



## *Allium parhamii* (Amaryllidaceae, Allioideae), a new species from Central Kopet Dagh Mountains, NE Iran

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### Abstract

*Allium parhamii* is described and illustrated as a new species from the Kopet Dagh mountain range, North Khorassan province in Iran. Based on morphological features, the new species belongs to *A.* sect. *Asteroprason*. It differs from the closely related species *A. monophyllum* in several floral and leaf characters as well as the micromorphological characters of seed coat and karyotype. The new species is illustrated and Red-Listed as critically endangered and notes on habitats and distribution are provided.

**Keywords:** *Asteroprason*, conservation, endemism, karyology, micromorphology, new species, taxonomy

### Introduction

*Allium* Linnaeus (1753: 294) (Amaryllidaceae, Allioideae) is mainly a perennial geophytic genus with currently accepted 1006 species (Govaerts *et al.* 2021). As one of the largest genera among the monocots, it is widely distributed over Holarctics and adapted to diverse habitats from the Mediterranean Basin to Central Asia (Friesen *et al.* 2006, Fritsch 2012). The Irano-Turanian floristic region is known as a main center of diversity of the genus *Allium* where it displays a high level of specific endemism (Matin 1992, Memariani *et al.* 2016a, 2016b). Of over 155 *Allium* species growing naturally in Iran (Dolatyari *et al.* 2020), about 42 species are native to the northeastern corner of the country, which is known as Khorassan-Kopet Dagh (KK) floristic province, with 36% of endemism in the genus (Memariani 2020). Moreover, the phytogeographical position of the KK has made it an area with disjunct distribution of several *Allium* species from the surrounding areas, especially Middle Asia (Memariani *et al.* 2007).

*Allium* subg. *Melanocrommyum* (Webb & Berthelot 1848: 347) Rouy (1910: 378) is the second-largest subgenus of *Allium* and comprises ten sections with about 83 species in Iran (Dolatyari *et al.* 2020). Several members of this subgenus have been listed as ornamental plants (Fritsch & Abbasi 2013). *Allium* sect. *Asteroprason* R.M.Fritsch in Fritsch *et al.* (2010: 184) is a section endemic to Khorassan-Kopet Dagh (KK) floristic province and Central-Eastern Alborz Mountains. Within it, *A.* subsect. *Christophiana* Tscholokaschvili (1975: 52) is narrowly endemic to the KK (Memariani *et al.* 2012). The species are typical xerophilous plants growing on rocky and stony exposed slopes with more or less flat star-like flowers, patent or later somewhat recurved and after anthesis longitudinally enrolled tepals.

During botanical excursions in Central Kopet Dagh Mountains in northeastern Iran, we collected a peculiar and distinct *Allium* that mostly resembles *A. monophyllum* Vvedensky (1934: 128) and believed to be a species new to science. In this paper, we aim to describe and illustrate this new species with notes on its seed coat micromorphology, chromosome number and karyotype, habitat, and conservation status.

### Material and methods

We examined the living and dried *Allium* specimens collected from Kopet Dagh Mountains and consulted the descriptions and identification keys in the relevant literature including Vvedensky (1935), Wendelbo (1971), Memariani *et al.* (2012),

Fritsch (2012), and Fritsch & Abbasi (2013). The features of gross morphology of the specimens were examined under a binocular stereomicroscope and compared with the specimens at FUMH, LE, and W herbaria (acronyms follow Thiers, 2022). We used the geographical range of the new species in the form of the extent of occurrence (EOO) and area of occupancy (AOO) to categorize the threat status (IUCN 2019), using the GeoCAT tool (Bachman *et al.* 2011). For micromorphological analysis, we placed the selected mature seeds on aluminum stubs using a double-sided adhesive tape, sputter-coated them with gold using an Emiteck K550, and then examined the seeds using the FEI Quanta 250 FEG and JEOL Neoscope JCM-5000 scanning electron microscope.

For karyotype analysis, the seeds were germinated at 4 °C for 6 months. Five to ten root tips of species were pretreated with 8-hydroxyquinoline (0.002 M) for three hours at room temperature. Then, they were fixed in Carnoy's solution (3:1 absolute ethanol – glacial acetic acid) for 24–48h at 4°C. The fixed root tips were hydrolyzed in 1N HCl at 60°C for 6–9 min followed by washing them in distilled water. Then they were stained with aceto-orcein (1% w/v) for 2 h. Finally, the root tips were squashed in acetic acid 45%. The metaphase chromosomes were counted for at least 10 cells of each seedling root. The examined metaphase plates were photographed using Olympus BX52 microscope with automatic camera. The numerical values were measured including total chromosome length (TCL) and arm ratio (AR) for each chromosome. Arm ratio (AR) was calculated according to Kutarekar & Wanjari (1983) by the formula  $AR = S/L$  (Short arm length of the chromosome/Long arm length of the chromosome). Furthermore, chromosomes were identified based on Levan *et al.* (1964). The idiogram was presented based on the data related to the chromosome length. Two parameters were used to determine the karyotype asymmetry including Coefficient of Variation of Chromosome Length ( $CV_{CL} = A_2 \times 100$ ) and Mean Centromeric Asymmetry ( $M_{CA} = A \times 100$ ). A is degree of karyotype asymmetry (Watanabe *et al.* 1999) and  $A_2$  is the interchromosomal asymmetry index (Romero Zarco 1986). Both parameters provide the most suitable methods for estimating karyotype asymmetry (Peruzzi & Erođlu, 2013). We used the chromosome data (long arms and short arms) of *Allium monophyllum* calculated by Fritsch (2018) to compare karyological parameters ( $CV_{CL}$  and  $M_{CA}$ ) of two related species. We also obtained Stebbins' categories (Stebbins 1971) to detect a symmetric karyotype.

## Description of the new species

### *Allium parhamii* Memariani, *sp. nov.* (Figs. 1–2, 3A)

Similar to *Allium monophyllum* in shape and color of flowers, but distinguished from it by its dense and many-flowered inflorescence, usually 2 leaves with much wider blades, and larger habit, capsules, and seeds.

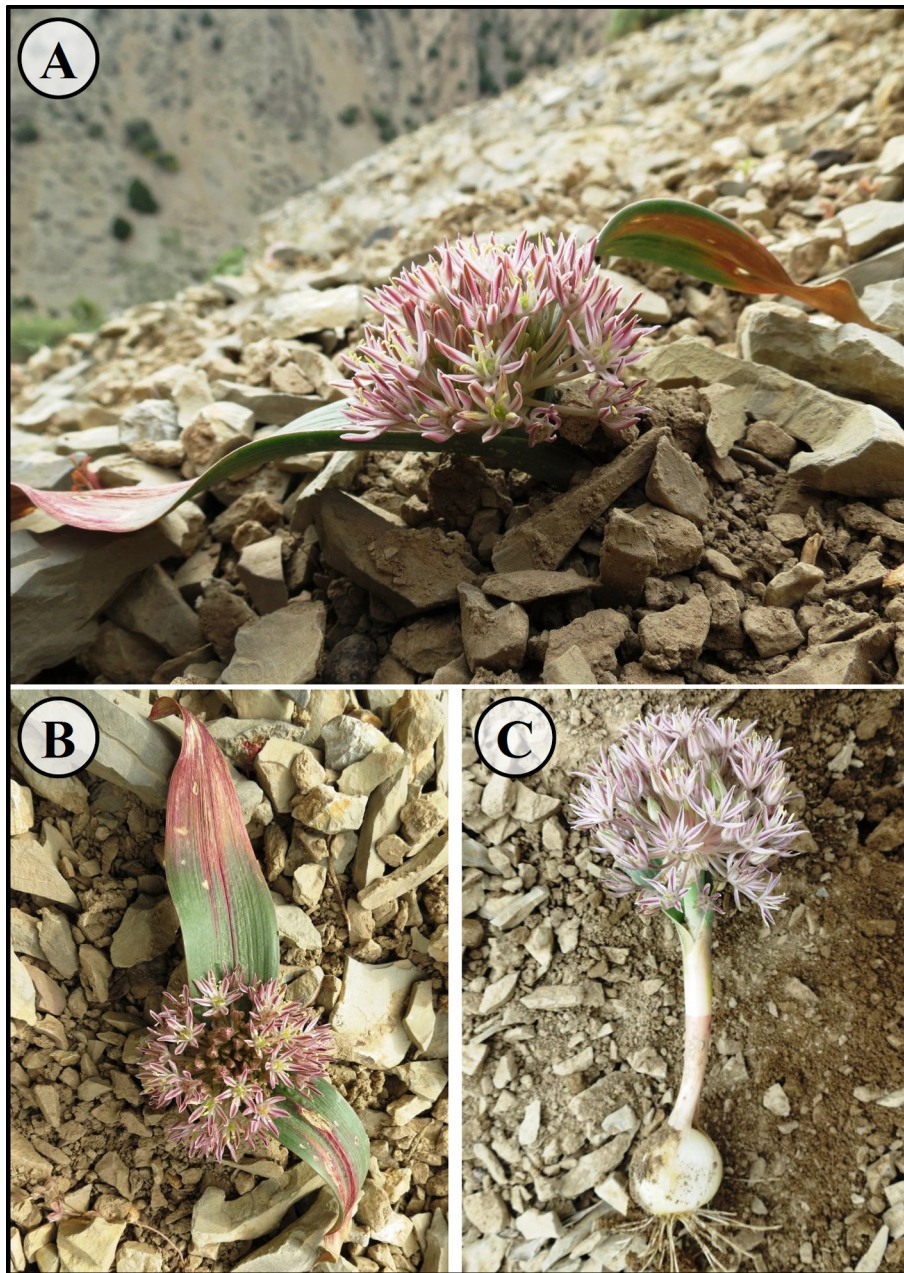
**Type:**—IRAN. North Khorassan: NE Shirvan, 6 km east of Namanlu towards Bajgiran, southern slopes of Sivak Mount, 2135 m, 8 June 2013, *Joharchi & Memariani 45135* (holotype: FUMH!; isotypes: FUMH!, W!).

Bulbs subovoid to subglobose, 1.5–2 cm in diameter, tunics greyish. Leaves (1–)2, oblong, almost thick and fleshy, basally enclosing the scape, the upper side sulcate, green and basally purplish, after flowering usually with wide reddish stripes in margin and surface, margin and sometimes in lower side scabrous, 10–20 cm long, 1.5–2.5(–3) cm wide. Scape cylindrical, erect, shorter than leaves, app. 2 cm above soil, 3–5 mm in diameter, pale green. Spathe membranous, splitting into 2–3 acuminate parts with brownish nerves. Umbel semi-globose, contracted, many-flowered, 3.5–4(–5) cm in diameter. Pedicels straight, up to 20 mm long, pale purple. Flowers flat funnel-like. Tepals long-triangular, longitudinally folded, acute, 6–7(–8) mm long, basally 1.5–2 mm wide, violet, pink to pale purple, with darker purple mid-vein, after anthesis convolute but not prickly stiff. Filaments 2/3 to 3/4 as long as tepals, whitish purple, subulate, basally connate. Anthers 1.5–2 mm long, yellow. Ovary subglobose- pyriform with three furrows, 2.5–3 mm in diameter, whitish green, coarsely tuberculate on surface. Style conical, 3–4 mm long, green to purplish, stigma undivided. Capsule depressed tripartite with furrows, 4–6 mm in diameter, in fresh state whitish green, in dry state brownish. Seeds one per locule, ovate, 2–2.3 mm long, 1.3–1.5 mm wide, 1 mm thick, shiny black.

**Additional specimen examined (paratype):**—IRAN. North Khorassan: NE Shirvan, *ca.* 5 km east of Namanlu towards Bajgiran, in *Juniperus polycarpus* woodland, 2040 m, 9 June 2008, *Memariani & Zangooei 40733-A* (FUMH).

**Eponymy:**—The specific epithet of the new species is commemorated to the late Saied Parham, an Iranian nature conservationist in North Khorassan province who was killed by the poachers on December, 1<sup>st</sup> 2010 when he was on his official duties in Sarani Protected Area. The type locality of *Allium parhamii* (Sivak Mount) is located at the

southeastern borders of Sarani Protected Area which has been protected since 1973. The area is a part of the Kopet Dagh Biosphere Reserve which was designated as one of the UNESCO worldwide inscribed areas in the Man and the Biosphere Programme (MAB) in 2018.

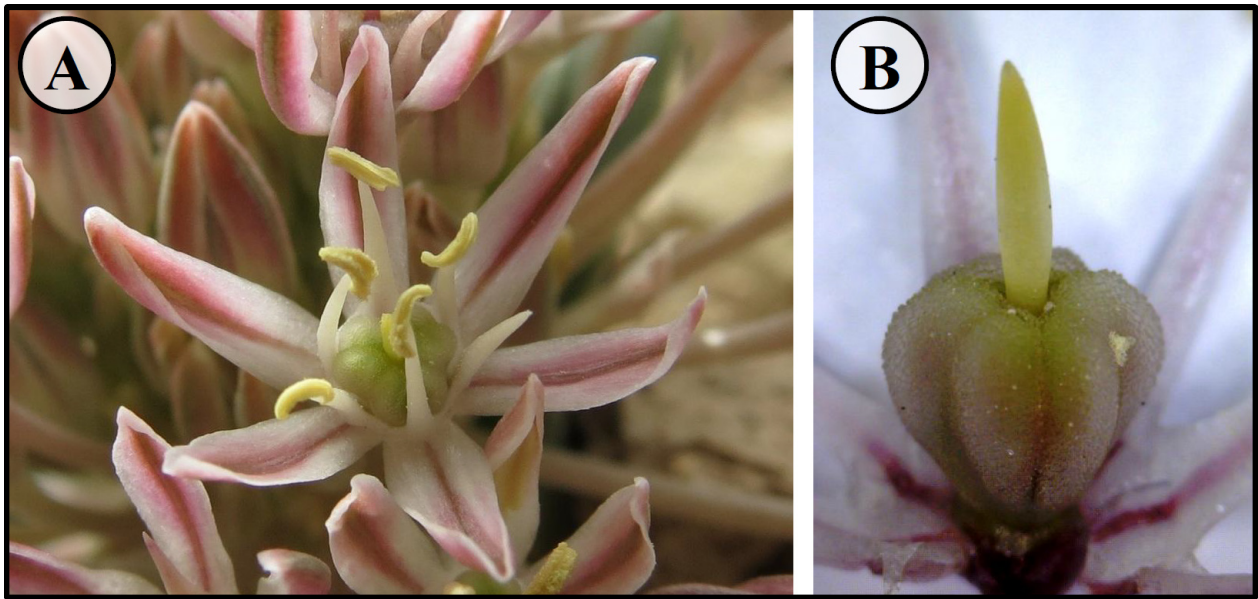


**FIGURE 1.** *Allium parhamii* in its natural habitat on exposed stony slopes. A–B. habit in early flowering stage with compact stature showing semi-globose inflorescence and two leaves, C. sub-globose bulbs and the grazed leaves by the livestock.

**Phenology:**—An ephemeroïd species, leaf sprouting is initiated in mid-May, flowering and fruiting is completed in mid-June.

**Distribution and habitat:**—So far, *A. parhamii* was found in higher elevations of Sivak Mt. in Central Kopet Dagh Mountains, NE Iran. It grows on south facing stony slopes, between 2000–2200 m a.s.l., in mountain steppes and very open *Juniperus polycarpus* var. *turcomanica* (B.Fedtsch.) R.P.Adams woodlands.

**Conservation status:**—According to IUCN Red List Categories and Criteria (IUCN 2019), *A. parhamii* is Critically Endangered (CR, B1+2ab(iii)). Its extent of occurrence is not more than 10 km<sup>2</sup> and the area of occupancy is less than 5 km<sup>2</sup>. The small populations are located at the borders between Sarani Protected Area and Gharajagheh P. A. It is highly recommended to extend the borders of these protected areas more southwards to the Aq-Kamar Mount which inhabits several narrow and regional endemic plants; however, the area is under high grazing by the livestock of local nomads which may enter illegally to the adjacent protected areas (Fig. 1C). Very peculiar habitats of fragmented populations of the new species in subalpine zone may be sensitive to climate change and global warming.



**FIGURE 2.** Flower details in *Allium parhamii*. **A.** close-up view of a flower; **B.** coarsely tuberculate ovary, conical style, and undivided stigma.

**Taxonomic relationships:**—Based on nuclear and plastid molecular markers, *Allium* sect. *Asteroprason* constitute a distinctive subgroup within *A.* subg. *Melanocrommyum* (Fritsch *et al.* 2010, Gurushidze *et al.* 2010). Morphologically, the new *Allium* species belongs to *A.* sect. *Asteroprason* subsect. *Asteroprason* which differs from the species of *A.* subsect. *Christophiana* i.e. *A. cristophii* Trautvetter (1884: 268) and *A. ellisii* Hooker (1903: t.7875) by its compact stature and crumpled and not prickly tepals in the fruiting stage. *Allium parhamii* is more similar to *A. monophyllum* especially in the shape and color of the flowers; however, it can be distinguished from the latter by its dense and many-flowered inflorescence, usually 2 leaves with considerably wider blades, and larger habit, capsules, and seeds (Fig. 3, Table 1). In dried herbarium specimens, several diagnostic characters of living material may be lost. So, *A. parhamii* may be confused with *A. aladaghense* Memariani & Joharchi in Memariani *et al.* (2012: 29) in dried material; however, it differs from the latter by its yellow (not violet) anthers (Fig. 2A, Table 1).

**Seed coat micromorphology:**—The seeds of the new species are oval-spherical, 1.3–1.5 mm wide and 2.0–2.3 mm long. The most frequent shape of testa cells is elliptic. The periclinal walls are convex with verrucate and granulate sculpture and the anticlinal walls are loosely undulated (Fig. 4). Based on Neshati & Fritsch (2009), the closely related *A. monophyllum* has different seed coat micromorphology with penta- to hexagonal testa cells, and very shallow S-undulation in anticlinal walls.

**Chromosome number and karyotype:**—The diploid chromosome number of  $2n = 16$  was found in *Allium parhamii*. The karyogram presented six pairs of metacentric chromosomes (m) and two pairs of satellited submetacentric chromosomes (sm) as well as one relatively long B chromosome (Fig. 5, Table 2). Fritsch (2018) reported the same chromosome number and symmetric karyotype for the closely related *A. monophyllum*; however, it differs from *A. parhamii* by its longer chromosome length, very short to invisible satellites, and absence of B chromosomes. Different ploidy levels i.e.  $2n = 16, 32, 48,$  and  $64$  have been reported for *Allium* species; however, most of taxa are diploid (Pastor *et al.* 1995, Fritsch & Astanova 1998, Peruzzi *et al.* 2017). Analysis of somatic metaphase spreads in *A. parhamii* confirmed the previous reports on the basic chromosome number of  $x = 8$ , a uniform karyotype, and presence of satellites and B chromosomes in *Allium* subg. *Melanocrommyum* (Fritsch & Astanova 1998, Genç *et al.* 2013). Table 3 shows the evaluation parameters for karyotype asymmetry in *A. parhamii* and *A. monophyllum*. According to Stebbins' categories (Stebbins 1971), the new species is placed in the class 2A, while *A. monophyllum* in 1A. Therefore, these results indicate a symmetric karyotype for both species. Genç *et al.* (2013) reported a similar symmetric karyotype in *Allium* subg. *Melanocrommyum* from Turkey. Based on the results, the karyotype of *A. parhamii* is more interchromosomally ( $CV_{CL} = 17.70$ ) and intrachromosomally ( $M_{CA} = 13.41$ ) asymmetric than that of *A. monophyllum* (Table 3).

**TABLE 1.** Morphological comparison of *Allium parhamii* with two closely related or similar species of *A.* sect. *Asteroprason*.

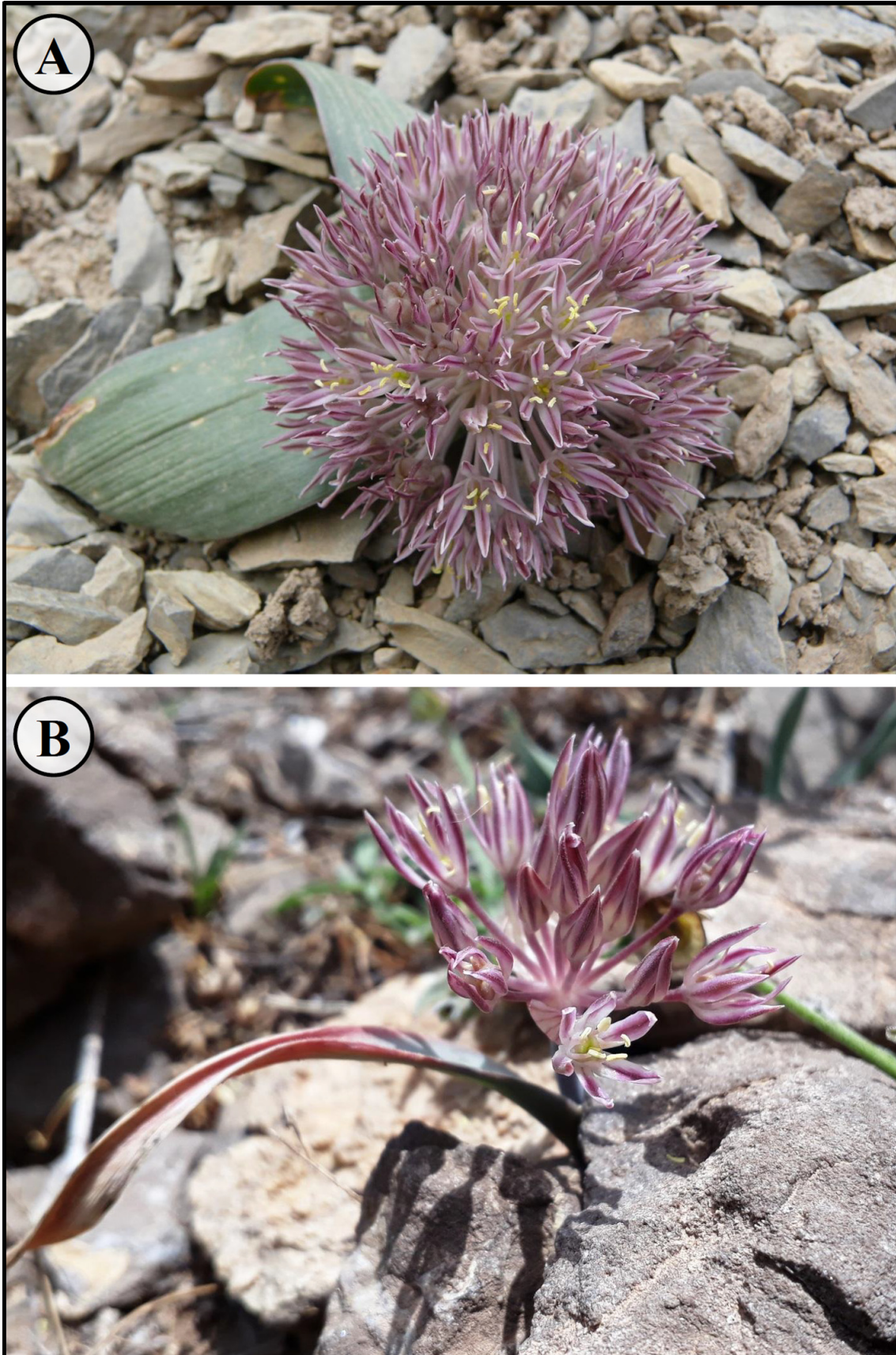
	<i>A. parhamii</i>	<i>A. monophyllum</i>	<i>A. aladaghense</i>
<b>Leaves</b>	(1–)2, 10–20 cm long, 1.5–2.5(–3) cm wide	1(–)2, 6–15 cm long, 0.3–1.1 cm wide	1–2, 7–20 cm long, 1.5–3 cm wide
<b>Inflorescence</b>	commonly ± 60 flowers	±20 flowers	± 50 flowers
<b>Flowers</b>	funnel-star like	funnel-star like	flat-star like
<b>Tepals</b>	6–7(–8) mm long, at base 1.5–2 mm wide, pink to pale purple (mid- vein dark purple)	5–7 mm long, at base 1–1.2 mm wide, dark violet, rose to pink (mid- vein purple)	5–7 mm long, at base 0.5–1 mm wide, white (mid-vein brownish)
<b>Filaments</b>	2/3 to 3/4 as long as tepals, whitish purple	2/3 to 3/4 as long as tepals, white to rose	4/5 to nearly as long as tepals, violet with white base
<b>Anthers</b>	yellow	yellow	violet
<b>Style</b>	green to purplish, 3–4 mm long	yellow ± purplish, 2–4 mm long	whitish violet, 3–4 mm long
<b>Capsule (diameter)</b>	4–6 mm	2–3 mm	5–6 mm
<b>Seed length</b>	2–2.3 mm	1.4–2 mm	2–2.5 mm

**TABLE 2.** Mean of satellites and arm length (µm) of the chromosomes in *Allium parhamii*.

Chromosome pair No.	Short arm (S)	Long arm (L)	Satellite length	Total length	Arm ratio (L/S)	Chromosome type
1	2.37	5.58	3.37	11.33 ± 0.23	2.35	sm/SAT
2	2.92	5.35	2.56	10.84 ± 0.18	1.83	sm/SAT
3	4.32	6.17	-	10.49 ± 0.26	1.43	m
4	4.52	5.48	-	10.00 ± 0.06	1.21	m
5	4.53	5.30	-	9.83 ± 0.02	1.17	m
6	4.54	5.08	-	9.62 ± 0.23	1.12	m
7	4.45	4.64	-	9.09 ± 0.06	1.04	m
8	4.36	4.47	-	8.84 ± 0.82	1.02	m
9	-	-	-	3.82 ± 0.01	-	B

**TABLE 3.** Karyotype symmetric indices calculated for *Allium parhamii* and *A. monophyllum*;  $CV_{CL}$ : coefficient of variation of chromosome length, and  $M_{CA}$ : mean centromeric asymmetry. The karyotype formula and chromosome data for calculating the indices for *A. monophyllum* are from Fritsch (2018).

Symmetric indices	Stebbins	$CV_{CL}$	$M_{CA}$	Karyotype formula
<i>Allium parhamii</i>	2A	17.70	13.41	$2n = 2x = 16 = 12m + 4sm(4SAT) + 1B$
<i>Allium monophyllum</i>	1A	11.04	11.83	$2n = 2x = 16 = 13m + 3sm(2SAT)$



**FIGURE 3.** **A.** *Allium parhamii* with dense many-flowered inflorescence and two wider leaves. **B.** the closely related species, *A. monophyllum*, with lower number of flowers in inflorescence and a single narrow leaf. Both species are endemic to Kopet Dagh Mountains, NE Iran.

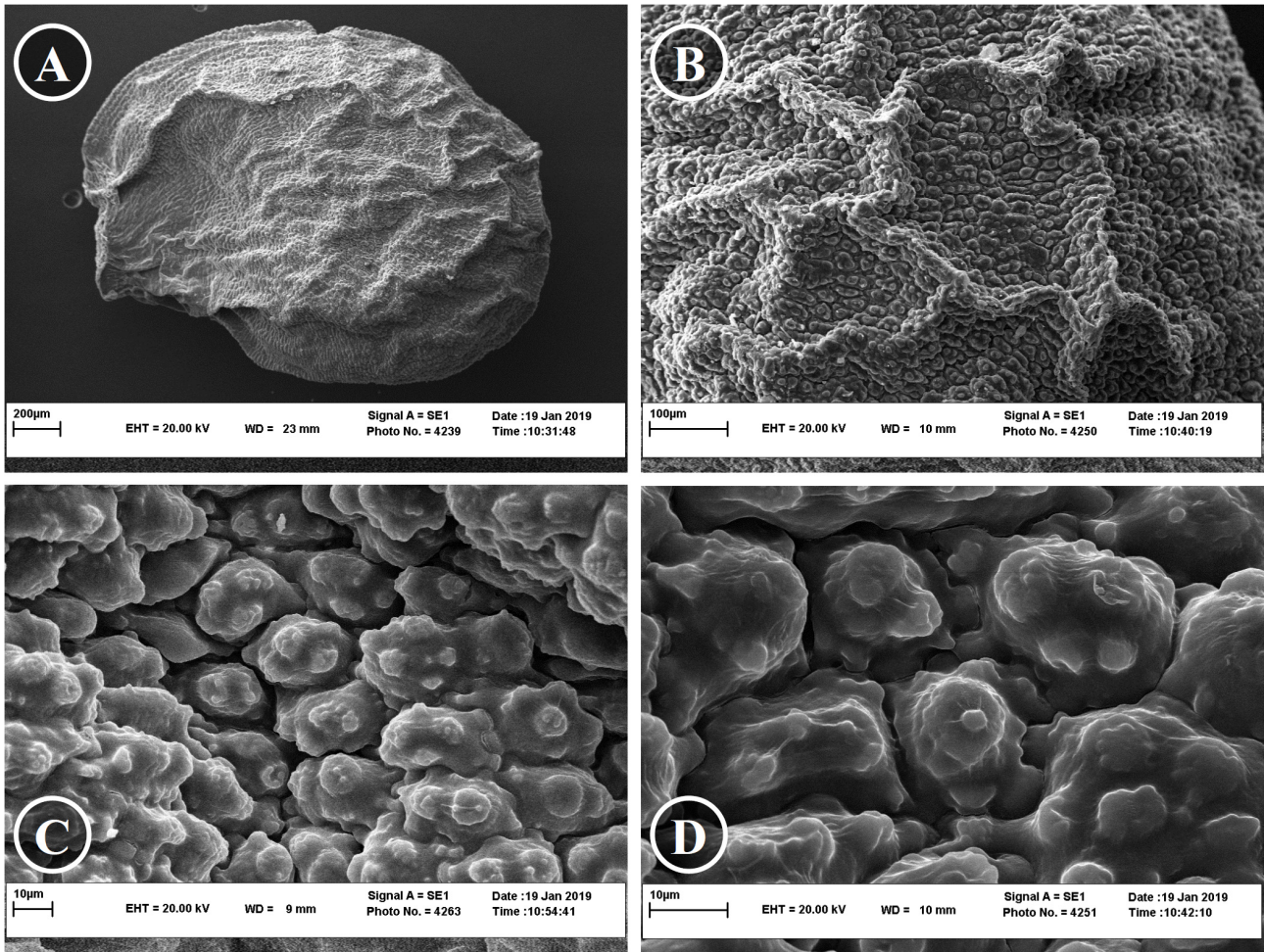
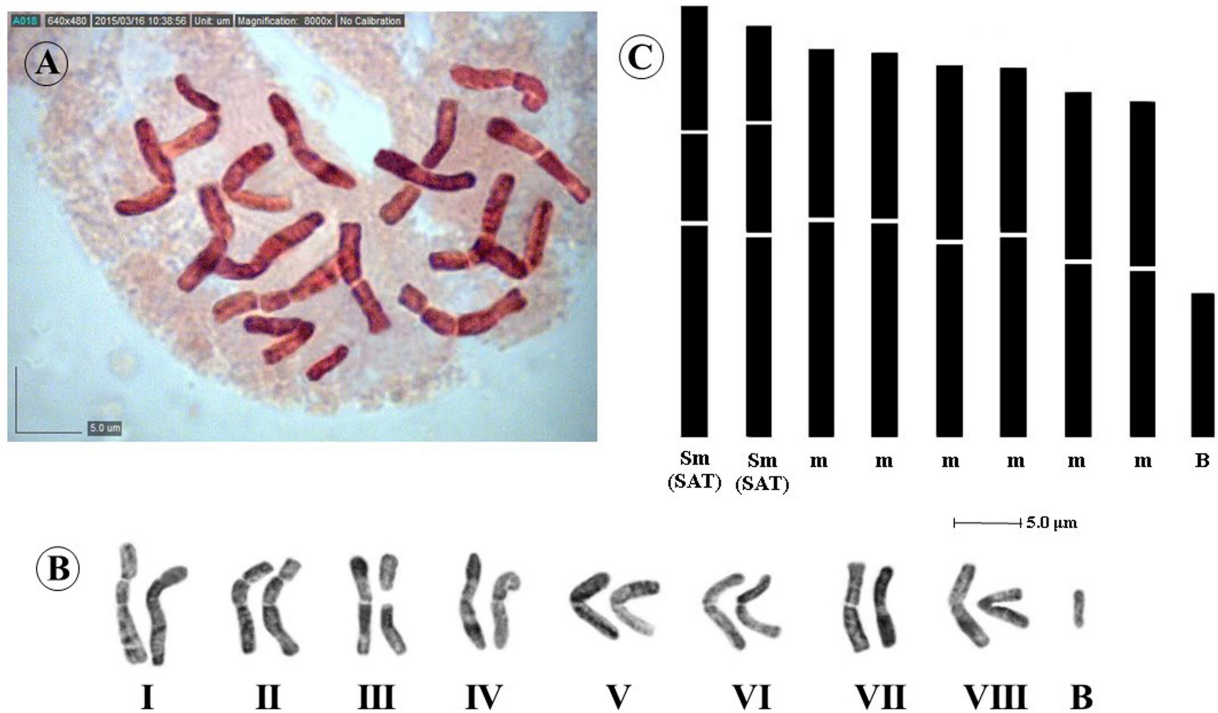


FIGURE 4. A–D. Scanning electron micrographs of *Allium parhamii* seeds and details of testa sculpture.

#### Identification key to *Allium* sect. *Asteroprason*

A revised and updated identification key to the species of *Allium* sect. *Asteroprason* (after Memariani *et al.* 2012) is provided here to facilitate identification of nine species of this section.

1. Plants of compact stature; tepals in fruiting stage crumpled and not prickly; leaves glabrous (Subsect. *Asteroprason*) .....2
- Plants of larger stature; tepals in fruiting stage stiff and prickly; leaves hairy or glabrous (Subsect. *Christophiana*) .....8
2. Scape stout, thickened, up to 10 mm in diameter; tepals after anthesis recurved; pedicels in fruiting stage up to 9 cm long; leaves in upper part spirally twisted ..... *Allium helicophyllum*
- Scape weak, up to 8 mm in diameter; tepals after anthesis patent; pedicels in fruiting stage not more than 4.5 cm long; leaves not twisted.....3
3. Tepals ± dark violet, after anthesis not much changing; leaves 0.3–1.1 cm wide; plants smaller in habit ..... *Allium monophyllum*
- Tepals violet, purple, pink, lilac, white, after anthesis longitudinally enrolled; leaves up to 8 cm wide; plants larger in habit.....4
4. Pedicels up to 4.5 cm long; scape up to 15(–20) cm long.....5
- Pedicels up to 2.5 cm long; scape up to 8 cm long.....6
5. Ovary surface glass-like glossy and colorless; filaments 3/5 to 2/3 as long as tepals..... *Allium elburzense*
- Ovary with coarse surface; filaments nearly as long as tepals ..... *Allium pseudobodeanum*
6. Leaves 1.5–3 cm wide .....7
- Leaves 5–8 cm wide ..... *Allium kuhsorkhense*
7. Flowers flat-star like; tepals white, at base 0.5–1 mm wide; anthers violet in early anthesis..... *Allium aladaghense*
- Flowers funnel-star like; tepals violet, pink to pale purple, at base 1.5–2 mm wide; anthers yellow in anthesis..... *Allium parhamii*
8. Leaves hairy, rarely subglabrous; pedicels up to 11 cm long; filaments 1/2 as long as tepals..... *Allium cristophii*
- Leaves glabrous; pedicels up to 6 cm long; filaments 2/3 as long as tepals ..... *Allium ellisii*



**FIGURE 5.** Karyotype and idiogram of *Allium parhamii*. **A–B.** Metaphasic plate, showing  $2n = 16 + 1B$  with two satellite chromosomes (I & II). **C.** Haploid idiogram based on the data of Table 2.

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