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Allium sunhangii – a new species from section *Brevidentia* F.O.Khass. & Iengal. (Amaryllidaceae) from Southern Pamir-Alay

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Abstract

Allium sunhangii, sp. nov., of the Middle Asiatic section *Brevidentia* F.O. Khass. & Iengal., (subgenus *Allium*, tribe Allioideae, Amaryllidaceae) is a small species from the Babatag area in the Surkhandarya region of Uzbekistan. It is morphologically close to *Allium brevidens* Vved. in having initially dark violet filaments and three-cuspidate inner filaments, but differs by its small size and visibly unequal tepals as well as in the phylogenetic analysis based on ITS data.

Key words

Allium, taxonomy, new taxa, molecular data, Middle Asia, *Brevidentia*.

Introduction

Allium Linnaeus (1753: 294), one of the largest genera in the Amaryllidaceae (Friesen et al. 2006; Li et al. 2010), has more than 1100 species worldwide (Govaerts et al. 2021). Members of the genus, such as garlic, leek, onion and shallot are used as food, medicine and ornament (Herden et al. 2016) and are characterized by bulbs enclosed in a membranous, fibrous or reticulate tunic, free or basally connate tepals and often a subgynobasic style (Friesen et al. 2006). *Allium* has two probable diversity centers, one in southwestern and central Asia and in the Mediterranean region, and a smaller center is in western North America (Friesen et al. 2006; Nguyen et al. 2008). The most recent classification of *Allium*, by Friesen et al. (2006), based on molecular phylogenetic analyses, includes 15 subgenera and 56 sections.

Subgenus *Allium*, with more than 375 species and 35 subspecies, is the largest subgenus within *Allium*, and is one of three main evolutionary lines within the genus (Friesen et al. 2006; Fritsch and Friesen 2002). Subgenus *Allium* consists of two main groups (Friesen et al. 2006; Hanelt 1992),

one has simple inner filaments while the other has three-cuspidate inner filaments. However, recently described sections show different morphological characteristics, such as types of filaments and different forms of bulblets (Khassanov 2018). The newly described sections are supported by nuclear molecular data (Friesen et al. 2006) and have revealed the presence of centers of recent speciation in the Middle Asia, Pakistan, Iran, Afghanistan and the Middle East (Khassanov 2018). Also, results from whole chloroplast genome analyses are continuing and being compared with morphology to determine whether morphology based taxonomy corresponds well to molecular data (Munavvarov et al. 2022)

Section *Brividentia* F.O. Khass. & Iengal., was previously a part of section *Allium* of subgenus *Allium*. Khassanov et al. (1997) divided section *Allium* into six section (*Allium* s. str., *Crystallina* F.O. Khass. & Iengal., *Filidentia* F.O. Khass. & Iengal., *Brevidentia* F.O. Khass. & Iengal., *Spathulata* F.O. Khass. & R.M. Fritsch and *Multicaulea* F. O. Khass. & Iengal.). According to the last revised and updated classification of subgenus *Allium* (Khassanov 2018), section *Brevidentia* includes 12 species, most of which are in central Asia and adjacent areas. The main characteristics are purple filaments, the inner ones three-cuspidate, as well as a rounded purplish ovary with pocket-like mounds of the nectary tubes. Most species show S-to U-type, U-type anticlinal walls and (globular) convex periclinal walls (Yusupov et al. 2022).

During field research in 2020, we collected an interesting species of *Allium* in Zarkasa, Babatag Mt., Surkhandarya region. Comparisons of molecular characteristics showed it to be a member of section *Brevidentia*. It resembles *A. brevidens* in its purple, three-cuspidate inner filaments, but differs in unequal tepals, which showed that it was previously unknown. Here, we propose if as new and provide a comprehensive description based on morphological and molecular approaches.

Material and methods

Plant material

All specimens were collected primarily by S.P. and O.T. in the summer of 2021. Material of the putative new species was collected from populations in the Zarkasa (Babatag range) area, Surkhandarya Region, Uzbekistan.

DNA extraction, PCR amplification and sequencing

Leaves for molecular analysis were dried in silica gel upon collecting. Total DNA was isolated by the CTAB protocol (Doyle and Doyle 1987) from 1 g of well-dried leaves. ITS primers were from White et al. (1990). Polymerase chain reaction (PCR) was performed under the following conditions: 5 min of initial denaturation at 94 °C, 35 cycles of denaturation for 45 secs at 94 ºC,

annealing for 45 secs at 55 ºC, and extension for 1–1.5 min at 72°C, then a final extension at 72°C for 5 min. PCR products were visualized using electrophoresis on 1.5% agarose TAE gel and sent to Beijing Genomics Institute (Shenzhen, China) for sequencing.

Phylogenetic analyses

To assemble and edit complementary strands, we used Sequencher 4.1.4 software (Burland 2000). Clustal X (Jeanmougin *et al*. 1998) was used to align DNA sequences, which were then manually adjusted using MEGA 7.0 (Kumar *et al*. 2016). Analysis of parsimony was conducted in PAUP* 4.0b10 (Swofford and Sullivan 2003) using heuristic searches with TBR and 1000 random addition sequence replicates. Bootstrap support (BS) (Felsenstein 1985) was estimated with 1000 replicates, each with 100 random addition sequence searches. The major consensus trees constructed from a maximum of 1000 trees were saved. RAxML v 8.2.8 (Stamatakis 2014) with GTR + G model and 1000 bootstrap replicates were used for performing Maximum Likelihood (ML) analyses. Based on the Akaike information criterion (AIC) implemented in jModelTest2 on XSEDE (www.phylo.org), the best-fitting nucleotide substitution model (GTR+G) was determined for each dataset. For Bayesian inference (BI) analyses, MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was utilized, with 10,000,000 generations with random trees sampled every 1000 generations. In the latter analysis, after discarding the first 25% of trees as burn-in. To estimate posterior probabilities (PP). we constructed a 50% majority-rule consensus tree from the remaining trees.

A total of 28 ITS sequences were downloaded from NCBI and used for phylogenetic reconstruction and to confirm the systematic position of the new species we selected 8 sections of subgen. *Allium* and two species from subgen. *Rhizirideum* (see Appendix). The classification system in this study follows the nuclear-based molecular phylogenetic classification of Friesen et al. (2006).

Allium sunhangii F.O. Khass., Tojibaev et Z. Yusup. sp. nov.

Description: Bulbs 0.4 – 0.8 cm wide, 0.7 - 0.9 cm long, ovoid; tunics reticulate, light brown, solitary; bulblets several, smooth, brownish. Scape terete, erect, 4.5 - 10 cm × 1.0 - 1.2 mm. Spathe bivalved, persistent, ca 4 mm long, with short beak. Leaves 2-4, narrowly linear, longer than inflorescence, 6 - 12 cm × 1-1.5 mm, semi-terete. Inflorescence lax, umbellate, hemispheric, 10- to 15-flowered. Flowers widely cup-shaped, nearly star-like, ca 5 mm long. Pedicels at base with bracts, 2 - 3 times longer than flowers. Tepals lanceolate-ovate, whitish with a dirty greenish-purple midvein, unequal 2.5 - 4 mm long, smooth, outer tepals slightly longer than inner ones. Filaments 1.5-2 times longer than tepals, inner ones 3-cuspidate, anther bearing cusp 2 times longer than basal teeth. Style exerted from flowers. Capsule 2 mm in diam.

Holotype: - UZBEKISTAN. Surkhandarya reg., Denau distr., Babatag Mt. R., Zarkasa peak, 37°98'6537"N, 68°16'6650"E, 2251 m a.s.l., 22th June 2021, collected by Pulatov S. O. and Turdiboev O. A. collection number: 22062021001.

Diagnosis: This species is most similar to *Allium brevidens* Vved. (Fig. 1), from which it differs in a more compact habit, remaining small spathe with a short beak, unequal tepals and strongly exserted, dark violet filaments (Fig. 2). The measurements of plant size are estimates based on 14 specimens collected from the wild but will likely not reflect the entire diversity within this species.

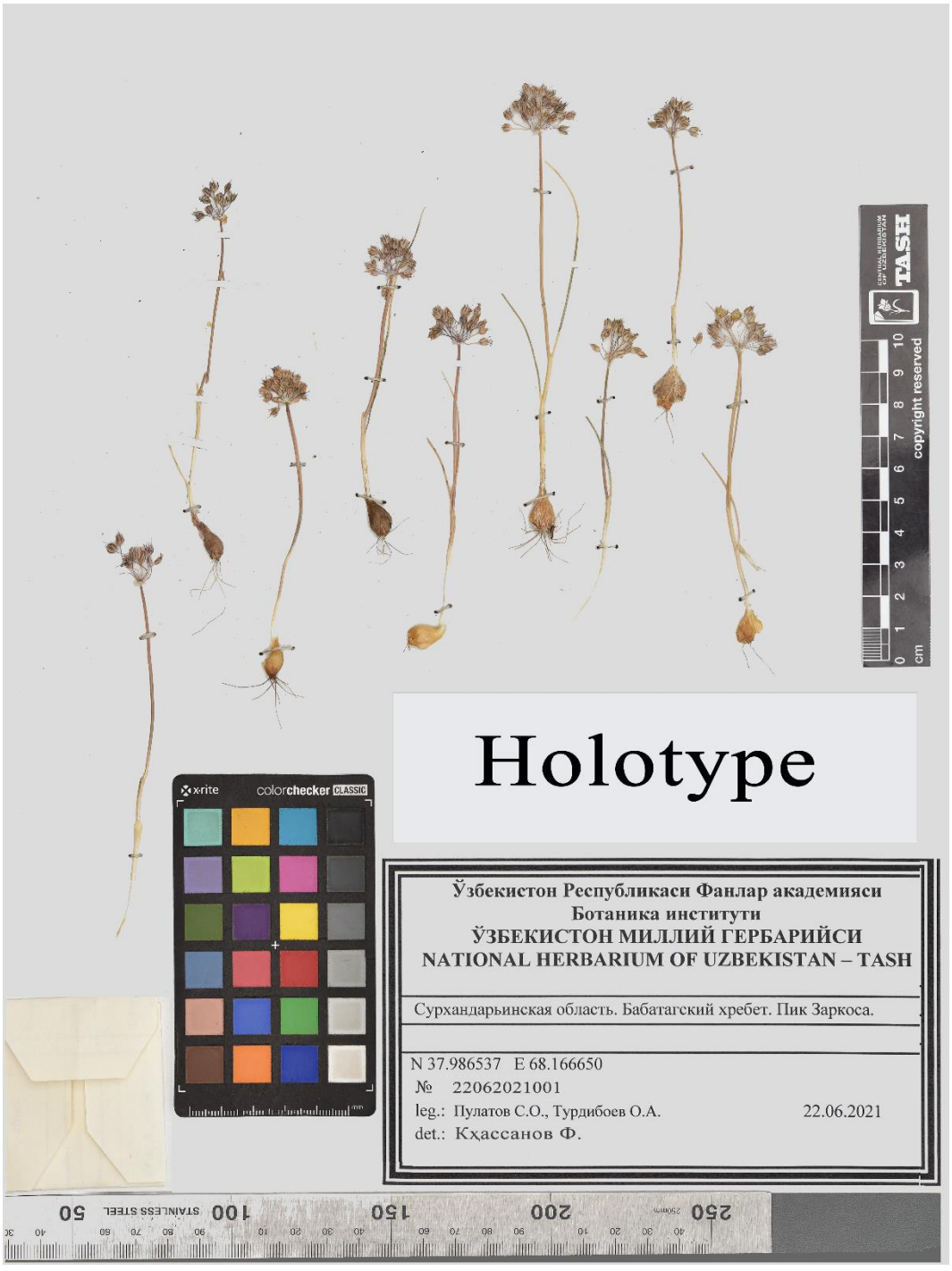

Figure 1. Holotype of *Allium sunhangii* F.O. Khass., Tojibaev et Z. Yusup. sp. nov.

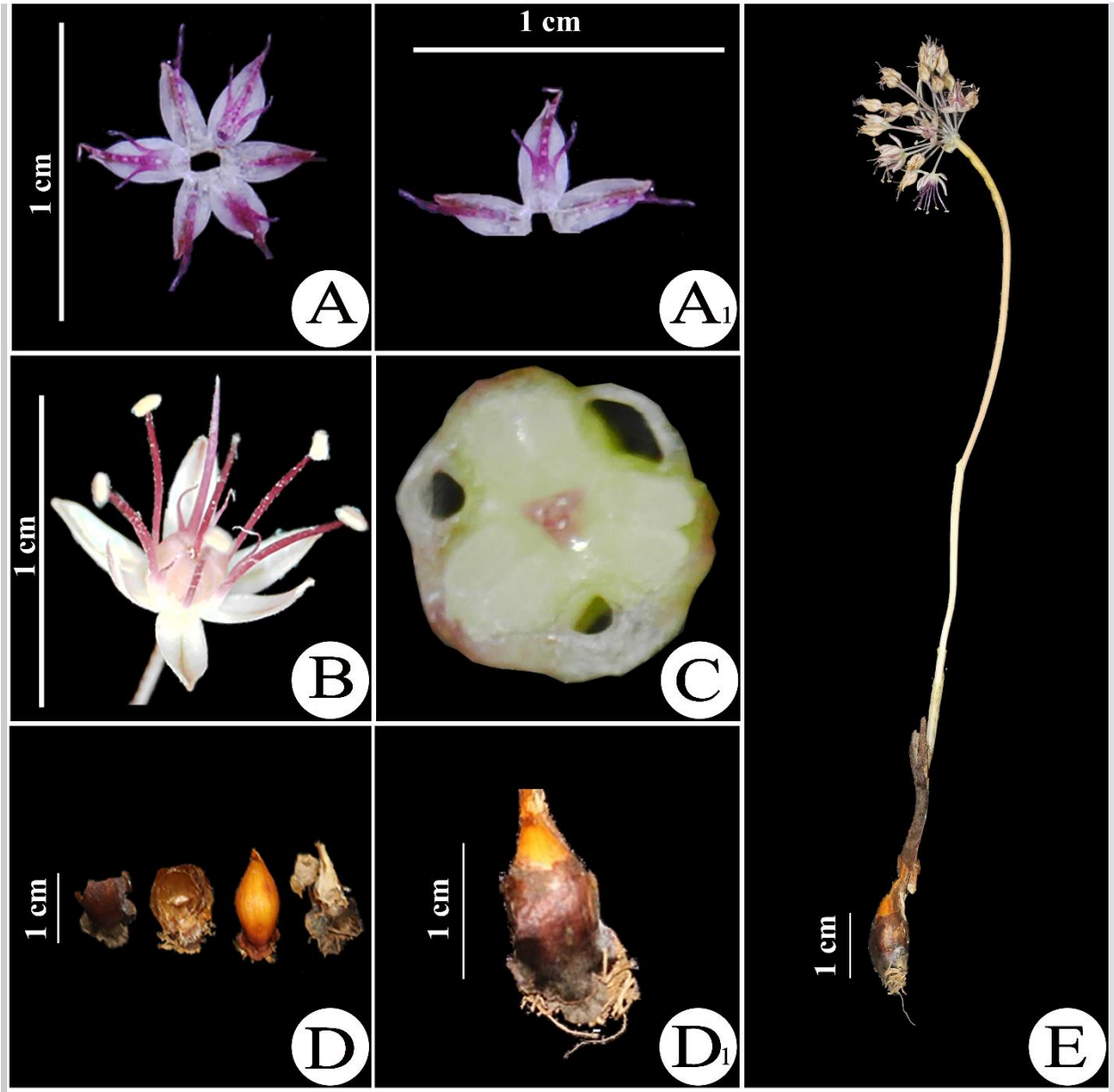

Figure 2. *Allium sunhangii* **A-A$_1$** whole and longitudinal section of flower with teeth **B** view of single flower **C** cross section of pistil **D-D$_1$** - bulb tunic and bulb **E** general view of species.

Etymology

Allium sunhangii is named after professor Sun Hang, one of the leading botanists at the Kunming Institute of Botany, Chinese Academy of Sciences, China, who actively promotes several projects within Central Asia.

Distribution and habitat

Allium sunhangii is known from 2 populations occurring to the south in the northwestern part of the Zarkasa range, at 2251 m a.s.l.

Phenology

Allium sunhangii was flowering on 22 June, 2022 and usually flowers around the same time as *A. brevidens*. Fruit begins to mature in the second half of June.

Ecology

Allium sunhangii grows in *Juniperus* forests, primarily on loamy soil, with *Convolvulus lineatus* L., *Cotoneaster nummularius* Fisch. & C.A. Mey., *Cousinia candicans* Juz., *Cousinia microcarpa* Boiss., *Daucus carota* L., *Dianthus tetralepis* Nevski ex Schischk., *Eremurus olgae* Regel, *Gentiana olivieri* Griseb., *Hypericum scabrum* L., *Juniperus seravschanica* Kom., *Lactuca serriola* L., *Lappula microcarpa* (Ledeb.) Giirke, *Lepyrodiclis holosteoides* (C.A. Mey.) Fisch. & C.A. Mey., *Lonicera nummulariifolia* Jaub. & Spach, *Malva neglecta* Wallr., *Marrubium anisodon* Koch, *Phlomis olgae* Regel, *Poa bulbosa* L., *Polygonum aviculare* L., *Primula baldshuanica* B. Fedtsch., *Rosa canina* L., *Rosa ecae* Aitch., *Taraxacum officinale* F.H. Wigg., *Veronica cardiocarpa* (Kar. & Kir.) Walp., *Ziziphora pamiroalaica* Juz. And is always surrounded by *Carex pachystylis* J. Gay (Fig. 3).

Figure 3. Habitat view of flowering *Allium sunhangii*.

Taxonomic relationship

Allium sunhangii was placed in the group of sect. *Allium* in all phylogenetic analyses (MP, ML and BI) (Fig. 5), but separate from morphologically and geographically similar species. *Allium sunhangii* belongs to sect. *Brevidentia*, subgen. The distribution of the new species and its relatives

are given in Fig. 4. *Allium sunhangii* is morphologically most similar to *A. brevidens*. The ITS based phylogenetic analysis is also congruent with this similarity.

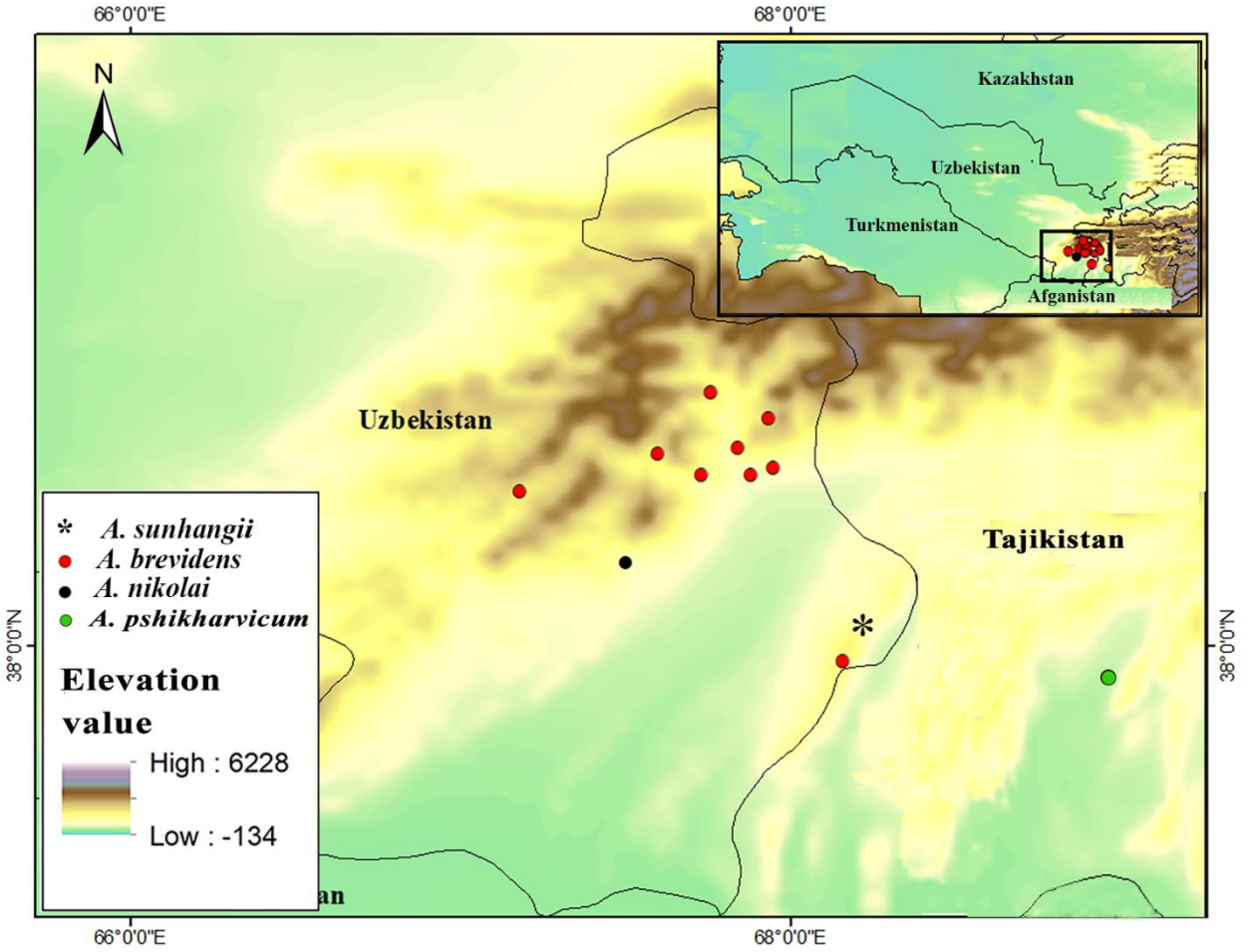

Figure 4. Distribution of *Allium sunhangii*, *A. brevidens*, *A. nikolai* and *A. pshikharvicum.*

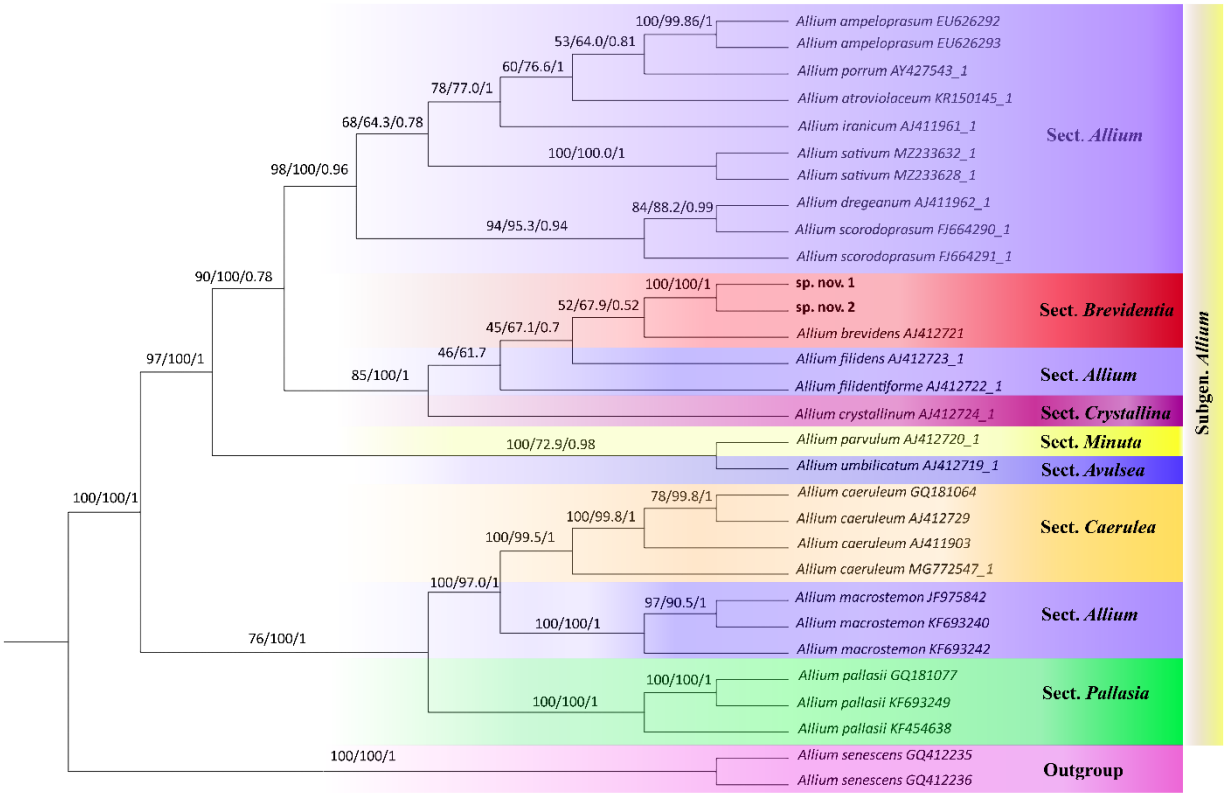

Figure 5. Phylogenetic tree inferred from MP, ML and BI (bootstrap support and posterior probabilities are given on branches, respectively), showing location of the *Allium sunhangii*.

Members of *Allium* sect. *Brevidentia*

Sect. ***Brevidentia*** F.O. Khass. et Yengal. in Ozturk, Seć̈men & Gork (Eds.) Plant Life in South West Asia. Ege Univ. Press, Izmir :147 (1996).

1. ***A. brevidens*** Vved., Not. Syst. Herb. Bot. Petrop. 5: 89 (1924). Holotype: Bukhara khanate, Hissar distr., hills on the southern slopes of Hissar range, near Karatag, (in Russian). 20.05.1913, n. 1721, collected by A.I. Michelson (lectotype LE; designated by Khassanov in Flora of Uzbekistan 1: 61 (2017)). Distribution: Middle Asia (Southern Pamir-Alai): Tajikistan, Uzbekistan. Represented in Fig. 6.

Figure 6. *Allium brevidens* Vved. (*locus classicus* Tajikistan, Karatagdarya, 1500 m., R. Fritsch, 22.05.2002.

2. ***A. pshikharvicum*** (R.M. Fritsch & F.O. Khass.) F.O. Khass & Z.Yusup. Type: Tajikistan, Darvaz range, road from pass Khoburabot between Robot and soldier post, steep stony-loamy slopes, in SE to SW exposition; 2200 m, 38°33'17"N, 70°48'07"E, leg. Fritsch, Keusgen, Hissoriev, Kudratov, 6199, (holotype GAT, isotypes GAT, TAD). Distribution: Southern Pamir Alay (Darwaz Range). Tajikistan. Represented in Fig. 7.

Figure 7. *Allium pshikharvicum* (R.M. Fritsch & F.O. Khass.) F.O. Khass & Z.Yusup. (*locus classicus* -Tajikistan, Darvaz range, road from pass Khoburabot between Robot and soldier post, steep stony-loamy slopes, in SE to SW exposition; 2200 m, 38°33'17"N, 70°48'07"E, leg. Fritsch, Keusgen, Hissoriev, Kudratov).

3. ***A. brevidentiforme*** Vved., Opred. Rast. Sred. Azii 2: 315, 78 (1971). Holotype: Kashkadarja valley, Igri-su river, right bank, Juniper forests (in Russian), 6.7.1955, fl., № 1617, Pjataeva, Tsukerwanik (TASH). Distribution: Western Pamir Alay (Hissar Range). Uzbekistan.

4. ***A. michaelis*** F.O. Khass. & Tojibaev, Linzer Biol. Beitr. 41(2): 1059 (2009). Holotype: Western Tien-Shan, Kurama range, near Ujgursaj village, 40°54'54"N, 71°03'27"E, h = 563 m.s.l., 24.05.2009, Khassanov, Tojibaev, Keusgen (TASH). Distribution: Endemic (Ferghana valley) Uzbekistan, Kyrgyzstan.

5. ***A. circumflexum*** Wendelbo in Acta Horti Gothob. 28: 22 (1966). Type: Prov. Bamian, Band-e-Amir, rich limestone steppe vegetation. Alt. 2900, 29 June 1962, leg. Hedge & Wendelbo 4803 (holotype BG, isotypes E; TASH 000456). Distribution: Afghanistan.

6. ***A. hedgei*** Wendelbo in Acta Horti Gothob. 28: 20 (1966). Type: Prov. Mazar-i-Sharif, Takht-i-Rustam, near Samangan (Aybak), dry slopes. Alt. 1200 m, 10 June 1962, leg. Hedge & Wendelbo, 3990 (type BG, isotypes E; TASH 000485). Distribution: Afghanistan.

7. ***A. ionandrum*** Wendelbo in Bot. Not. 121: 270 (1968). Type: E- Afghanistan, Urgun. 35 km NW Urgun, 32 27’ N, 690 07’ E, versus Surmat, 33°27"N, 69°02"E, 2200-2400 m. 10.06.1967, 35915 (W, isotype B, MUN). Distribution: Afghanistan.

8. ***A. micranthum*** Wendelbo in Biol. Skr. 10, No. 3 (Symb. Afghan. 4): 178 (1959) (as cited in Nasir 1975, 22. p). Type: Kurram valley, Afghanistan, 1879, Coll. & Comm. Dr. J.R.T. Aitchison 228, December 1879 (holotype K). Distribution: Afghanistan.

9. ***A. miserabile*** Wendelbo in Nytt Mag. Bot. 14: 104 (1967). Type: Flora of West Pakistan, Kohat, Kohat to Thal, c. 20 km from Kohat, Rocky slope on small hillock. Alt. c. 675 m. 26 May 1965. Coll. Jennifer Lamond, 1549 (holotype E). Distribution: Afghanistan.

10. ***A. nikolai*** F.O. Khass. & Achilova in Opred. Rast. Sred. Azii 11: 497 (2015). Neotype: Uzbekistan, 25 km eastern Bajssun town, gypsaceous slopes under the shrubs, 23.06.2013, Yusupov et al. (TASH). Distribution: Uzbekistan (Kelif-Sherabad mountain range). Uzbekistan. Represented in Fig. 8.

Figure 8. *A. nikolai* F.O. Khass. & Achilova (*locus classicus* - Uzbekistan, 25 km eastern Bajssun town, gypsaceous slopes under the shrubs, 23.06.2013, Yusupov).

11**. *A. ophiophyllum*** Vved. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 3: 8 (1928) (as cited in Khassanov and Yusupov 2022, 415. p). Type: Montes Meridionales: Sogdiano-transoxanae. Ad declivia argilloso-arenosa gypsacea, elevationis Chaudak-tau haud procul a pago Dzharkurgan, 30.04.1928, Vvedensky (TASH, isotype K, W, MBG, LE, MW). Distribution: Middle Asia: Southern Pamir-Alay. Uzbekistan, Tajikistan.

12. ***A. sunhangii*** F.O. Khass., Tojibaev & Z. Yusup. sp. nov. Holotype: - UZBEKISTAN. Surkhandarya reg., Denau Distr., Babatag Mt. R., Zarkasa peak, 37°9865'37"N, 68°16'6650"E, 2251 m a.s.l., 22th June 2021, collected by Pulatov S. O. and Turdiboev O. A. collection number: 22062021001. Distribution: Middle Asia: Southern Pamir-Alay (Babatag ridge). Uzbekistan, ? Tajikistan.

sect. *Brevidentia* key for determination of species from

1. Inner filaments simple, triangular-subulate……..*A. miserabile* Wendelbo

   \+ Inner filaments 3(or 5)-cuspidate, the lateral sterile cusps shorter than the median anther-bearing cusp...…… 2

2. Outer filaments with two obtuse teeth at base……..……..…*A. hedgei* Wendelbo

   \+ Outer filaments simple…….……..3

3. Leaves normally twisted……..4

   \+ Leaves normally straight

4. Perianth (6-)7 mm long…….…….. *A. ophiophyllum* Vved.

   \+ Perianth 3-4.5 mm long…….……5

5. Perianth lilac with purple midvein; filaments violet, twice as long as tepals……...*A. circumflexum* Wendelbo

   \+ Perianth lilac-greenish with green midvein; filaments whitish, shorter than tepals…….*A. michaelis* F.O. Khass. & Tojibaev

6. Filaments ciliate at base, bracteoles present……..……7

+ Filaments glabrous, bracteoles absent

7. Bulblets with subcrystalline tunic ……...……A. *brevidentiforme* Vved.

+ Bulblets without subcrystalline tunic…….8

8. Plants to 60-80 cm tall; inflorescence dense, globose, flowers 30-50……..……...…….……9

+ Plants 10-30 cm tall; inflorescence lax, umbellate, flowers 10-25 ……..……...…..10

9. Scape ca 80 cm tall; inflorescence dense, tepals greenish yellow with green midvein, …… *A. pshikharvicum* (R.M. Fritsch & F.O. Khass.) F.O. Khass & Z.Yusup.

+ Scape ca 30 cm tall; inflorescene loose, tepals white with purple midvein…….*A. brevidens* Vved.

10. Leaves shorter than scape; spathe with beak to 1 cm long; tepals rose colored, with purple midvein……..…….……..*A. nikolai* F.O. Khass. & Achilova

+ Leaves longer than scape; spathe with beak ca 3 mm long; tepals whitish with greenish midvein……..……...….*A. sunhangii* F.O. Khass., Tojibaev & Z. Yusup.

11. Outer tunic reticulate-fibrous; perianth urceolate-campanulate, whitish ……..……..……..……..……A. *ionandrum* Wendelbo

+ Outer tunic coriaceous; perianth, widely bell – shaped, purple or viole……..……....…….*A. micranthum* Wendelbo

Conclusion

Allium sunhangii is an endemic species from the western Pamir Alay area of Uzbekistan. It grows on mountains with trees of *Juniperus* at about 2251 m a.s.l. The flora of the Babatag range includes ca. 12 endemic species of vascular plants (including *Allium gypsodictyum* Vved., *A. incrustatum* Vved. and *A. tojibaevii* F.O. Khass., Turginov & S. Pulatov ined.), now enriched by another species of *Allium*.

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Appendix 1. GenBank accessions of species used in this study

Species	ITS
Allium ampeloprasum L.	EU626292
Allium ampeloprasum L.	EU626293
Allium atroviolaceum Boiss.	KR150145.1
Allium dregeanum Kunth	AJ411962.1
Allium iranicum (Wendelbo) Wendelbo	AJ411961
Allium macrostemon Bunge	JF975842
Allium macrostemon Bunge	KF693240
Allium macrostemon Bunge	KF693242
Allium porrum L.	AY427543.1
Allium sativum L.	MZ233628.1
Allium sativum L.	MZ233632.1
Allium scorodoprasum L.	FJ664290.1
Allium scorodoprasum L.	FJ664291.1
Allium umbilicatum Boiss.	AJ412719.1
Allium brevidens Vved.	AJ412721
Allium caeruleum Pall.	AJ411903
Allium caeruleum Pall.	AJ412729
Allium caeruleum Pall.	GQ181064
Allium caeruleum Pall.	MG772547.1
Allium filidens Regel	AJ412723.1
Allium filidentiforme Vved.	AJ412722.1
Allium crystallinum Vved.	AJ412724.1
Allium parvulum Vved.	AJ412720.1
Allium pallasii Murray	GQ181077
Allium pallasii Murray	KF454638
Allium pallasii Murray	KF693249
Allium senescens L.	GQ412235
Allium senescens L.	GQ412236
Allium sunhangii F.O. Khass., Tojibaev et Z. Yusup. sp. nov.	OP642456

Allium sunhangii F.O. Khass., Tojibaev et Z. Yusup. sp. nov.	OP642457