

## ***Leucojum vernum* IN ÎNTORSURII MOUNTAINS (SOUTH-EASTERN CARPATHIANS)**

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**Abstract:** *This study investigates the population structure and ecology of the species *Leucojum vernum* (spring snowflake) in Întorsurii Mountains, South-Eastern Carpathians, where was supposed to be extinct. The research objectives were: (1) to identify the species in Popii Valley, (2) to estimate its population size, (3) to map the species extent, (4) to delineate the species ecological niche, (5) to identify and describe the vegetation types where the species grows, (6) to identify the co-occurring species as indicators of suitable habitat for *L. vernum*, and (7) to assess the species conservation status in Popii Valley and in Romania. Identification and description of the species population relied on an intensive survey in the whole watershed of Popii Valley. A detailed vegetation map of the Popii Valley watershed was created. For the description of the species habitat, 27 sampling plots were used. The species ecological preferences were drawn by means of the indicator species analysis, climate and geological maps. Climate suitability was checked by projecting the analyzed population in the bioclimatic envelope of *L. vernum* in Romania, using principal components analysis (PCA) ordination and classes of aridity index. The assessment of the conservation status followed the principles and methodological standards for monitoring protected species at the European Union and global level. In the studied area *L. vernum* was found in four forest associations, on moist and nutrient-rich soils. The indicator species of the suitable / unsuitable habitats were identified with the help of the phi coefficient of association between species. The population size and species habitat are in favorable conditions, although signs of bulbs collection for gardening were observed. These findings were discussed in a national and global context. An updated map for species extent in Romania was provided in order to support the assessment of the global and national IUCN category.*

**Key words:** *Leucojum vernum, chorology, indicator species, IUCN criteria, vegetation mapping.*

### **1. Introduction**

*Leucojum vernum* (spring snowflake) is an herb species from the Amaryllidaceae

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family, monocots, endemic to Europe. It has ovoid bulbs, 3-5 linear leaves of 15-35 cm, a solitary unbranched stem, 1-2(3) flowers with six tepals 15-22 mm long of white color except the tip which has a yellowish-green spot, and obovoid fruit; it blooms in March-April [30]. It spreads in the temperate climate of Central Europe and the Carpathians area, avoiding the continental zones of Pannonian, Pontic or Sarmatic regions. In Romania, the species reaches the easternmost border of its area [1, 26]. Its closest relative is *Leucojum aestivum*, which can be distinguished by its smaller (9-17 mm) and more numerous (3-5) flowers and by their occurrence in late spring to early summer (May-June). In Romania, this species grows in the wild only outside the Carpathian arch, in lowland or alluvial forests whereas *L. vernum* also occurs in Transylvania up to mountain beech or spruce forests [28, 30].

Although the species is not listed in the Romanian Red List of vascular plant species [18], it may represent local concerns for conservation due to bulbs removal [2, 7, 21, 25]. It is included in the Red List of Carpathians as near threatened (NT), being vulnerable (VU) in Ukraine, and endangered (EN) in Serbia [22]. In the global Red List [2], the species was assessed as least concern (LC).

The presence of the species in Întorsurii Mountains is documented only by old herbarium material - the years 1972 and 1976 (Herbarium of the Faculty of Silviculture in Brasov, vouchers no. 26187 and 55999). Further observations lead to the conclusion that it has disappeared from the known site - Popii Valley near Budila village, Brasov county, the "Coasta potecii" site (professor M. Danciu, personal communication). Therefore, the aim of the study was to investigate the

population of spring snowflake in Popii Valley, following the objectives: (1) to identify the species in Popii Valley, (2) to estimate its population size, (3) to map the species extent, (4) to delineate the species ecological niche, (5) to identify and describe the vegetation types where the species grows, (6) to identify the indicator species of suitable habitat for *Leucojum vernum*, and (7) to assess the species conservation status in Popii Valley and Romania.

## 2. Materials and Methods

### 2.1. Study Area

The survey was conducted in the watershed of Popii Valley (2274 ha), near Budila village of Brasov county, belonging to Întorsurii Buzăului Mountains. In the study area the altitudes vary between 550 and 1,190 m, the geological substrate is made of flysch, conglomerates, sandstones, loessoid deposits, and rarely limestone [27]. The climate belongs to the Dfb type, which means temperate continental with warm summers and no dry season [1]. The average temperature ranges from 5.1 to 7.4°C and the average annual precipitations range from 575 to 625 mm. The coldest month is January, with -4.1°C at lower altitudes (due to thermic inversions), -3.6°C at middle altitudes, and -4.7°C at higher altitudes [10].

### 2.2. Inventory

Field investigation was done in February - May 2023, following a stratified preferential sampling. The criteria for stratification were dependent on the available maps (forest management plans, geology, digital elevation model) and

aimed to cover as much of the ecological gradients of the studied area as possible. They were: dominant species (xeric, mesic or meso-higric woody species - alder, beech, hornbeam, oak, silver fir, willows; grasslands), altitude (above / below 800 m), relief (sunny slopes / shady slopes / valleys / ridges), geology (flysch / sandstone / conglomerates / limestone or marl). *Leucojum vernum* occurrences were recorded along several transects covering all the forest units and grasslands from the Popii Valley catchment area. Complete inventories of plant communities were done on 27 sampling plots (phytosociological relevés) of 400 m<sup>2</sup>, of which 11 had spring snowflakes and 16 did not. The sampling aimed to cover all vegetation types observed in the area with at least three plots; exceptionally there was only one plot of shrubby vegetation having an area too small to place inside three non-overlapping plots. The plots without *Leucojum* were required for the identification of unsuitable sites or unfavorable plant assemblages. All species abundances were estimated on the Braun-Blanquet scale (+, 1, 2, 3, 4, 5) [5]. In 11 plots, the flowering or fruiting stems of *L. vernum* were counted in March-April when the full development of the snowflake was attained. We did not conduct detailed investigations in all species locations due to the relatively short-time persistence of the flowering individuals; besides, we wanted to reduce habitat degradation by trampling and to avoid marking the site for potential human "predators". Evidence of threats was recorded along all transects.

Information about species chorology was extracted from the literature [7, 28, 30], from the phytosociological database of Romanian woody vegetation [19], from

the herbarium of the Faculty of Silviculture in Braşov (RO-BVS code in *Index Herbariorum*), and from web databases ([www.iNaturalist.org](http://www.iNaturalist.org), [www.gbif.org](http://www.gbif.org) [17]).

The ecological niche of *Leucojum vernum* was outlined based on indicator species, geological and climate maps [1, 10, 27]. The ecological indicator values for every species recorded in the sampling plots were extracted from Romanian Flora [28] and simple average values were computed at plot level. The indicator values are available for six ecological factors - light (L), temperature (T), continentality (K), soil humidity (U), soil reaction (R) and nutrients (N), ranging from 1 (lowest level of the factor) to 9 (highest level of the factor). These values were adapted and rescaled for Romania's flora based on Ellenberg's methodology [9].

Mapping of species populations and vegetation types was performed in QGIS v.3.16 ([qgis.org/en/site](http://qgis.org/en/site) [13]). To differentiate between sunny and shady slopes, the potential incoming solar radiation was computed in SAGA GIS v.8.1 ([saga-gis.org](http://saga-gis.org) [14]) based on the digital elevation model ([srtm.csi.cgiar.org](http://srtm.csi.cgiar.org) [15]).

The vegetation map for Popii Valley was created based on correlations observed between field observations, geology [27], broad ecosystem types, and topography.

To compare the climate in Întorsurii Mts. with that of other sites, an ordination of all known occurrences of *Leucojum vernum* in Romania was created considering the bioclimatic variables [10] as independent variables. The ordination, as a method of showing multivariate similarities between sites, followed the principal components analysis (PCA) approach [24]. Of the 19 available

bioclimatic variables, only those were kept which in the study's context were not highly correlated ( $r < 0.95$ ) with others. The variables used were: Bio\_01 = annual mean temperature, Bio\_02 = mean diurnal range of temperature, Bio\_03 = isothermality, Bio\_06 = min temperature of the coldest month, Bio\_07 = temperature annual range, Bio\_12 = annual precipitation, Bio\_13 = precipitation of wettest month, Bio\_15 = precipitation seasonality, Bio\_19 = precipitation of coldest quarter. The future climate aridity was derived from CHLSA data for the SSP3-7.0 scenario of the CMIP6 models [23].

Tabular analysis of vegetation data, computation of average indicator values for plots and identification of co-occurring

species based on the phi coefficient of association [3] were performed in Juice software [29].

The box-plot graphs and PCA ordination were created in PAST v.4.16 [12].

The species names followed the Romanian Flora [28]. Associations names are in accordance with [4].

### 3. Results and Discussion

#### 3.1. Results

*Leucojum vernum* was observed in seven places in the watershed of Popii Valley (Figure 1). The Romanian names for these places are Coasta Potecii, Pârâul Ursului, Seci, Cărbunari, Pârâul Boului, Pârâul Căldării, and Valea Popii.

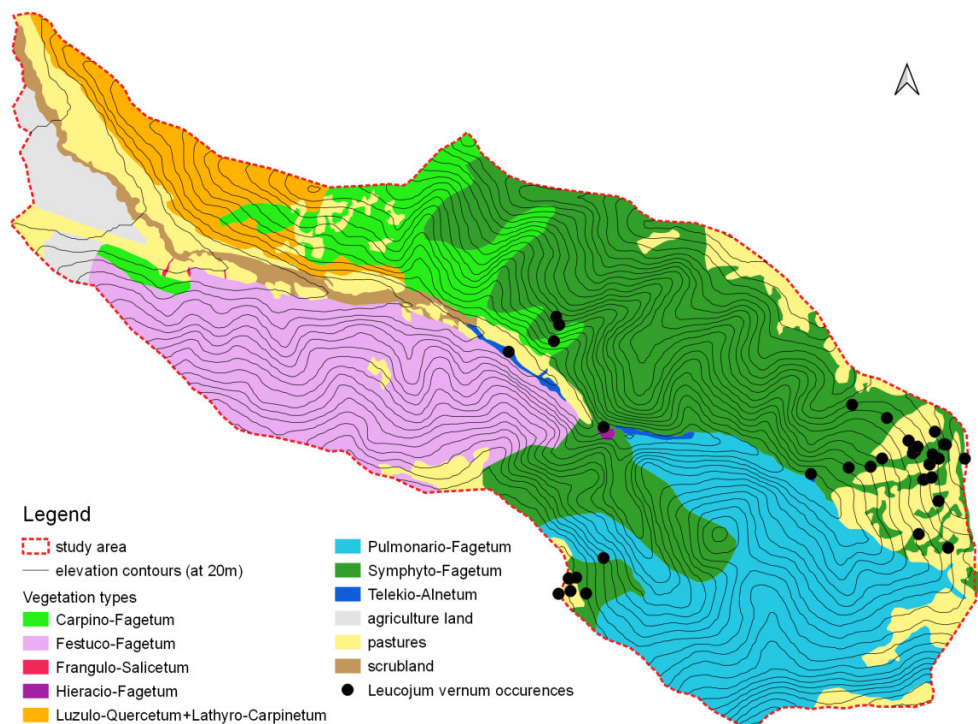


Fig. 1. Forest associations in the watershed of Popii Valley near Budila (BV) and *Leucojum vernum* occurrences, mapped based on field inventories, topography, geology, and satellite images (Google)

The maximum distance between the occurrence locations is more than 4 km and the convex hull for these occurrence has 617 hectares. In accordance with the IUCN mapping standards [20], the population in Popii Valley covers five squares of 2x2 km, meaning plus 20 km<sup>2</sup> to the species' area of occupancy (AOO).

In the whole watershed of Popii Valley, nine woody associations were identified and mapped (Figure 1): (1) acidic dry-mesic oak forests - *Luzulo luzuloidis-Quercetum petraeae*, (2) mesic oak-hornbeam forests - *Lathyro hallersteinii-Carpinetum*, (3) beech-hornbeam forests - *Carpino-Fagetum*, (4) oligotrophic beech forests - *Hieracio rotundati-Fagetum*, (5) mesotrophic beech forests - *Festuco drymeae-Fagetum*, (6) eutrophic beech forests - *Symphyto cordati-Fagetum*, (7) eutrophic beech-fir forests - *Pulmonario rubrae-Fagetum*, (8) alder forests - *Telekio speciosae-Alnetum incanae*, (9) grey willow shrubs - *Frangulo-Salicetum cinereae*.

The spring snowflake was present in four woody associations: (3) hilly beech forests, (6) eutrophic mountain beech forests, (8) alder forests, (9) grey willow shrubs. In these plant communities the coverage of herb species is relatively high (40-90%) and the sites are defined by altitudes in the range 650-980 m, gentle slopes (2-15 degrees), calcareous conglomerates and flysch. Some individuals grew in the pastures, near the forest edge. Plant species highly associated with *Leucojum vernum* are eutrophic and mesohigrophilous: *Caltha palustris* (65), *Chrysosplenium alternifolium* (54), *Corydalis solida* (47),

*Allium ursinum* (41), *Dentaria bulbifera* (41). Negatively associated species are among those tolerating acidic and dry soils: *Festuca drymeja* (-54), *Quercus petraea* (-54), *Calamagrostis arundinacea* (-44), *Galium schultesii* (-44), and *Dryopteris filix-mas* (-40). These species could be used for future identification in the field of the suitable (positive values) or unsuitable (negative values) habitat for *Leucojum vernum* in Întorsurii Mts. or neighboring mountains. Complete species composition is given in Table 1.

As shown in Figure 2, the spring snowflake grows on moister and nutrient richer soils, shady sites with a mesothermic and less continental climate. Most often, it occurs near temporary springs, rivulets or shallow terrain where the soil is more fertile and richer in nitrogen. Although such conditions are present in the main valley, the species is almost missing here most probably due to intensive harvesting (personal communication from local villagers and foresters).

The overall number of *Leucojum vernum* individuals in the sampled plots was 580, as shown in Table 2. The average number of individuals on a plot was 52 (ranging from 10 to 187). The extrapolation of this value to the 30 identified locations led to an estimated population size of *Leucojum vernum* in Întorsurii Mts. of 1,560 individuals. The real population size is probably much higher due to the inherent bias of the survey, and also because vernal species like those of *Leucojum* genus may have many dormant individuals not bursting every year [8].

Table 1

*Synthetic species composition of forest vegetation with Leucojum vernal in Popii Valley*

Frequency class	Species
81-100%	<i>Anemone nemorosa, Fagus sylvatica, Leucojum vernal</i>
61-80%	<i>Allium ursinum, Anemone ranunculoides, Dentaria bulbifera, D. glandulosa, Euphorbia amygdaloides, Pulmonaria officinalis</i>
41-60%	<i>Acer pseudoplatanus, Aegopodium podagraria, Caltha palustris, Carex pilosa, C. sylvatica, Chrysosplenium alternifolium, Corydalis solida, Fragaria vesca, Gagea lutea, Mercurialis perennis, Picea abies, Rubus hirtus</i>
21-40%	<i>Adoxa moschatellina, Ajuga reptans, Carpinus betulus, Corylus avellana, Crataegus monogyna, Crocus banaticus, Daphne mezereum, Festuca drymeja, Galium aparine, G. odoratum, Geranium robertianum, Geum urbanum, Glechoma hederacea, Isopyrum thalictroides, Lamium galeobdolon, Lathyrus vernus, Luzula luzuloides, Pulmonaria rubra, Ranunculus carpaticus, R. ficaria, Sambucus nigra, Stellaria nemorum, Symphytum cordatum, Urtica dioica, Viola reichenbachiana</i>
1-20%	<i>Abies alba, Acer platanoides, Alliaria petiolata, Alnus glutinosa, A. incana, Anthriscus sylvestris, Arum maculatum, Asarum europaeum, Brachypodium sylvaticum, Cardamine amara, Carex digitata, C. flacca, C. pendula, C. riparia, C. spicata, Circaea lutetiana, Cirsium oleraceum, C. palustre, Clinopodium vulgare, Cornus sanguinea, Corydalis cava, Deschampsia cespitosa, Dryopteris carthusiana, Equisetum hyemale, Erythronium dens-canis, Euonymus europaeus, Fraxinus excelsior, Galanthus nivalis, Glyceria notata, Helleborus purpurascens, Hordelymus europaeus, Hypericum maculatum, Juncus effusus, Lamium maculatum, Lathraea squamaria, Lysimachia nummularia, Miliium effusum, Myosotis sylvatica, Oxalis acetosella, Paris quadrifolia, Petasites albus, P. hybridus, Plagiomnium undulatum, Platanthera bifolia, Polygonatum multiflorum, Polystichum aculeatum, Populus tremula, Ranunculus auricomus, R. repens, Rosa canina, Rubus idaeus, Salix caprea, Salvia glutinosa, Stachys alpina, S. sylvatica, Symphytum tuberosum, Ulmus glabra, Veronica chamaedrys, V. montana, V. officinalis, Viburnum opulus, Viola odorata, Waldsteinia ternata</i>

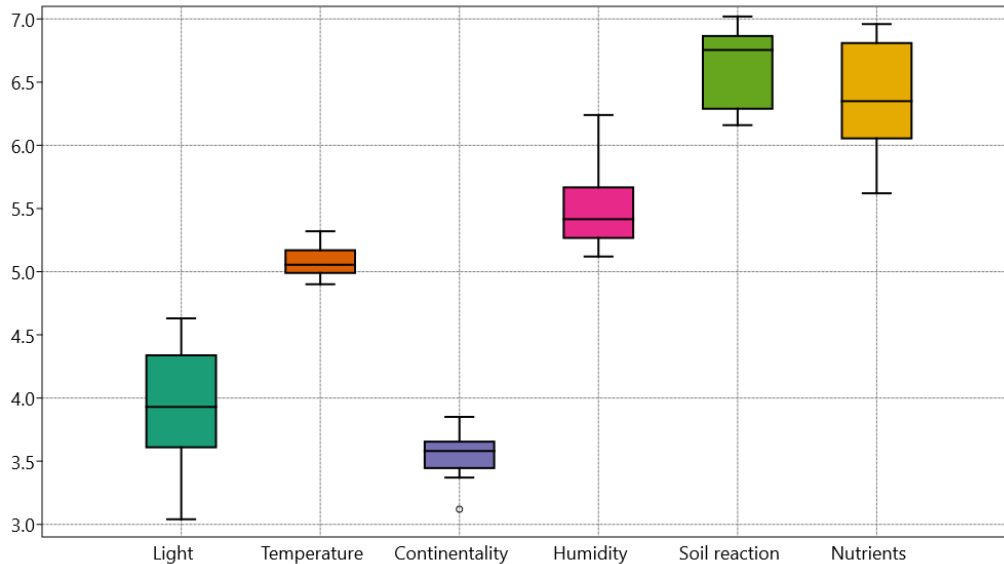


Fig. 2. Variation of indicator values for the main ecological factors of plant communities with *Leucojum vernum* in Popii Valley. Indicator values were given on the Ellenberg scale (1=minimum, ..., 9=maximum). The box-plot represents: the median (the line inside the box), 50% of the values (the box), 99% confidence interval (whiskers), and the outliers (dots)

Since a minimum viable population size is not available for *Leucojum vernum*, our analysis used the more general rule of 100/1,000 individuals - 100 to avoid inbreeding depression and 1,000 to maintain evolutionary potential [11]. Considering these values, the population of *Leucojum vernum* in Întorsurii Mts. seems to be in good conditions, without major risks for population viability.

Besides, due to the species being located in seven sites and several vegetation types, the extinction risk is diminished [20]. Most of the flowers were fertile, setting viable fruits. Regarding threatening factors, only isolated removals of bulbs or flowers for gardening or selling purposes were observed. Signs of disease were not noticed.

Table 2

Population size on sampled plots from Popii Valley, Întorsurii Mountains

Plot ID	1	2	3	4	5	7	11	15	16	17	18	Total
Individuals	78	81	59	187	51	22	10	19	20	28	21	580

In Romania, *Leucojum vernum* has a wide range, stretching along 590 km from west to east and 630 km from north to south, at altitudes between 50 and 1370 m (Figure 3). It occurs in the geographic

units [19, 30]: the plains - Boianu, Burnas, Ialomița, Pitești, Tecuci; depressions - Brașov, Hațeg; hills - Bistrița, Bulza, Mureș; mountains - Almăj, Bihor, Codru-Moma, Gurghiu, Gutâi, Harghita, Igniș, Întorsurii

Buzăului, Maramureș, Meseș, Metaliferi, Nemira, Pădurea Craiului, Parâng, Perșani, Poiana Ruscă, Rodna, Șureanu, Țibleș, Trotuș, Vlădeasa, Zarand; piedmonts - Getic, Sucevei, Homoroadelor. According to the De Martonne aridity index (Figure 3), 10% of the locations belong to the semi-humid class, 26% to humid, 45% to very humid, and 19% to extremely humid. The sites from Întorsurii Mts. belong to the very humid class, and they did not

appear in the margin of the climatic envelope of *Leucojum vernum* in Romania as depicted by the PCA ordination (Figure 4). The separation of the sites inside the "very humid" class along the bioclimatic variable of precipitation seasonality (B.15) is well correlated with the geographical west-east gradient - Întorsurii Mts. and other locations from the Eastern Carpathians have an increased precipitation seasonality.

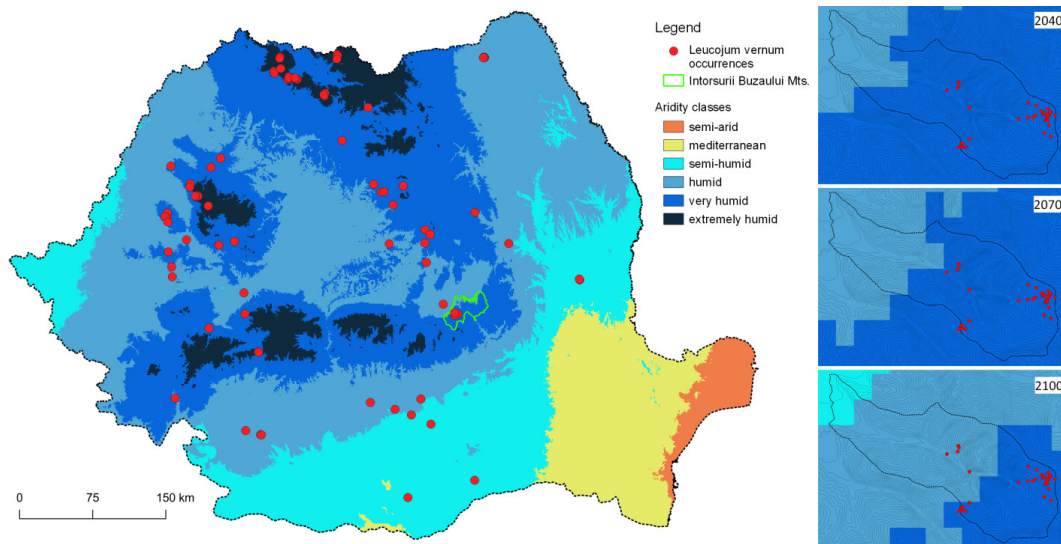


Fig. 3. Records of *Leucojum vernum* in Romania. In the background are the aridity classes according to the De Martonne index. On the right side are details for the Popii Valley watershed with the aridity classes predicted for 2040, 2070, and 2100 in accordance with CHELSA data [23]

Some sites outside the Carpathian arch, reported only in the literature without herbarium material [19], could represent misidentifications of the species with *L. aestivum*, if one considers the surveying period (July to September) too late for the recognition of *Leucojum vernum*.

According to the reported locations, the area of occupancy (AOO) measured in the IUCN's standard 2x2 km grid is 588 km<sup>2</sup>. This value is inside the thresholds of vulnerable category (500-2000 km<sup>2</sup>).

However, the requested additional criteria related to habitat fragmentation, continuing decline or extreme fluctuations are not supported by available data. The extent of occurrence is 52330 km<sup>2</sup> which is outside the thresholds of any threatening category. Besides, our own observations along the Carpathians (for instance in Parâng and Șureanu Mts.) indicate population sizes of thousands of individuals. Therefore, there are no reasons to consider this species



threatened in terms of occupied area, population size, and vitality.

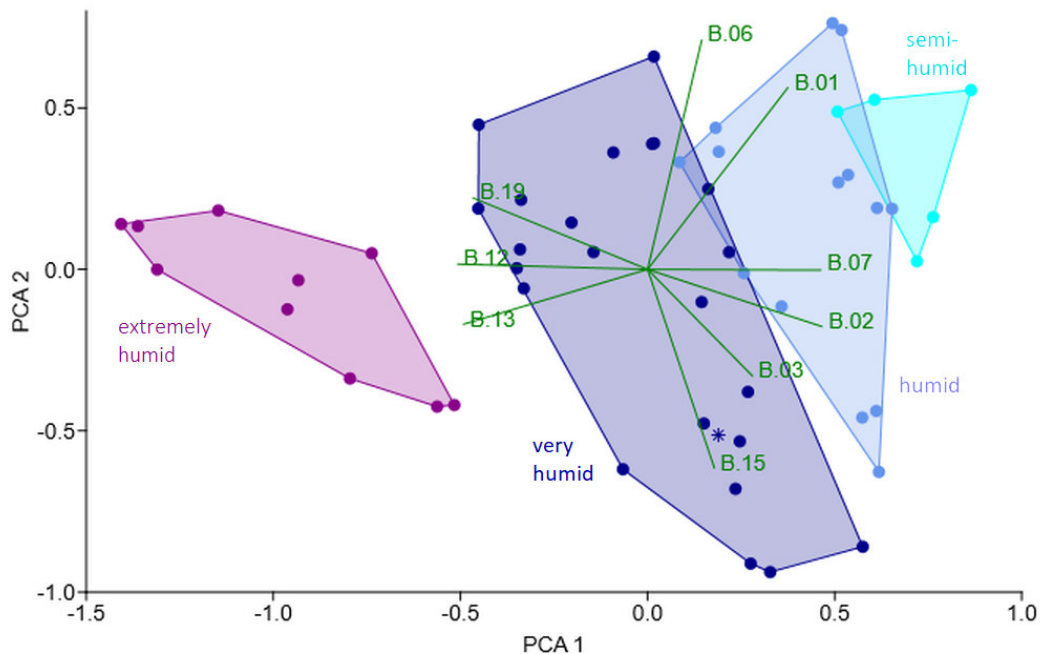


Fig. 4. *Principal components analysis (PCA) ordination of the sites with *Leucojum vernum* in Romania. The independent variables were: B.01 = annual mean temperature, B.02 = mean diurnal range of temperature, B.03 = isothermality, B.06 = min temperature of the coldest month, B.07 = temperature annual range, B.12 = annual precipitation, B.13 = precipitation of wettest month, B.15 = precipitation seasonality, B.19 = precipitation of coldest quarter. The sites are grouped according to the De Martonne aridity classes, as in Figure 3. The site of Întorsurii Mts. is marked with a star (\*) inside the very humid class. The variability explained by the first two components is 89% (58.2% + 30.8%)*

### 3.2. Discussion

The population of *Leucojum vernum* from Popii Valley is the only one known in Întorsurii Buzăului Mountains and the second in Braşov county. The closest wild populations are those from Prejmer Forest (at 25 km) and Racoş Valley in Perşani Mts. (at 85 km). In Romania, the species grows in different vegetation types, from lowland ash-oak forests to mountain fir-beech forests [19]; in part, such diversity is due to its development in early spring

when competition for light is weaker; however, the requirements for soil conditions are narrower. In the studied area, it was found in two associations that were not reported previously as a habitat of the species in Romania, namely *Telekio speciosae-Alnetum incanae* and *Frangulo-Salicetum cinerae*.

Based on the observed population size, on its occurrence in more than one site, and on testimonials of local villagers, we can assume the species wasn't extinct in Popii Valley. The extinction hypothesis

could be derived from incomplete surveys, a mismatch with species flowering time, a condensed or inhibited development of the above ground shoots.

Besides the observed impact due to bulbs removal, in Popii Valley other potential (although unlikely) long-term threats could be: (1) habitat reduction due to the land use change in some of the forest patches hosting *Leucojum vernum* that are not in the forest fund and thus could be subjected to pasture recovery; (2) changes in habitat suitability due to severe droughts and drying up of springs. A potential erosion of genetic diversity due to geographic isolation is less plausible since there are cultivated plants in a range of 4-5 km. To prevent these threats, species monitoring could be a feasible solution. If necessary, bulbs could be transplanted from other wild populations or gardens. The vegetative regeneration of this species in the surrounding climate is very easy, as demonstrated by the presence of the species in the gardens of many villages and cities in the whole of Romania (including drier areas or areas outside wild population occurrences). Neither the current nor the future climate will make the habitat unsuitable for the species in Întorsurii Mts. (Figures 3 and 4). The species is less dependent on zonal climate, but rather more on soil conditions or micro-climate. Variation in soil moisture is greater than observed on the climate maps [10, 23]; not even the vegetation map (Figure 1) or digital elevation models can reflect all soil moisture variability. The combination of micro-topography and vegetation cover offers many suitable niches that could compensate for climate aridity fluctuations. So far, there is no evidence that the population of *Leucojum*

*vernum* in the studied area or in 90% of the other locations in the country is directly or seriously susceptible to climate change.

A severe impact on the species due to trees felling is less likely, since the species also grows in young forests, forest edges, forest gaps, scrub-land. On the other hand, substituting beech with conifers may reduce habitat favorability; in all available inventories of the plant communities with spring snowflakes [19], the tree layer is dominated by broadleaved trees.

In the global assessment of *Leucojum vernum* [2] a large part (about 90%) of *Leucojum vernum* occurrences in Romania was neglected - Figure 5. The data in our study, together with that from [21], may represent the baseline for an updated evaluation. Since the actual assessment places the species in the category of least concern (LC), an increased area of occupancy, the extent of occurrence and population size due to the inclusion of Romanian data will lead to an equal or even more favorable status.

In Întorsurii Mts., *Leucojum vernum* is in favorable conservation status. It is not quite clear if the observed sub-populations represent remnant or recovered sites escaped from the intensive removal activities with commercial or gardening purposes existing in the past. Testimonials of local inhabitants indicate that many home gardens in the neighboring villages have spring snowflakes originated from the wild. For the remaining sites, the threat of harvesting is weaker because of their remoteness or poor accessibility. Of course, the threat should be carefully considered, but there is no efficient method to eradicate it. The area is not

inside a protected area and the species is not legally protected, but the increasing offers of ornamental plants on the market and the increasing awareness of younger generations towards environmental protection [25] may diminish this pressure. Since our study did not confirm

species extinction or severe reduction in the area, and due to the lack of previous measurements of species population, based on the recommendation of [6], it can be assumed that population size and area have a stable trend.

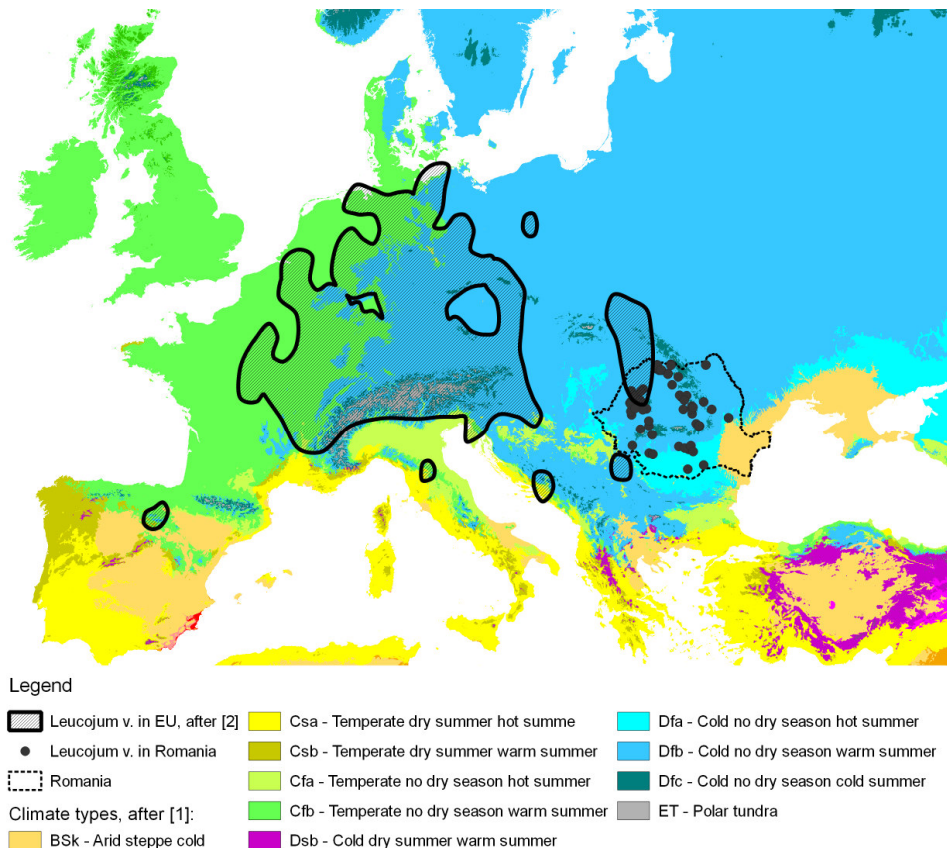


Fig. 5. Global area of *Leucojum vernum* according to the IUCN evaluation [2] and to data from the present study. In the background - climate types after [1]

#### 4. Conclusions

The presence of *Leucojum vernum* was confirmed in the watershed of Popii Valley, Întorsurii Mts. This population has significant value for the conservation of species in the south-eastern Carpathians. The status of this population is good in terms of occupied sites, population size

and vitality, quality of habitat, lower level of impact. The risk of extinction induced by major hazards is reduced due to the existence of six distant sub-populations (at 1-6 km apart) on various sides of the watershed, altitudes and vegetation types. The particular conditions of topography and soil, together with several highly associated species allow the identification

of potential sites in the neighboring areas. The species distribution in Romania was updated and some challenges for future assessments were identified.

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## Supplementary material



Fig. 6. *Leucojum vernum* on March 4, 2023 (left) and the shrubby habitat of the species (right) in the Popii Valley watershed



Fig. 7. The most common habitat for *Leucojum vernum* in Popii Valley - beech forests with patches of *Allium ursinum* and temporary springs