



# *Galanthus subalpinus* (Amaryllidaceae): a new species of snowdrop from the Western Balkans

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**Summary.** *Galanthus subalpinus* (Amaryllidaceae), a new snowdrop species endemic to the Western Balkans (North Macedonia and Kosovo) is described and illustrated. Morphological differences between the new species and the similar *Galanthus* species, *G. nivalis* and *G. graecus*, are reported and discussed, in combination with DNA sequence data from plastid (*trnL-trnF*, *matK*) and nuclear (ribosomal ITS) markers and genome size. Line drawings, photographs of habitat and morphology, a distribution map and a preliminary conservation assessment are provided. The genome size (1C-value) of *G. bursanus*, *G. samothracicus* and *G. × valentinei* nothosubsp. *subplicatus* are reported for the first time.

**Key Words.** Balkan flora, conservation, genome size, 1C-value.

## Introduction

Numerous variants of *Galanthus* are found (and indeed arise) in cultivation, including many hybrids (Davis 1999, 2001; Bishop *et al.* 2001). A plant in cultivation called *Galanthus* ‘Mt. Korab’ was found to be of particular interest, as it could not be assigned to any known species (Davis 1999, 2001). Mount Korab is in north-west Macedonia, in the Western Balkans. The plant in question combines leaf characteristics of *G. graecus* Orph. ex Boiss. and the floral morphology of *G. nivalis* L. (see Recognition), but these species do not occur in the Western Balkans. A field visit (I. McEnergy) was made to Mount Korab (North Macedonia) in 2019, to see if there might be any *Galanthus* at this locality. *Galanthus* plants matching *G.* ‘Mt. Korab’ were found at high elevation (c. 1900 m) in subalpine grassland.

There are nine indigenous *Galanthus* (snowdrop) taxa in the Balkans: *G. gracilis* Čelak., *G. graecus*, *G. nivalis*, *G. plicatus* M.Bieb. subsp. *plicatus*, *G. plicatus* subsp. *byzantinus* (Baker) D.A.Webb, *G. reginae-olgae* Orph. subsp. *reginae-olgae*, *G. reginae-olgae* subsp. *vernalis* Kamari, *G. samothracicus* Kit Tan & Biel and *G. × valentinei* Beck nothosubsp. *subplicatus* (Zeybek) A.P.Davis (Zeybek & Sauer 1995; Davis 1999, 2001; Davis *et al.* 2001; Biel & Tan 2013; Tan *et al.* 2014). Fieldwork, molecular data (Rønsted *et al.* in prep.) and re-evaluation of herbarium specimens show that *G. graecus* is a separate species, and not conspecific with *G. elwesii* Hook.f., as reported by Davis (1999, 2001). None of these nine species occurs in high elevation (mountainous) locations in the Western Balkans (Davis 1999, 2001). Populations of *G. graecus* may occur in similar habitats in the SE Balkans, but *G. nivalis* is absent at elevations above 1500 m (Davis 1999). *Galanthus elwesii*

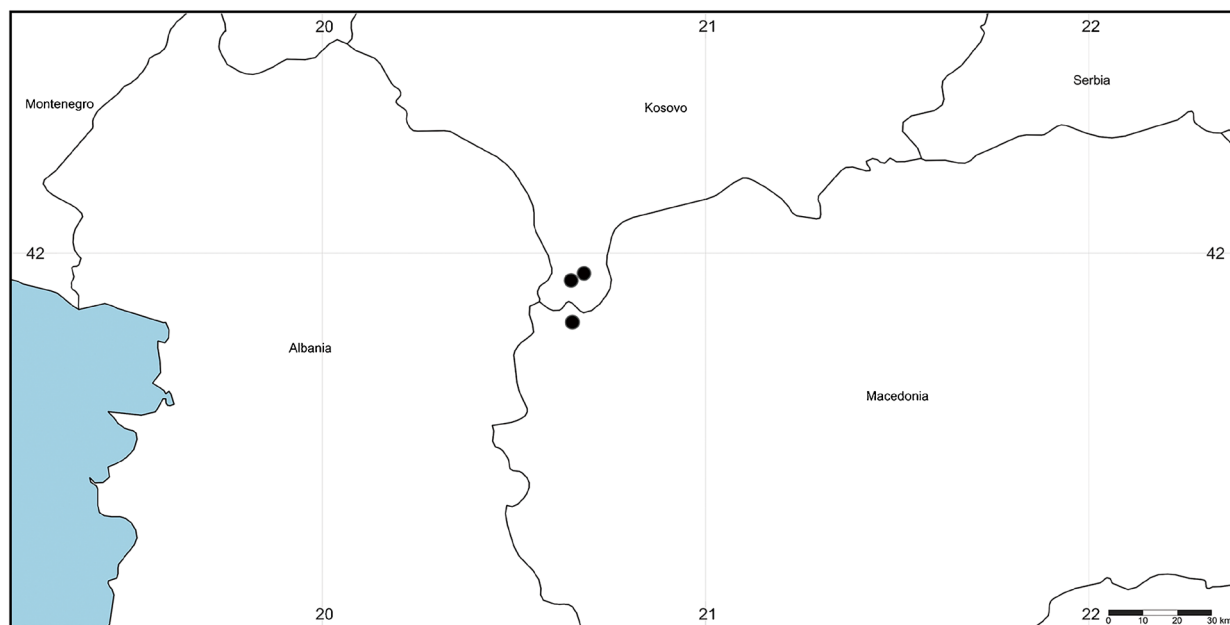
has been recorded from the Western Balkans, in the mountainous region of southern Kosovo (Hashani *et al.* 2019), close to Mount Korab in north-west Macedonia. The plants studied by Hashani *et al.* (2019) correspond to *G.* ‘Mt. Korab’ in their morphology, distribution and ecology and undoubtedly represent the same species.

Further investigation of the original *Galanthus* ‘Mt. Korab’ garden material using plastid (*trnL-trnF*, *matK*) and nuclear ribosomal (ITS) DNA markers, showed a relationship with *G. nivalis* and the Nivalis clade (fide Rønsted *et al.* 2013), with no evidence of hybridisation. Measurements of its genome size (1C-values) showed no overlap with either *G. nivalis* or *G. graecus*, or indeed any other *Galanthus* species whose genome size has been estimated to date. Based on its distinct morphology and difference in genome size, together with the DNA sequence data, we hereby describe a new species of *Galanthus*: *G. subalpinus*.

## Materials and Methods

### Morphology and distribution

Measurements, colours and other details are based on cultivated living material of *Galanthus* ‘Mt. Korab’ and plants observed in the wild (in North Macedonia). Morphological examinations in the laboratory were made using a Leica MZ95 stereo-microscope. Morphological terminology follows Beentje (2010). Distribution maps were plotted using coordinates recorded in the field and from the literature (Hashani *et al.* 2019). The distribution map (Map 1) was produced using SimpleMapp (Shorthouse 2010). A preliminary conservation status



**Map 1.** Distribution of *Galanthus subalpinus* based on collection and observed localities.

assessment of *Galanthus subalpinus* was informally determined following the IUCN's Red List Categories and Criteria (IUCN 2024); extent of occurrence (EOO) and area of occupancy (AOO) were calculated using ground point data and ShinyGeoCAT (Moat *et al.* 2023) based on the recommended grid size of  $2 \times 2$  km. Herbarium acronyms follow Thiers (2024, continuously updated).

### Genome size analysis

Genome size was estimated by flow cytometry using the 'one-step' method outlined in Pellicer *et al.* (2021). Approximately  $1\text{ cm}^2$  of leaf tissue of the *Galanthus* specimen was co-chopped with a similar sized piece of leaf from the internal calibration standard *Allium cepa* 'Ailsa Craig' (assumed genome size of  $34.89\text{ pg}/2\text{C}$ ; Clark *et al.* 2016) in 2 ml of General Purpose Buffer (GPB) supplemented with 3% PVP and 0.08% (v/v) beta-mercaptoethanol (Loureiro *et al.* 2007). The sample was then passed through a  $30\text{ }\mu\text{m}$  nylon mesh, stained with propidium iodide ( $1\text{ mg}/\text{ml}$ ; Sigma) to give a final concentration of  $50\text{ }\mu\text{g}/\text{ml}$ , and finally analysed on a Partec CyFlow Space (Sysmex GmbH, Munster, Germany) flow cytometer fitted with a  $100\text{ mW}$  green solid state laser (Cobalt Samba, Sona, Sweden). The resulting histograms were evaluated using the FlowMax software (v.2.7; Partec GmbH) to determine the mean relative fluorescence of the 2C-peaks (i.e.  $G_0/G_1$  stage of the cell cycle) of the *Galanthus* and *A. cepa* to convert these values into an absolute DNA amount in pg ( $[\text{mean relative fluorescence of the 2C-peak for } \textit{Galanthus} / \text{mean relative fluorescence of the 2C-peak for } \textit{A. cepa}] / 2\text{C-value (pg) of } \textit{A. cepa}$ ). The 2C-value (pg) was divided by

2 and multiplied by 0.978 to convert the genome size estimate into a 1C-value expressed in giga base pairs (Gbp). The conversion factor of  $1\text{ pg} = 0.978\text{ Gbp}$  is taken from Doležel *et al.* 2003.

### DNA sampling, extraction, sequencing, and data analysis

To infer the genetic identity of the unknown *Galanthus* sp. we sequenced the plastid regions *trnL-trnF* (*trnL* intron and *trnL-trnF* intergenic spacer) and *matK*, and the nuclear Internal Transcribed Spacer (ITS1/ITS2). These markers have previously been successfully used to distinguish between other *Galanthus* species and identify recently formed hybrids via differential inheritance of plastid and nuclear genomes (i.e. Lledo *et al.* 2004; Rønsted *et al.* 2013).

DNA was extracted from dried leaves using a modified CTAB protocol (Doyle & Doyle 1987) and purified using a QIAquick purification kit (QIAGEN). DNA quality was checked on an agarose gel by electrophoresis. Polymerase chain reactions and Sanger sequencing and alignments were conducted as described in Rønsted *et al.* (2013). The *trnL-trnF*, *matK* and ITS sequences were matched against sequences deposited in the NCBI hosted GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) repository (Sayers *et al.* 2022) using BLAST (Basic Local Alignment Search Tool) to find similar nucleotide sequences. Most of the sequences found in GenBank were produced in three studies: Lledo *et al.* (2004), Rønsted *et al.* (2013) and Margoz *et al.* (2013), each for multiple *Galanthus* species. The Genbank numbers for *McEnery* s.n. are: *matK* PQ002606; *trnL-trnF* PQ002607; ITS PP988692.

## Taxonomic Treatment

***Galanthus subalpinus* A.P.Davis & I.McEnery sp. nov.** Type: U.K., cultivated material growing in Sutton Coldfield, West Midlands, originally collected from Mt Korab (North Macedonia), c. 1900 m, fl., 25 March 2024, McEnery s.n. (holotype K!).

<http://www.ipni.org/urn:lsid:ipni.org:names:77352294-1>

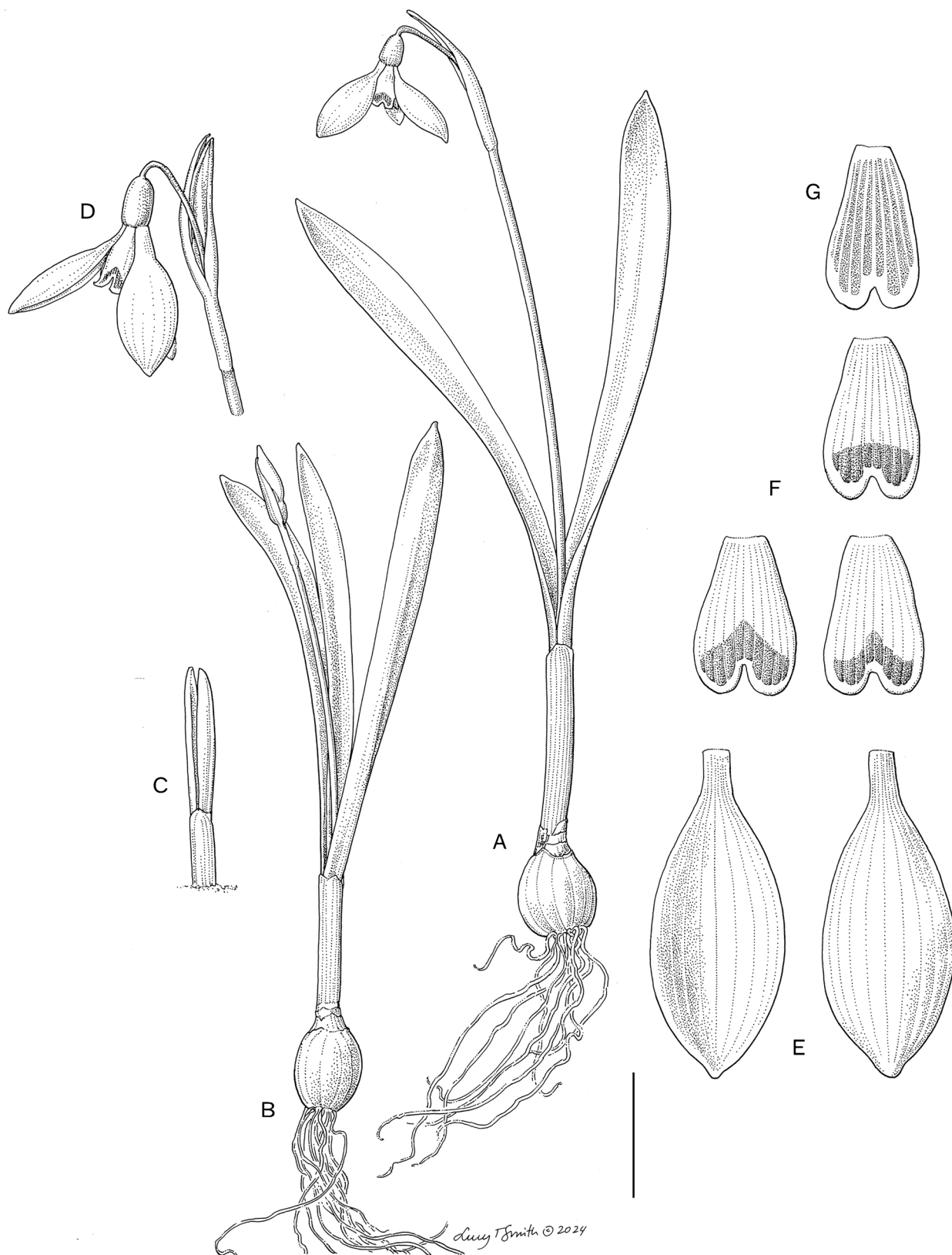
*Bulb*, ovoid to narrowly ovoid, 3.2 – 4.7 × 1.4 – 2.1 cm, scales whitish, fully or partially covered with a light brown papery tunic; adventitious roots whitish. *Basal sheath* ± tubular, tubular-conical, circular or oval in cross section, 3 – 6 × 0.5 – 0.7 cm, whitish to greenish. *Leaves* synanthous, applanate or subrevolute in vernalization; leaf blades linear (strap-shaped) to narrowly oblanceolate, indistinctly canaliculate, at flowering time 9.8 – 13.5 × 0.95 – 1.4 cm, slightly lengthening after flowering, abaxial surfaces with a prominent keel, dull matt green to bluish grey-green (semi-glaucous) on both surfaces; margin subrevolute to flat; apices obtuse, flat, with a small white point at tip. *Scape* 8.2 – 13.7 (– 14) cm long, 1.6 – 2.8 mm in diam., light green, dark green in upper part, slightly glaucous. *Pedice*l 2.2 – 3.9 cm long, 1 – 2 mm in diam., light green. *Spathe* papery, slightly arcuate, with 2 thick ribs (up to 1.5 mm in diam.) on margins, 2.5 – 3.6 × 0.4 – 0.7 cm, ± equal in length to the pedicel at flowering, mid green. *Flowers* narrowly ovoid to pyriform when closed (i.e. in outline shape), fragrant; perianth segments 6, separated, in two whorls. *Outer perianth segments* 3, narrowly obovate to obovate, indistinctly ribbed, 1.9 – 2.8 × 0.8 – 1.3 cm, white, bases unguiculate, claws 2 – 4 × 1 – 3 mm; apex acute, slightly cucullate. *Inner perianth segments* 3, ± ½ the size of the outer perianth segments, broadly to narrowly obovoid to obtriangular, white, each segment with an apical sinus (notch) and a single Λ-shaped, green apical mark, the mark often narrower above the sinus and thicker at the ends, the green mark on the adaxial surface composed of a series of lines covering nearly the entire surface of the inner segment, and almost extending to the base of the segment; margins near sinus slightly curled upwards (flared). *Stamens* 6, in two whorls; anthers basifixed, ± sagittate, orange, connective c. 2.5 × 0.5 mm; anthers sacs 4 per anther, c. 5.5 mm long and c. 1.1 mm in diam., latrorse in the middle part and introrse to the apex; anther apex sharply acute with multiple villi; filaments c. 2 mm long, white. *Pollen* orange. *Gynoe*cium (ovary and receptacle) syncarpous, tricarpellate; ovary superior, globose or ellipsoid to narrowly ellipsoid, indistinctly triangular in cross section, bright green, 3 – 11 mm long, 3 – 7 mm in diam.; placentation axile, ovules 12 – 18 per locule. *Pistil* filiform, style 6 – 9.5 mm long and 0.3 mm in diam., greenish, with

numerous villi (c. 0.4 mm long) in the upper ½ – ⅓; stigma capitate, trilobed, c. 0.3 mm in diam. *Fruit* a capsule, at maturity indistinctly triangular in cross section, globose to ellipsoid, or narrowly ellipsoid, at maturity 0.8 – 1.5 (– 2.1) × 0.5 – 1.3 cm, bright to light green. *Seeds* ± orbicular to ovoid, c. 3.5 × 2.5 mm, with shiny light to dark brown reticulate seed coat and ± developed whitish elaiosome, 1 – 3 mm long. Figs 1 and 2.

**RECOGNITION.** *Galanthus subalpinus* has leaf characteristics like those of *G. graecus* and flowers like those of *G. nivalis*. The leaves are either applanate ('flat') in vernalization, i.e. when the leaves are enclosed in the sheath at bud stage they are held flat against one another, or one leaf may fold around the other (partially subrevolute) as the leaves start to emerge and during maturation. Generally, seedlings and young plants are applanate (Fig. 1C) and mature plants more likely to be partially subrevolute (Fig. 1A), particularly during and after flowering. It is possible to find both applanate and partially subrevolute plants in the same population. In *G. nivalis*, the leaves are always strictly applanate. The leaf surfaces of *G. subalpinus* are evenly dull grey-green (semi-glaucous) on both surfaces, or sometimes slightly more glaucous on the lower surface (Fig. 2). In *G. nivalis* the leaf surfaces are discolorous, green-glaucous on the lower (abaxial) surface and green with a light glaucous longitudinal, median stripe on the upper (adaxial) surface. The inner perianth segments of *G. subalpinus* have single apical markings (Figs. 1F, 2C) like *G. nivalis*; *G. graecus* has either two markings per inner perianth segment (one at the base and one at the apex), or a single, more or less X-shaped mark.

DNA sequences of *trnL-trnF*, *matK*, and ITS showed that *Galanthus subalpinus* is related to *G. nivalis* and species of the Nivalis clade (fide Rønsted *et al.* 2013) with no evidence of hybridisation with other *Galanthus* species.

The genome size of *Galanthus subalpinus* was estimated to be 32.74 Gbp/1C (SD 0.041), which is larger than *G. graecus* (27.6 Gbp/1C; Zonneveld *et al.* 2003, based on specimen Davis 353) and smaller than *G. nivalis* (35.31 Gbp/1C; mean value of 19 samples, Zonneveld *et al.* 2003). The only species' that have comparatively similar genome sizes are *G. alpinus* Sosnowsky, *G. cilicicus* Baker, and *G. ikariae* Baker, which are morphologically unlike *G. subalpinus* and occur in the Caucasus, southern Turkey and the Aegean, respectively. We also measured three species that lacked reports for genome size, but none of these was comparable to *G. subalpinus*, i.e., *G. samothracicus* (28.13 Gbp/1C; SD 0.024), *G. bursanus* Zubov, Konca & A.P.Davis (26.38 Gbp/1C; SD 0.008), and *G. × valentinei* nothosubsp. *subplicatus* (25.37 Gbp/1C; SD 0.012).



**Fig. 1.** *Galanthus subalpinus*. **A** habit, flowering; **B** habit, flower bud stage; **C** young emerging leaves, showing applanate vernation; **D** flower; **E** outer perianth segment, abaxial view (right) and adaxial view (left); **F** inner perianth segments, with alternative marking types (below), all in abaxial view; **G** inner perianth segment, adaxial view. Scale bar: **A** – **C**=4 cm; **D**=2.5 cm; **E** – **G**=1 cm. All from McEneaney S.N. DRAWN BY LUCY T. SMITH.



**Fig. 2.** *Galanthus subalpinus* in situ and in cultivation. **A** habitat, subalpine grassland; **B** small clump, situated in an ephemeral water pool; **C** flowers; **D** leaves at early fruiting stage. **A – B** North Macedonia; **C – D** UK, in cultivation. PHOTOS: I. MCENERY.

**DISTRIBUTION.** Europe: North Macedonia, Mount Korab; Kosovo, Dragash-Restelica mountain range and probably NE Albania. Map 1.

**HABITAT.** Hilly, subalpine grasslands, with seasonally wet soils (from snowmelt); elevation 1500 – 1900 m; mainly on salicaceous substrates.

**CONSERVATION STATUS.** *Galanthus subalpinus* is provisionally assessed as Critically Endangered [CR B1ab(iii)+2ab(iii)] using the IUCN Red List Categories and Criteria (IUCN 2024). EOO was estimated to be 17 km<sup>2</sup> and AOO to be 8 km<sup>2</sup>. *Galanthus subalpinus* is known from only three populations, based on three geolocated data points (Map 1). In Kosovo, the subpopulations are reported to contain a small number of individual (50 – 100 plants), with threats including overgrazing, landslides, fires, and unauthorised plant

collecting for the horticultural trade (Hashani *et al.* 2019). Further field work in North Macedonia, Kosovo and NE Albania is required before an authoritative and formal conservation assessment can be made.

**ADDITIONAL SPECIMENS EXAMINED.** UK. Cultivated material growing in Sutton Coldfield, West Midlands, originally collected from Mt Korab (North Macedonia), fl., 24 March 2024, McEnery s.n. (holotype K!).

**PHENOLOGY.** Flowering late March – May in the wild and in cultivation in the UK. Fruiting, wild and cultivated in the UK, May – June.

**ETYMOLOGY.** *Galanthus subalpinus* is named after the subalpine zone (1500 – 1800 m) where the species is found in the wild.

**NOTES.** With the inclusion of *Galanthus subalpinus*, the genus *Galanthus* now comprises 25 species.

### Key to the identification of *Galanthus subalpinus*, *G. nivalis* and *G. graecus*

1. Flowers: each inner perianth segment with two distinct green marks, one at the apex and one at the base, or one large, more or less X-shaped green mark ..... **G. graecus**
1. Flowers: each inner perianth segment with a single, green apical mark ..... **2.**
2. Leaves held flat together at the interface with the soil, or one leaf partially curved around the other; leaf blades evenly grey-green (semi-glaucous) on both surfaces, or slightly more glaucous on the abaxial (lower) surface ..... **G. subalpinus**
2. Leaves held flat together at the interface with the soil; leaf blades green-glaucous on the abaxial (lower) surface, green with a light glaucous, longitudinal, median central stripe on the adaxial (upper) surface ..... **G. nivalis**

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### Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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