



## *Allium matinae*—a new species from northwestern Iran

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### Abstract

*Allium matinae* from northwestern Iran is newly described based on morphological and molecular characters. The new species belongs to *Allium* sect. *Daghestanica*, which is distributed in three main disjunctive areas: Europe, Caucasus and China. *Allium matinae* shows morphological similarity with the European species of this section, despite being more related to the Caucasian species according to molecular results. The Caucasian species together with the new Iranian species show an intermediate position in *A.* sect. *Daghestanica*, which is evidenced by incongruence between *nrDNA* (ITS) and *cpDNA* trees.

**Keywords:** *Allium* sect. *Daghestanica*, Caucasus, disjunctive distribution, molecular species diagnosis

### Introduction

During our study of morphological and molecular variability in *Allium xiphopetalum* Aitch. & Baker in Aitchison (1888: 118) we have examined all herbarium specimens in the herbarium IRAN (Tehran, Iran; acronym follows Thiers [2019]) determined as such. However, one herbarium specimen from northwestern Iran was determined doubtfully as *A. xiphopetalum*, because the inflorescence was not completely developed yet (Fig. 1). Particularly, preliminary results showed that the ITS sequence obtained from this specimen was very different from sequences of *A. xiphopetalum* available in GenBank. BLAST analysis showed instead a close affinity to species from *A.* sect. *Daghestanica* Tscholokashvili (1965: 83) N.Friesen in Friesen *et al.* (2006: 390), i.e. *A. ericetorum* Thore (1803: 123) (97.3%) and *A. daghestanicum* Grossheim (1928: 208) (96.9%). *Allium* sect. *Daghestanica* consists of three geographical alliances: the typical alliance containing the Caucasian species *A. daghestanicum* and *A. gunibicum* Miscz. ex Grossheim (1928: 208), having thin thread-like leaves; another European alliance comprising *A. ericetorum*, *A. ochroleucum* Waldstein & Kitaibel (1805: 204), *A. kermesinum* Reichenbach (1848: 25), *A. suaveolens* Jacquin (1789: 305) and *A. palentinum* Losa & P.Montserrat (1953: 423), which are distributed from the eastern Alps to the Pyrenees and show semi-cylindrical or narrowly linear leaves and bulb tunics splitting into longitudinal stripes (instead of fibers); lastly, an alliance consisting of six Chinese species, which are primarily distributed in the southeastern fringe of the Qinghai-Tibet plateau, and includes *A. chrysanthum* Regel (1875: 91), *A. chrysocephalum* Regel (1887: 335), *A. herderianum* Regel (1887: 324), *A. rude* J.M.Xu (1980: 286), *A. xichuanense* J.M.Xu (1980: 285), and *A. maowenense* J.M.Xu in Xu *et al.* (1994: 356) (Li *et al.* 2010, Xie *et al.* 2019).

Morphologically, the specimen from northwestern Iran showed characters intermediate between Caucasian and European species, with a certain degree of similarity with *A. ericetorum*, especially for the linear leaves and outer bulb tunics with dense parallel fibers. Unfortunately, only the larger plant on the herbarium sheet contained two sufficiently developed flowers that allowed us evaluating the proportion between the tepals and the shape of the stamens (Fig. 3).

The incompleteness of the specimens investigated prevented us to provide a more detailed description of these plants. Some identical features such as the division of bulb tunics in longitudinal stripes, the leaf shape and the bud shape of the plants on the herbarium sheet attest for their circumscription under the same taxon.

All the visible characters of the plants from northwestern Iran did not match with any known species of *A.* sect. *Daghestanica*. Here we describe these plants as a new species and compare it to species from Caucasus, Europe and China using ITS marker (nuclear ribosomal DNA) and three regions of plastid DNA (*cpDNA*; *trnL-trnF*, *trnL-rpl32*, *trnQ-rps16*) on species belonging to all the three geographical alliances. Unfortunately, the collection of more plants at the type location was impossible. We supplement the description of the species with molecular diagnoses, as it was exemplified by Filipovich *et al.* (2012).

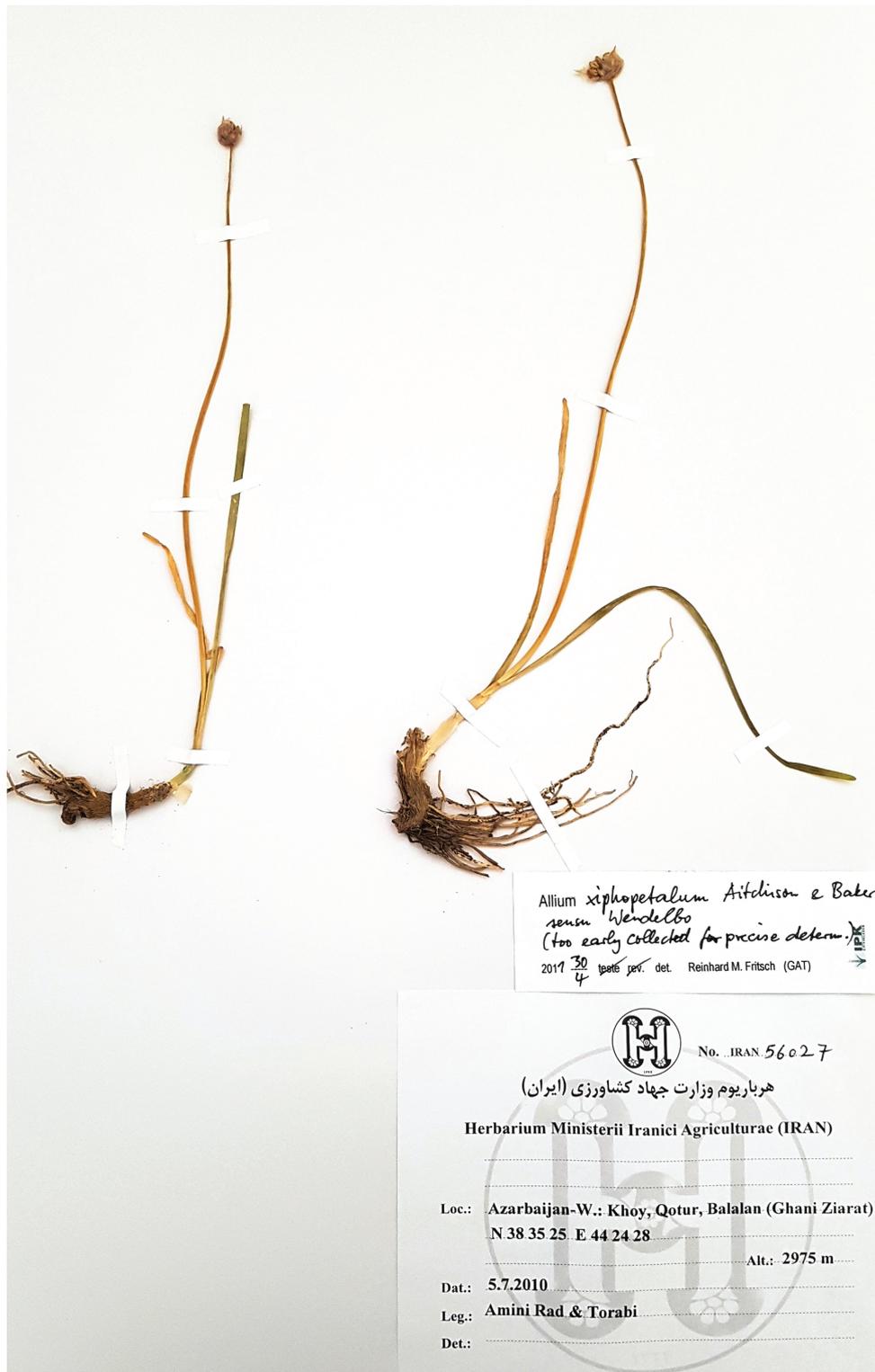


FIGURE 1. Holotype of *Allium matinae* (specimen on the right).

## Material and methods

**Material sampled:**—Sequences from two species of the section *Daghestanica* from Caucasus (*A. daghestanicum* and *A. gunibicum*), from two European species (*A. ericetorum* and *A. suaveolens*), and from four Chinese species (*A. chrysocephalum*, *A. chrysanthum*, *A. xichuanense* and *A. rude*) were used for the phylogenetic analysis. *Allium hymenorrhizum* Ledeb. in Ledebour *et al.* (1830: 12) and *A. carolinianum* Redouté (1804: t. 101), as representatives of *Allium* sect. *Falcatifolia* N. Friesen in Friesen *et al.* (2006: 390) within the phylogenetically related *A.* subgen. *Polyprason* Radić (1990: 250, 253), were chosen as outgroup. The voucher number and the corresponding ENA/GenBank accession numbers of specimens used are listed in Table 1.

**DNA extraction, amplification and sequencing:**—Total genomic DNA was isolated from herbarium specimens using the InnuPREPP Plant DNA Kit (Analytic Jena AG) according to the instructions of the manufacturer, and used directly in PCR amplification. The complete 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 were as follows: for *trnL-trnF* as described in Taberlet *et al.* (1991), whereas for the *rpl32-trnL* (UAG) and *trnQ-rps16* spacers as described in Shaw *et al.* (2007). PCR products were sent to SeqLab (Göttingen, www.seqlab.de) for sequencing. Forward and reverse sequences from all individual were manually edited in Chromas Lite 2.1 (Technelysium Pty Ltd) and combined in single consensus sequences. The sequences of all samples were aligned with ClustalX (Thompson *et al.* 1997), and the alignment was manually corrected using MEGA7 (Kumar *et al.* 2016).

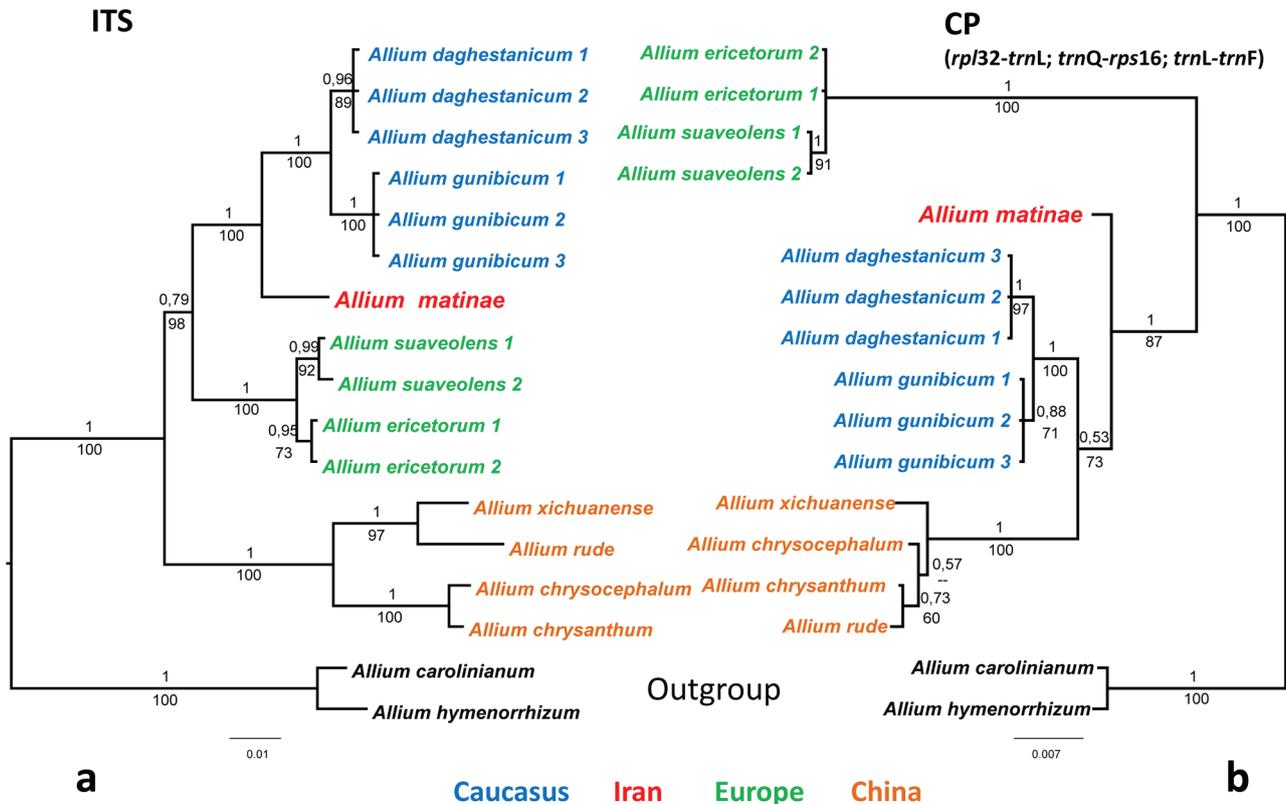
**Phylogenetic analyses:**—Both data sets (ITS and the combined *cpDNA* regions) were analyzed separately by means of FITCH parsimony with the heuristic search option in PAUP version 4.0b10 (Swofford 2002) with MULTREES, TBR branch swapping and 100 replicates of random addition sequence. Gaps were treated as missing. 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 using 1000 pseudo replicates were performed to assess the support of the clades (Felsenstein 1985). Bayesian phylogenetic analyses were performed using MrBayes 3.1.23 (Ronquist & Huelsenbeck 2003). The sequence evolution model was chosen following the Akaike Information Criterion (AIC) obtained from jModeltest 3.7 (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).

## Results

**ITS sequences:**—The alignment of ITS sequences of the 17 taxa (Table 1) generated a matrix of 642 characters, 97 of which were parsimony informative. Parsimony analysis resulted in one most parsimonious tree of 137 steps (CI = 0.9051). For the Bayesian analyses, the substitution model TVM+G was chosen. The phylogenetic trees derived from Bayesian inference were topologically similar to those from parsimony analyses. Therefore, only the Bayesian trees supplemented by bootstrap values (BS) are shown (Fig. 2a). Species of the section *Daghestanica* were divided in two clades: a strongly supported clade including Chinese taxa (1.0 PP, 100% BS) and a European-Caucasian clade (0.79 PP, 100% BS) including the Iranian species. The European-Caucasian clade was in turn divided into two strongly supported subclades—European and Irano-Caucasian—where the Iranian species clade was sister to the Caucasian subclade.

***cpDNA* sequences:**—The sequence lengths of the three regions analyzed for this study were: 804–828 bp for *trnQ-rps16*; 792–826 bp for *rpl32-trnL* and 163–168 bp for *trnL-trnF*. Therefore, the combined alignment is 1869 bp long, with 1733 characters that were constant, 12 were variable but parsimony-uninformative, and 124 were parsimony informative. Parsimony analysis returned three most parsimonious trees of 153 steps (CI = 0.9020, RI = 0.9620). For the Bayesian analyses, the substitution model GTR+I was chosen. The phylogenetic trees obtained were topologically similar to those obtained from parsimony analyses. Therefore, only the Bayesian trees supplemented with BS are shown (Fig. 2b). Species of the section *Daghestanica* were divided in two clades, with European species forming a strongly supported clade (1.0 PP, 100% BS) sister to all the other Asian (Caucasian, Iranian and Chinese) species. In the latter clade, the new species was sister to all the other Caucasian and Chinese taxa (PP = 1.0, BS = 87%). There was only one single nucleotide variant (SNV) in *rpl32-trnL* spacer (at the position 1315 in the combined alignment)

that was exclusively shared between the Iranian and Chinese species, and there were several common positions shared by the Iranian, Caucasian and Chinese species (see Alignment in Suppl. 1). However, the sisterhood of Caucasian and Chinese clades was only weakly supported by PP (0.53) and moderately supported by BS (73%).



**FIGURE 2.** ITS (a) and plastid DNA (CP) (b) Bayesian trees of *Allium* sect. *Daghestanica* species. Posterior probabilities (PP) and parsimony bootstrap support (BS)  $\geq 50\%$  are given above and below branches, respectively. See Table 1 for further details on the accessions.

## Discussion

Molecular data from both organelles (plastid and nuclear) showed the distinct position of the Iranian plant within the section *Daghestanica*, confirming its specific rank. However, some incongruence between the trees obtained from different genomes (*nrDNA* and *cpDNA*) can be found. Indeed, in the ITS tree the Iranian species is clearly related to the Caucasian clade and the Irano-Caucasian clade is sister to the European clade, whereas in the *cpDNA* the Iranian species is related to the Caucasian + Chinese clade despite sisterhood of Caucasian and Chinese clades is not strongly supported. This incongruence may be due to the unstable topology of *cpDNA* tree (low statistical support), possibly caused by a sampling bias and/or the confused phylogenetic signal yielded by the markers used. Nevertheless, the new species may be a remnant of a depauperated lineage testifying that Iran may have served as a bridge between the European and the Sino-Caucasian groups. However, it is also possible that the lack of molecular and taxonomic knowledge concerning *Allium* sect. *Daghestanica* species in other Asian areas, such as Tianshan Mountains, may account for the large disjunction gap between the Caucasian and Chinese species. Therefore, only the use of upcoming molecular studies including all species of this section and species from the related sections *Falcatifolia* and *Oreiprason* Hermann (1939: 57) can clarify the phylogenetic and phylogeographic relationships within *A.* sect. *Daghestanica*.

**TABLE 1.** Accessions of *Allium* species used in the study. Herbarium codes of voucher numbers follow Thiers (2019).

Species	Accession	Origin	Voucher	nrITS	trnQ-rps16	rp132-trnL	trnL-trnF
<i>Allium gunicicum</i> 1	Am503	Russia, Daghستان, Avgali, Kochali	DAG 20.08.2008 R. Murtazaliev	LR700265	LR700278	LR700250	LR700293
<i>Allium gunicicum</i> 2	Am504	Russia, Daghستان, Verchnij Gumib	DAG 16.07.2012 R. Murtazaliev	LR700266	LR700279	LR700251	LR700294
<i>Allium gunicicum</i> 3	Am737	Russia, Daghستان, Karabudachkensky distr., Gubden	DAG 17.08.2014. R. Murtazaliev	LR700267	LR700280	LR700252	LR700295
<i>Allium daghestanicum</i> 2	Am860	Russia, Daghستان, Shamilsky Distr.	GAT 2541969	LR700268	LR700281	LR700253	LR700296
<i>Allium daghestanicum</i> 3	Am861	Russia, Daghستان, Unzukul'sky distr	LE 13.09.1987, G. Leonova	LR700269	LR700282	LR700254	LR700297
<i>Allium daghestanicum</i> 1	Am938	Russia, Daghستان, Danuch	DAG 22.07.2017 R. Murtazaliev, Z. Guseinova	LR700270	LR700283	LR700255	LR700298
<i>Allium matinae</i>	Am1009	Iran, Azarbaiyan-W, Khoy, Qotur	IRAN 56027	LR700271	LR700284	LR700256	LR700299
<i>Allium ericetorum</i> 1	Am540	Slovenia, Caven	OSBU 25972	LR700272	LR700285	LR700257	LR700300
<i>Allium ericetorum</i> 2	Am542	Slovenia, Nanos	BOGOS 2008-1099-W	LR700272	LR700286	LR700258	LR700301
<i>Allium suaveolens</i> 1	Am888	Germany, Bavaria, Kochel	BOGOS 2016-0201-W	LR700274	LR700287	LR700259	LR700302
<i>Allium suaveolens</i> 2	Am935	Slovenia, Ankaran	27.10.2016. Petra Sladek	LR700275	LR700288	LR700260	LR700303
<i>Allium xichuanense</i>	Am977	China, Nanjiang, Qinghai	OSBU 21163	LR700276	LR700289	LR700261	LR700304
<i>Allium chrysocephalum</i>	Am911	China, Gansu, near Luqu	OSBU 26219	LR700277	LR700290	LR700262	LR700305
<i>Allium chrysanthum</i>		China		MH383259	MH992108	MH383269	MH383289
<i>Allium rude</i>		China		MH066485	NC042158	MH383267	MH383287
<i>Allium hymenorrhizum</i>	Tax 3135	Tajikistan, Saravshan Range, Lake Iskanderkul	GAT 0014707	AJ411879	LR700291	LR700263	LR700306
<i>Allium carolinianum</i>	Tax 2570	Tajikistan, Hissar Range, Ansob Pass	GAT 0012395	AJ250290	LR700292	LR700264	LR700307

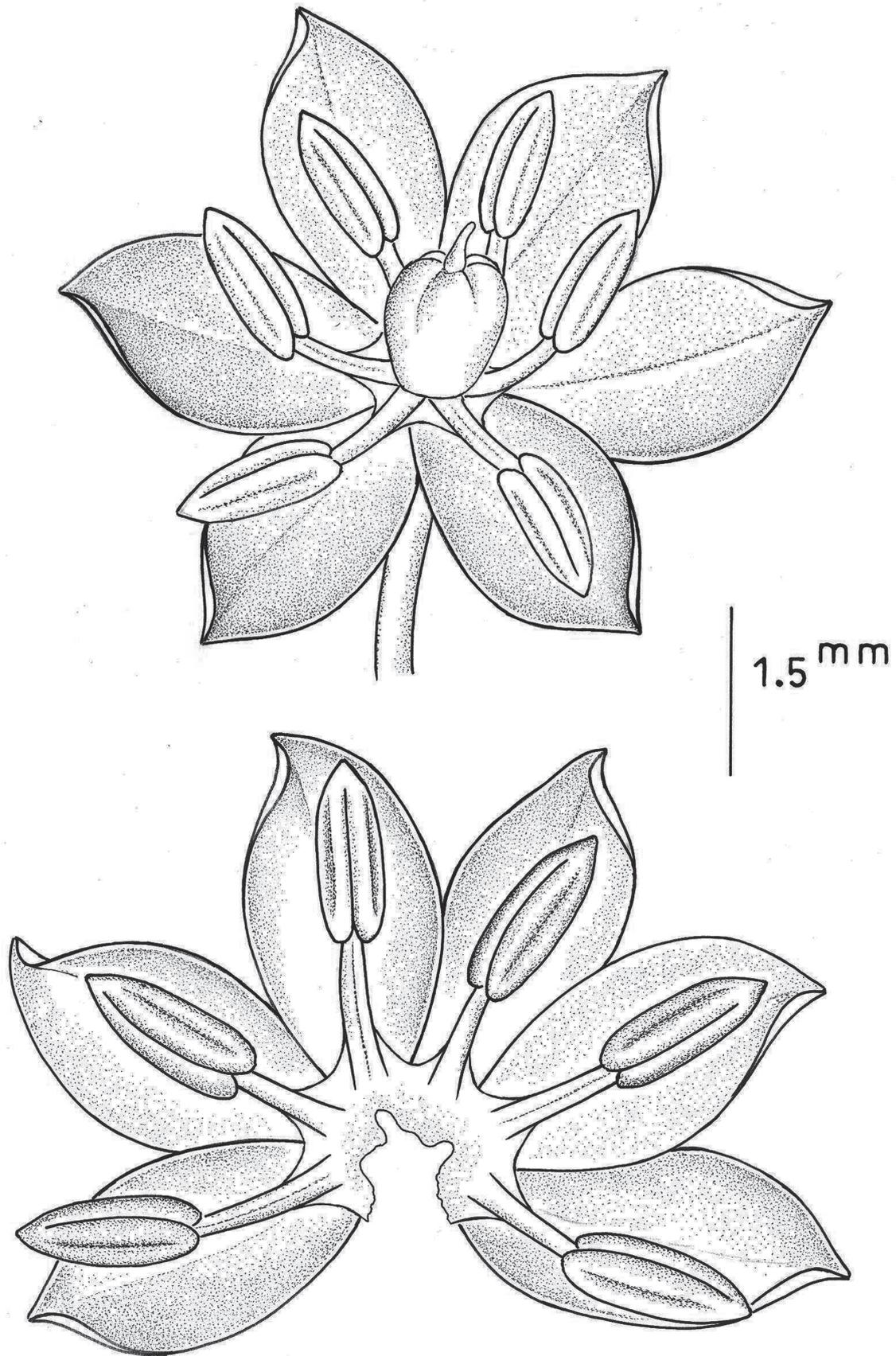


FIGURE 3. Flower of *Allium matinae*.

## Taxonomic treatment

*Allium matinae* N.Friesen & M.Abbasi *sp. nov.* (Figs. 1, 3)

**Type:**—IRAN. Western Azarbaijan: Khoy, Qotur, Balalan (Ghani Ziarat). N 38°35'25", E 44°24'28", alt. 2975 m. Leg. 5.07.2010 Amini Rad & Torabi (holotype right specimen IRAN 56027!).

**Description:**—Stems 1, 19–21 cm high, 1.5 mm in diameter, terete. Bulb 1.0 cm in diameter, 3 cm long, almost cylindrical; outermost tunics dust-coloured, paper-like, split into longitudinal stripes; inner tunics golden brown, shining. Leaves 2(–3), linear, 2–2.5 mm wide, sheathing the lower part (1/4–1/5) of the stem. Spathe bivalved, persistent; umbel compact, globose in anthesis, ca. 11 mm in diameter. Number of flowers 10–20. Pedicels ± equal in length at anthesis, 1–4 mm long; flowers equal to or longer than pedicels. Perianth cup-shaped; tepals slightly unequal, 3.7–4.1 mm long, 1.6–1.8 mm wide, white with a purple midvein, distinctly attenuate toward apex, rose in buds. Stamens not exerted; filaments filiform, yellowish to pale brown, shortly triangular at base.

Thus, slightly different ratios between the size of stamens, pistil and tepals can be found, whenever flowers at full anthesis would be later compared.

**Diagnosis:**—It differs from the closely related *A. daghestanicum* and *A. gunibicum* (East Caucasus) by linear leaves and yellowish to yellowish-brown stamen filaments and whitish tepals (not rose or purplish). The new species differs from the European *A. ericetorum*, by the lower number of flowers per inflorescence and whitish tepals (not yellowish).

**Molecular diagnosis:**—The new species differs from all the other species of *Allium* sect. *Daghestanica* at the following nucleotide positions in the nuclear ITS region (EMBL accession number LR700271): at position 30—cytosine not thymine; 35—cytosine not thymine; 1117—adenine not thymine; 124—thymine not adenine; 511—cytosine not thymine.

**Distribution and habitat:**—Northwestern Iran (known from the type locality only), on stony ground in alpine belt.

**Flowering period:**—July to August.

**Etymology:**—This species is named after Ms. Farideh Matin who was dealing with *Allium* taxonomy and other bulbous plants in Iran for almost thirty years (1970–2000).

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