated by Friesen (1995). In Siberia *A. obliquum* is distributed only from West Sayan Mountains in the east to the West Altai in the west, therefore the studied accessions from Altai are probably the closest to the type. Morphological comparison of the accessions Am898 and Am1095 (herbarium vouchers OSBU 26005 and 27817, respectively) of *A. obliquum* from Dzungar Alatau Mountains with *A. obliquum* examples from other distribution area (Romania, South Ural, Altai and Tarbagatai) do not show any significant morphological differences, as well as no significant similarity with *A. toksanbaicum*.

In the cpDNA tree (Fig. 5), however, all accessions of *A. obliquum*, *A. toksanbaicum* and *A. dshungaricum* are grouped together in a monophyletic, but rather weakly supported, clade (PP 0.65; BS 61). Nevertheless, the disjunct geographical pattern of all *A. obliquum* accessions is confirmed: Europe with South Ural; South Dzungaria (together with *A. toksanbaicum*); and Altai + Tarbagatai (including *A. dshungaricum*) (see also Alexeev 1967, and the distribution map of *A. obliquum* provided in Didukh *et al.* 1982). The phytogeography of *A. obliquum* with its disjunctive range extending from Romania to the west to the West-Sayan Mountains (Russia) to the east and Mongolian Altai to the south requires a more thorough study. Except for Dzungarian accessions, ITS data distinguish *A. toksanbaicum* from most of the *A. obliquum* accessions very well. Possibly, a hybridogenic event involving *A. obliquum* and *A. toksanbaicum* could have taken place in the Dzungarian area. At the moment we can only hypothesize that hybridisation and/or introgression events may have occurred, maybe also involving the Southeastern + Western clade of *A. obliquum*, which indeed show incongruences between the two phylogenies. However, the effect of incomplete lineage sorting on accession of *A. obliquum* cannot be ruled out. In the case of the *A. obliquum*, in order to answer these phylogenetic questions, the accessions from Mongolia, Northwest China and Kyrgyzstan should also be analyzed. We will try to do this in the near future.

The isolation of *Allium toksanbaicum* from all the other *A. obliquum* accessions, including Dzungarian ones, in the fingerprint analysis (SCoT) also supports its circumscription as a new species (Fig. 6). Contrarily to the nrITS tree, all the three examined accessions of *A. obliquum* (Am1125 – Romania, Am1099 – Tarbagatai, and 1095 - Dzungar Alatau) cluster together in SCoT analysis, congruently with the plastid tree (Fig. 5). As a fingerprint method, SCoT follows a full genome approach, albeit in our case is apparently more similar to the plastid result than to the nrITS result.

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