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THE SECRETS OF THE EDELWEISS (*LEONTOPODIUM* R. BR. EX CASS.): A REVIEW ON THIS SYMBOL, SCIENTIFIC OBJECT AND A NATURAL TREASURE

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Abstract. The edelweiss (*Leontopodium* R. Br. ex Cass), a symbol of endurance and beauty, captivates attention far beyond its delicate appearance. Found in the harsh alpine environments of Europe and Asia, this remarkable plant thrives where life faces its greatest challenges. Its cultural significance is rivaled only by its unique adaptations, making it a subject of fascination in both folklore and science. This literature review explores the multifaceted nature of the edelweiss, delving into its ecological role, biochemical properties, genetic diversity and potential applications. Why edelweiss? The answer lies in its rarity, its resilience in extreme conditions, and the unique phytochemicals it produces, which have demonstrated intriguing medicinal properties. By synthesizing existing research, this review seeks to provide a comprehensive understanding of the edelweiss, highlighting its ecological, cultural, and scientific importance. As research on alpine flora continues to expand, the edelweiss has emerged not only as a botanical emblem, but also as a source of untapped scientific potential.

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The plant name edelweiss comes from the German words "edel" (noble) and "weiss" (white), while the genus name *Leontopodium* derives from the Greek "leon" (lion) and "podion" (foot), referring to a lion's paw. Linnaeus initially classified the genus as *Gnaphalium* (from the Greek "gnafallon," meaning wool shavings) before Cassini renamed it in 1819 (Cassini 1819; Flann et al. 2010)

Morphology: The genus *Leontopodium* consists of perennial herbaceous plants typically covered with whitish hairs. The stems may be single or multiple, with alternating leaves. Basal leaves are on petioles, while stem leaves are sessile. The flowers, with a double perianth, are arranged in complex inflorescences at the top of the stem, surrounded by snow-white stem leaves. The funnel-shaped corolla consists of five fused petals, encircled by transparent, multi-branched filamentous structures – modified calyces retained as a pappus for the achenes. The flowers contain fused stamens and a pistil column with lobes, and linear stigmas. The fruits are smooth, fibrous, cylindrical-obovate seeds without endosperm. Pollination is entomophilous, with insects like flies (Muscidae family) attracted by the white stem leaves (Kuzmanov 2012; Metodiev 2021; Pavlova et al. 2023). The achene trichomes and the carpopodium exhibit taxonomic significance (Ma et al. 2022).

The Bulgarian edelweiss populations (*L. nivale* (Ten.) Hand.-Mazz) have a diverse rhizome, one or several erect stems, and oblong or linear leaves that are hairy on both sides. Its inflorescences consist of 5–12 globose capitula with white or yellowish tubular flowers. The outer flowers are female, while the inner ones are functionally male. The fruit seeds (achenes) are cylindrical or obovate, with numerous white hairs (pappus) (Kuzmanov 2012).

In Bulgaria, two subspecies are recognized: *L. nivale* subsp. *nivale* (syn. *L. alpinum* var. *pirinicum* Velen.), found in Pirin Mt (2170–2605 m a.s.l.), and *L. nivale* subsp. *alpinum* Greuter, found in the Balkan Mts (**Figure 1**). Subspecies *nivale* has shorter stems, spread trichomes, and white-woolly leaves, while subsp. *alpinum* has taller stems, closely adhering trichomes, and greenish upper surface of the leaves (Kuzmanov 2012; Kozuharova et al. 2018). It blooms from July to August and bears fruit from September to October (Bancheva 2015).

Ecomorphology: The hairs on the bracts, flowerheads, and with less density on the whole plant, serve to limit excessive water loss through transpiration, a vital adaptation for its survival in dry habitats, and protection against ultraviolet rays. Some scientists suggest that this defense occurs through absorption within

the protective fibrous layer and energy dissipation, aided by diffraction effects, which is possible because of the fibrous nature of the plant's filaments. The plant's fibrous surface functions as a photonic device, controlling light interactions similarly to

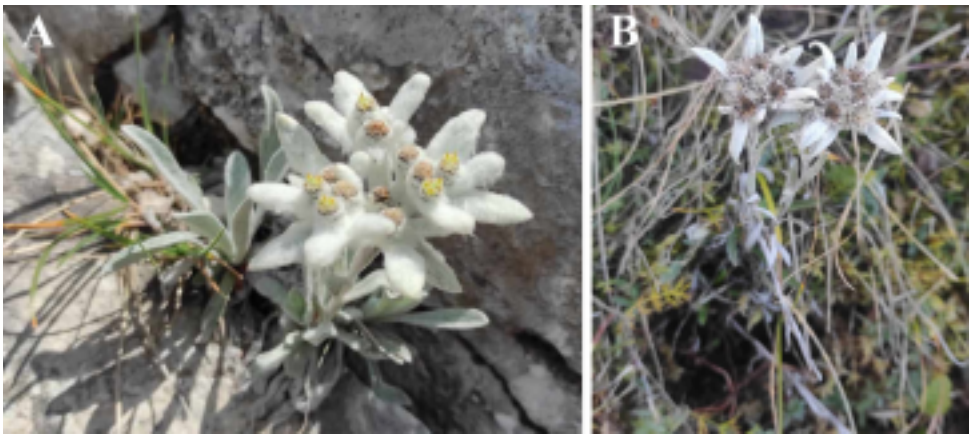

Fig. 1. Bulgarian species of edelweiss.**A** – *L. nivale* subsp. *nivale*, Pirin Mts., Kazanite area (August, flowering phase). **B** – L. *nivale* subsp. *alpinum*, Balkan Mts., Kozya Stena reserve (September, seeds, senescing). Source: A. A. Pozumentshtikov

engineered optical devices such as waveguides or diffraction gratings (Vigneron et al. 2005). These unique structure adaptations make the edelweiss highly resistant to harsh conditions, ensuring its survival in challenging mountainous environments.

Ecology, habitats and biogeography: The edelweiss comprises of endemic and stenobiont plants, thriving in high-altitude sunny rocky slopes, grassy steppe like regions, and alpine pastures at elevations between 1800–3400 m a.s.l. It predominantly grows on limestone and humus-rich soils (Ischer et al. 2014). Its distribution extends from Asia (Hindu Kush, Pamir-Altai, Tian Shan, Sino-Tibetan Mountains, Qinghai-Tibet Plateau) to Europe (Pirin, Balkan Mts., Carpathians, Alps, Prokletija). Edelweiss plants inhabit hard-to-reach areas like rock crevices and stony glades in coniferous, subalpine, and alpine belts. Populations are sparse, consisting mainly of individual plants or small tufts, characterized by low reproductive potential and limited migratory abilities (Bancheva 2015).

In Bulgaria, the edelweiss plants grow at 1700–2800 m altitude in the marble karst of northern Pirin Mt (including areas around Vihren Peak, Kazanite, Bayuvi Dupki-Djindzhiritsa Reserve, and Kamenititsa), and the limestone Balkan Range (it is found in locations such as the Kozyata Stena Reserve, Korudere, Triglav, Mazalat, and Zli Vruh). Protected under the Biological Diversity Act, edelweiss

habitats fall within the Pirin and Central Balkan National Parks, various reserves, and NATURA 2000 sites (Tzonev et al. 2009; Roussakova 2009). Major threats to the species include its habitat specificity, low abundance and density, fragmented distribution, weak regeneration, and destruction by tourists (Bancheva 2015).

Autecological studies conducted in the Swiss Alps indicate that moisture

index, summer temperature, slope, and topographic location are key factors for edelweiss habitats. The plant thrives in areas with low average temperatures, high altitudes, and steep-slopes, primarily on calcareous or limestone-rich rocks, while avoiding silicate rocks. It occupies grass-poor, open pasture communities, scree, and other growth-limiting environments, classifying it as a heliophyte and making it vulnerable to competition. Edelweiss plants prefer low-humidity environments with well-aerated soils and average summer temperatures below 10°C, consistent with its high-mountain distribution. Since they coexists with thermophilic species (*e.g.*, *Galium lucidum* All., *Astragalus monspessulanus* L. and *Phleum phleoides* (L.) H. Karst), this suggests that the current distribution may not fully represent the realized niche of the genus. Although it inhabits steep slopes, studies indicate that this factor is not of great importance and most likely this is also part of its niche, due to continuous tourist harvesting, but it may also be due to its preference for dry habitats with prolonged erosion, which can help reduce competition (Ischer et al. 2014).

In Bulgaria, *L. nivale* subsp. *nivale* is calciphilic, growing in neutral to slightly alkaline soils (pH ~7.5) with poor morphology and quality (Kozuharova et al. 2018). As a chasmophyte, it inhabits areas with 50–80% plant cover, often dominated by *Sesleria korabensis* (Kumm. & Jav.) Deyl and *Carex kitaibeliana* Degen ex Bech., along with other psychrophytic and cryophytic hekistothermal vegetation in the alpine treeless zone. It participates in unique plant communities in Pirin, such as *Leontopodio-Potentilletum stojanovii* (Mucina et al. 1990), and contributes to southern European alpine tundra alliances like *Leontopodio nivalis Elynion myosuroidis* (Chytrý et al. 2015).

Ex situ studies by Kozuharova et al. (2018) reveal that *L. nivale* subsp. *nivale* dies off after four years when transplanted to lower altitudes near the town of Dobrinishte. This highlights the incompatibility of wild edelweiss with garden cultivation and underscores the threats posed by rising temperatures and climate change, including the loss of snow cover, which is essential for maintaining humidity and providing protection. These findings emphasize the vulnerability of this species to environmental changes critical to its survival and development.

Symbiotic associations – rhizosphere and endosphere: The rhizosphere, in direct contact with plant roots, is enriched by rhizodeposition, which attracts and

shapes specific microbial communities. These communities are influenced by factors such as soil type, plant genotype, and root system architecture, all of which modify the rhizosphere environment (Hütsch et al. 2002; Bais et al. 2006; Minz et al. 2013; O□ek-Lalzar et al. 2014; Saleem et al. 2018). The endosphere, closely linked to the rhizosphere, houses microorganisms that are transmitted horizontally or vertically. This includes non-pathogenic endophytes that thrive in nutrient-rich, protected environments under strong selective pressures (Compant et al. 2010; Hardoim et al. 2015; Wang M. et al. 2016b). These environments

facilitate interactions via secondary metabolites and processes such as antibiosis and biofilm formation (Compant et al. 2010; Abisado et al. 2018). Actinobacteria, a type of Gram-positive bacteria, are widely recognized for their production of secondary metabolites, including antitumor, antiparasitic, insecticidal, antibacterial, antifungal, immunomodulatory agents, and herbicides. They are the primary source of most antibiotics in use today (Barka et al. 2016). Oberho□er et al. (2019) proposed that *L. nivale* subsp. *alpinum* might host novel actinobacteria. From rhizosphere soil, they isolated 77 actinobacterial strains, identifying genera such as *Actinokineospora*, *Kitasatospora*, *Asanoa*, *Microbacterium*, *Micromonospora*, *Micrococcus*, *Mycobacterium*, *Nocardia* and *Streptomyces* using the 16S rRNA molecular marker. Plant tissue analysis revealed diverse operational taxonomic units, with microbial diversity decreasing from roots to rhizomes, leaves, and inflorescences. The performed metagenomic study also highlighted a significant presence of unculturable actinobacteria, including taxa like Rubrobacteridae, Thermoleophilales, Acidimicrobiales, and unclassified taxons. These findings suggest the potential for isolating bioactive compounds for pharmaceutical applications (Oberho□er et al. 2019).

A strain of rhizobacterium, *Sphingomonas* sp. Cra20, was isolated from the roots of *L. leontopodioides* which grows at elevation 3800 m in Tianshan, China (Luo et al. 2020). This bacterium performs nitrogen fixation and produces siderophores. Studies on its effect on *Arabidopsis thaliana* demonstrated increased root fresh weight and lateral root development. Initially attributed to auxin synthesis, it was later discovered that the bacterium lacks auxin synthesis pathways; instead, the growth enhancement is due to volatile organic compounds it releases.

Pollination: Species differentiation within the genus *Leontopodium* is challenging due to apomixis, a form of asexual reproduction where seeds are produced without gamete fusion. This process complicates species identification as it limits genetic variation, making it more difficult to use genetic markers for classification. In *L. nivale* subsp. *alpinum*, a specific form of apomixis called diplospory has been identified. Diplospory involves the development of the

embryo sac from the megaspore mother cell without undergoing meiosis, resulting in a diploid embryo sac. This diploid egg can develop into an embryo via parthenogenesis, bypassing fertilization (Erhardt 1993; Noyes 2007; Blöch et al. 2010). This subspecies reproduces both sexually and asexually, with sexual reproduction typically prevailing (Hörandl et al. 2011). Populations exhibit diverse reproductive systems, including hermaphroditic, gynomonoecious (both hermaphroditic and female flowers on one plant), and andromonoecious (hermaphroditic and male flowers on one plant) types. The proportions of these systems vary geographically, further complicating species differentiation and blurring distinctions between populations (Kozuharova et al. 2018).

Field studies have revealed that *L. alpinum* emits both a sweet and a sweat-

like odor. The sweat-like scent is most likely due to the presence of compounds such as 3-methyl-2-pentenoic acid, butyric acid, and other fatty acids, while the sweet aroma is attributed to 2-phenylethanol and phenylacetic acid. Compounds like 3-hexanol and 3-hexenyl acetate contribute to a green, grassy note, while 5-dien-4-ol imparts a woody, herbaceous scent. Nectar analysis has identified glucose, fructose, 16 types of amino acids (including some non-proteinogenic ones), lipids, phenols, and proteins. Interestingly, nectar is primarily secreted by hermaphrodites and male flowers. The most common pollinators belong to the order Diptera, predominantly flies from the family Muscidae, along with insects from Hymenoptera, Coleoptera and Lepidoptera. Although the inflorescences of edelweiss may not appear specialized for attracting specific pollinators, the unique combination of its sweet yet sweat-like odor acts as a specific attractant for flies. Additionally, the white, hairy stem leaves serve as optical landmarks that further attract pollinators. An intriguing observation involves the butterfly *Erebia tyndarus* Esper, a pollinator known to land on sweaty human skin or clothing to drink sweat for its nitrogen and dissolved salts. The ubiquity of flies and the adaptation to fly pollination provide a significant ecological advantage in the harsh environments where the edelweiss thrives. This form of pollination is both ecologically functional and critical for the plant's survival under such conditions (Erhardt 1993).

Chromosome number and karyotype studies: The polyploidy assures increased genetic material that provides a diverse genetic base on which evolution can act, offering a broader range of adaptive options and facilitating evolutionary leaps (Payne & Wagner 2019; Van de Peer et al. 2020). The genus *Leontopodium* has been reported to encompass varying ploidy cytotypes. According to most chromosome number studies, two basic chromosome numbers are described: x=12 and x=13 (Meng et al. 2012; Russell et al. 2013). The relatively small and numerous chromosomes are the likely reason for discrepancies in the reported

chromosome numbers of species within the genus (Russell et al. 2013) (**Figure 2**). The tribe Gnaphalieae, to which the genus belongs, exhibits a wide variety of karyotypes (Watanabe et al. 1999), however, in the study by Meng et al. (2012), the karyotypes of the *Leontopodium* species were found to be unimodal, indicating no significant differences in chromosome length. While variations in basic chromosome numbers are common in the Gnaphalieae tribe, further research is needed for a better understanding of these differences (Stille et al. 2014).

Using flow cytometry, Russel et al. (2013) reported a relatively small genome size ranging from 0.93 pg to 1.14 pg (1C=3000Mb), along with notable differences in chromosome structure. For instance, *L. artemisiifolium* (Léveillé) Beauv. was found to possess a pair of heteromorphic chromosomes, which vary in size relative to each other and the other chromosomes (based on five species from a single population). Typically, heteromorphic chromosomes are observed in dioecious

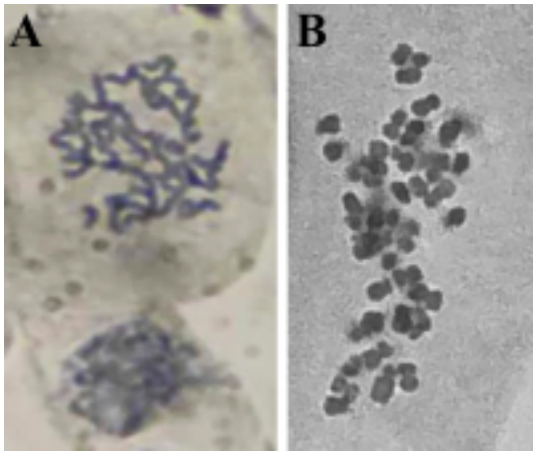

Fig. 2. Chromosomes of commercially available edelweiss assigned as *L. alpinum* (*Stella alpina*, by Hortus Sementi, https://www.agrogradina.bg/cvetq-edelvais hort) stained with Gomori's hematoxylin, pretreated in: **A** – 0,01% colchicine for 5 min; **B** – 0,05% colchicine for 2 h at RT. Magnification 1000x (immersion oil). Source: A. A. Pozumentshtikov, M. Kitanova.

plants (Charlesworth 2002). According to the authors, this polymorphism is most likely the result of homoploid hybridization or represents a transitional stage of chromosome rearrangements within an actively evolving taxon (Russell et al. 2013). Although European species share the same basic chromosome number as some Asian species, further analyses are needed to determine whether the basic chromosome numbers x=12 and x=13 evolved independently multiple times or originated from a single evolutionary event.

Cytological variation at the diploid karyotype level, as demonstrated by Russell et al. (2013), supports the hypothesis of rapid speciation within the genus, accompanied by changes in basic chromosome numbers as suggested by Sa□er et al. (2011). According to Sa□er et al. (2011), the low genetic variation observed within the genus indicates a rapid rate of diversification, which complicates resolving evolutionary relationships and understanding how chromosome numbers

evolved. Further research is required to clarify whether speciation occurred after large-scale genomic events in diploid species or whether such events were drivers of speciation. Previous studies have suggested that frequent changes in chromosome number may precede noticeable morphological changes or differences in DNA sequences (Russel et al. 2013), highlighting the significant role of polyploidy in the evolution of the genus. Interestingly, polyploid species are more common in regions such as Europe and Russia, which may indicate that polyploids possess superior dispersal abilities or greater tolerance for diverse environmental conditions, as observed in other genera (Van de Peer et al. 2020).

An earlier study by Meng et al. (2012) reported a basic chromosome number of x=14, though they also observed plants with a basic chromosome number of x=12. They noted that the Gnaphalieae tribe typically has chromosome numbers derived as multiples of 7, where n=14 is the most common (Anderberg 1994; Watanabe et al. 1999). The authors suggested that the basic chromosome numbers of x=12 and x=13 likely resulted from secondary reduction leading to dysploidy (Watanabe et al. 1999). Dysploidy is characteristic of many genera within the Asteraceae family, including *Leontopodium*, and is believed to have evolved as an adaptation to

diverse habitats. In angiosperms, it is generally thought that symmetric karyotypes evolve first, with asymmetric ones developing later (Stebbins 1971; Stace 1989). However, changes in ploidy levels and karyotype asymmetry in the genus do not come together. This indicates that both polyploidy and dysploidy play critical roles in karyotype evolution and speciation, though environmental factors may also influence karyotype asymmetry (Meng et al. 2012).

According to Stille et al. (2014), the basic chromosome numbers studied in 16 *Leontopodium* species are x=6, 8, 9 and 11, with x=8 being the most common. They concluded that the basic chromosome numbers might be useful for systematic and phylogenetic analyses but identifying them is challenging due to the high intraspecific variability within the genus. Additionally, they suggested that the large differences in basic chromosome numbers within species likely have limited relevance for phylogenetic inferences, as these differences may not carry biologically significant information.

Tantray et al. (2021) conducted an insightful study examining the meiotic division of species from geographically isolated regions of the Himalayas. In *L. jacotianum* Beauv. (2n=4x=48), they identified meiotic abnormalities such as cytomixis and structural heterozygosity, which directly affected pollen viability. Their findings confirmed that the species is tetraploid, whereas previous studies have defined it as diploid (2n=24) or having a dysploid chromosome set (2n=28) (Khatoon & Ali 1988). The species is described as “evolutionarily active” due to its intraspecific variation in chromosome number. Cytomixis is believed to contribute to aneuploidy and polyploidy by producing hypoploid and hyperploid

cells, which can lead to uneven gametes and impact fertility. Up to 5–7 pollen mother cells (PMCs) were involved in the chromatin transfer in *L. jacotianum*, making it one of the most affected plants. Notably, *L. jacotianum* exhibited the highest percentage of PMCs involved in cytomixis, with 32% of its PMCs displaying this phenomenon. This high rate of cytomixis significantly impacted the plant's pollen fertility, as chromosomal abnormalities caused by the process disrupted normal pollen development. These findings suggest that *L. jacotianum* may experience a higher rate of chromosomal aberrations, likely influencing its evolution and reproductive success (Tantray et al. 2021). In *L. jacotianum*, structural heterozygosity was observed to result from reciprocal translocations, which can lead to unbalanced chromosome segregation during cell division. This imbalance produces daughter cells with chromosome duplications or deletions. While cells with deletions are typically eliminated by natural selection, they can persist in polyploid individuals (Schubert & Lysak 2011; Tantray et al. 2021). Such structural changes are essential for the speciation and adaptation of plant species. In *L. jacotianum*, these changes form an integral part of its genetic system, aiding in evolutionary processes (Rieseberg 2001; Rieseberg & Willis 2007; Tantray et al. 2021).

It is possible that meiotic anomalies, like those observed in *L. jacotianum*, occur in other *Leontopodium* species and play a role in the evolution and adaptation

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of the genus, potentially influencing speciation.

Taxonomy and phylogeny of the genus *Leontopodium:* The genus *Leontopodium* R. Br. ex Cass. (Asteraceae) belongs to the subtribe Gnaphaliinae of the major tribe Gnaphalieae the latter ones including 178 genera and about 2,100 species (Smissen et al. 2020). *Leontopodium* comprises 58 species that are distributed across Asia and Europe (Bayer et al. 2007; Chen et al. 2011). Russell et al. (2013) reported the division of the genus into two sections: *Leontopodium*, which is more widely distributed across Eurasia, and *Nobilia* (Beauverd) Hand.-Mazz., which is found in the Himalayas, China and Japan. The infrageneric taxonomy system, species delimitation, and interspecies systematic relationships of *Leontopodium* remains controversial due to incomplete taxon sampling, incongruence among DNA markers and morphology and influenced by the high rate of hybridizations between species (Stille et al. 2014). In Europe two species occur, *L. alpinum* Cass. and *L. nivale* (Ten.) Huet ex Hand.-Mazz (Sa□er et al. 2011). China has the largest number of *Leontopodium* species in the world (37) and the West and Southwest regions of China (*e.g.,* Qinghai-Tibet Plateau) are the centers of speciation and diversification of this genus (ca. 20 species) (Chen et al. 2011; Chen & Bayer 2011; Meng et al. 2012). For example, two morphologically

similar species, *L. caespitosum* (from the Hengduan Shan) and *L. microphyllum* (Taiwan), were basal, suggesting they are part of an ancestral stock within the genus (Blöch et al. 2010). The speciation and diversification of edelweiss in the Tubetan plateau is influenced by climatic and geomorphological patterns in this region coupled with repeated, partial re-colonisations of large high-altitude areas (i.e. during the Pleistocene glaciation cycles during the last cold snap (120,000 years ago). New species have been constantly described as *L. nyingchiense*, a new species from Xizang (Tibet), China (He et al. 2024)

The genus *Leontopodium* is part of the so-called FLAG clade named after the four large genera *Filago*, *Leontopodium*, *Antennaria*, and *Gamochaeta* (Galbany-Casals et al. 2010). Nie et al. (2016) and later Xu et al. (2023) have also confirmed the presence of this clade and that the genus *Leontopodium* is monophyletic. The latter study, based on chloroplast genomes and nuclear genes, addressed the complex phylogenetic relationships within this genus with observed phylogenetic ambiguity and incongruence between chloroplast and nuclear genes. *Leontopodium* species were divided into three main clades in the chloroplast genome phylogenetic tree and six main clades in the nuclear gene phylogenetic tree. Chloroplast trees had higher support values and were more effective for phylogenetic resolution also supporting the distinction of the sections *Nobilia* and *Leontopodium* as reported by Russell et al. (2013). Moreover, the authors found out that the characteristics of the leaf base, stem types, and carpopodium base were phylogenetically correlated and may have potential value in the taxonomic study of *Leontopodium*.

Blöch et al. (2010) explored phylogenetic relationships between 22 *Leontopodium* species from a broad geographic range and with a good representation from the Himalayan/Tibetan center of diversity based on chloroplast markers and nuclear genes. The recently described Southeast Tibetan monotypic *Sinoleontopodium* (*S. lingianum* Y. L. Chen Dickoré) was shown to belong to *Leontopodium*. In another study based on Amplified Fragment Length Polymorphism (AFLP) analysis of 38 populations of 16 different species, Sa□er et al. (2011) distinguished 10 distinct groups with clear boundaries within the genus, confirming that the species are indeed very closely related with a short evolutionary history.

Few studies explored the population divergence of Far East Asian *Leontopodium populations* (*e.g.*, *L. japonicum*), a species restricted to the temperate regions of China, Korea, and Japan. Jeon et al. (2015) and Lee et al. (2016) reported distinct genetic isolation of Korean populations (Korea *vs.* China and Japan). By comparison, a non-significant level of differentiation, but a high degree of genetic diversity, was detected between Chinese and Japanese populations. These data support the notion that, rather than migrating southward

from more northern latitudes, current populations in Korea are distributed due to colonization via East China Sea land bridges, similar to movement by warm-temperate species. Furthermore, geographical isolation because of an oceanic barrier has probably led to allopatric speciation for Korean populations.

The chloroplast markers showed low rates of sequence divergence within this genus *Leontopodium* despite the presence of morphologically diverse species (Blöch et al. 2010). They have perhaps arisen due to rapid radiation and hybridisation triggered by multiple, severe climatic changes, and habitat fragmentation and rejoining. Ecological constraints might also have been responsible for the occurrence of similar morphological characters in unrelated lineages, resulting in the grouping of taxa with few morphological similarities.

According to Blöch et al. (2010) and Sa□er et al. (2011), *L. alpinum* and *L. nivale* comprise a genetically distinct group of the European section of *Leontopodium*. The genetic diversity (Diversity-Weighted (DW) values) of Asian populations (*e.g.,* the Yunnan group) is higher compared to European ones, which further supports the idea that this is an ancient and long-term isolated population from which the genus originated. Despite the wide geographic distance, European species show surprisingly little divergence from its Asian relatives. The question is still unresolved as to whether the European taxa comprise two distinct species, subspecies, or a series of varieties or unclassifiable forms. Currently, *L. alpinum* and *L. nivale* are rather recognized as separate species, however, a combined approach, including additional genetic analyses, ecological studies and morpho taxonomic methods, should be used to confirm these hypotheses. Although the evolution of these two European species is under debate, they have most likely migrated from Asia (Sino-Himalayan region) via Middle Asia during the last

cold snap (Pleistocene; 120,000 years ago) along with widespread herb-grass and Artemisia-grass steppe formations (Grichuk 1992). Sa□er et al. (2011) showed that the populations from Bulgaria (Pirin and Balkan Mts.) are with higher diversity among the European ones and is probably the most ancient and genetically distinct, suggesting that it may have served as a long-term refuge for the species during past climate changes.

Secondary metabolites and bioactivities: Numerous compounds of different classes have been identified in different edelweiss species (**Figure 3**).

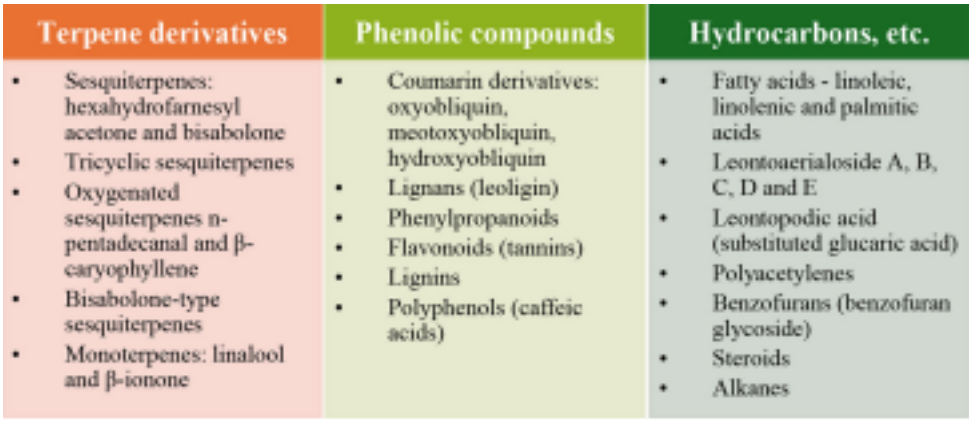

Fig. 3. Classes of secondary metabolites identified in edelweiss according to Bicchi et al. 1975; Schwaiger et al. 2002, 2004;Stuppner et al. 2002; Dobner et al. 2003b; Ganzera et al. 2012; Chen et al. 2018.

Antibacterial activity: Dobner et al. (2003a) investigated the antibacterial activity of *L. alpinum*. The authors tested crude extracts of aerial parts and roots obtained using dichloromethane, methanol, or 70% aqueous methanol as solvents. Dichloromethane extracts demonstrated significant inhibition against the strains: *Streptococcus pyogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Sesquiterpenes and other compounds selectively inhibited strains of *Enterococcus faecium*, *S. aureus*, *Streptococcus pneumoniae*, and *S. pyogenes*. Linoleic and linolenic acids were the most potent compounds, with a minimal inhibitory concentration (MIC) of 4 μg ml^{-1}, supporting the traditional use of these metabolites for gastrointestinal issues and dysentery.

Gao et al. (2017) tested the antibacterial activity of essential oil of *L. leontopodioides* (Will.) Beauv. that showed strong activity against the Gram positive strains of *S. aureus* (MIC = 0.039 mg ml^{-1}) and *B. subtilis* (MIC = 0.313 mg ml^{-1}), while no inhibition was observed for the Gram-negative *E. coli* and P. *aeruginosa* (MIC > 5 mg ml^{-1}). Qian et al. (2018) tested the essential oil of *L. longifolium* against 8 microorganisms, including four bacterial strains of *S. aureus, E. coli*, *B. subtilis*, *P. aeruginosa*, and four fungi (*Candida albicans*, *Aspergillus flavus*, *Mucor mucedo*, *Phytophthora parasitica*). The most sensitive to the oil was

S. aureus, whereas *E. coli* showed limited inhibition, and no antifungal activity was established.

Anti-inflammatory activity: Dobner et al. (2004) reported that extracts, fractions, and pure constituents of *L. alpinum* applied topically significantly reduced croton oil-induced ear edema in mice. Dichloromethane extracts of aerial

parts exhibited concentration-dependent anti-inflammatory effects. Bisabolane-type sesquiterpenes reduced leukocyte accumulation and inflammation by 46%, while the coumarin (obliquin), lignan (leoligin), and tricyclic sesquiterpenes reduced inflammation by 50%. Lariciresinols were identified as TNF-α inhibitors, and linoleic and linolenic acids demonstrated anti-inflammatory effects by inhibiting the cyclooxygenase/lipoxygenase pathways in the arachidonic acid metabolism. This inhibition prevents the synthesis of pro-inflammatory eicosanoids from arachidonic acid, including prostaglandins, thromboxanes, and leukotrienes. Additionally, the coumarin and the 7α-siliferol-5-ene sesquiterpene inhibited IL-8- induced leukocyte chemotaxis by up to 58% at a low concentration.

Antioxidant activity: The antioxidant activity of essential oils from *L. leontopodioides* and *L. longifolium* was evaluated using a DPPH radical scavenging assay in comparison with a synthetic antioxidant. The results indicated that the edelweiss essential oils required much higher concentrations for comparable effects (Gao et al. 2017; Qian et al. 2018).

Cytotoxic activity: Gao et al. (2017) investigated the cytotoxic effects of *L. leontopodioides* essential oil on HepG2 (liver cancer cell lines) and MCF-7 (breast adenocarcinoma cell lines) using the MTT assay. The oil, dissolved in DMSO, was tested alongside doxorubicin as a positive control. The essential oil inhibited cell growth in a dose- and time-dependent manner over 72 h. Although much less potent than doxorubicin, the oil exhibited notable activity. The authors attributed its effects to phenols, aldehydes, and alcohols, particularly β-caryophyllene, which may inhibit tumor cell motility, invasion, and aggregation.

The studies emphasized the importance of screening for beneficial substances from edelweiss and emphasized the need to evaluate individual compounds as well as their synergistic effects when tested in fractions.

Applications: There are many reported uses of edelweiss' biological potential (**Figure 4**).

Accordingly, numerous patents were generated using edelweiss as few of them are shown in **Table 1**.

The diseases traditionally treated with edelweiss are often related to bacterial infections and inflammations (Dobner et al. 2003a). In European folk medicine, extracts from one of the edelweiss species, *L. alpinum*, also known as "Herba

Leontopodii", have been used to treat diarrhea, dysentery, angina, bronchitis, cancer, and other conditions in both humans and animals. For oral use, the herb was boiled in wine, and milk was then added. When used for breast cancer, it was

boiled in water, and the resulting extract was applied as a compress.

Fig. 4. Applications of edelweiss bioactivity. According to: He (1994); Wu (2002); Dobner et al. (2003a); Schwaiger et al. (2004); Hornick et al. (2008); Duwensee et al. (2011); Li et al. (2012); Yang et al. (2015); Scharinger et al. (2016); Wang et al. (2016b); Yu et al. (2016); Qi et al. (2017); Zhao et al. (2019); Campiche et al. (2022); Aguchem et al. (2024).

The diseases traditionally treated with edelweiss are often related to bacterial infections and inflammations (Dobner et al. 2003a). In European folk medicine, extracts from one of the edelweiss species, *L. alpinum*, also known as "Herba Leontopodii", have been used to treat diarrhea, dysentery, angina, bronchitis, cancer, and other conditions in both humans and animals. For oral use, the herb was boiled in wine, and milk was then added. When used for breast cancer, it was boiled in water, and the resulting extract was applied as a compress.

The aerial parts of *L. leontopodioides*, have been used in Chinese folk medicine to treat albuminuria, hematuria, and vaginitis (Li et al. 2012). In the Tibetan Plateau and Inner Mongolia regions of China, the aerial parts of several edelweiss species, such as *L. leontopodiodes*, *L. haplophylloides* Hand.-Mazz., *L. nanum* (Hooker & Thomson ex Clarke) Hand.-Mazz., *L. longifolium*, and *L. dedekensii* (Bureau & Franchet) Beauv., have been used in folk medicine to treat acute nephritis and proteinuria (Schweiger et al. 2004).

The species *L. leontopodioides* has been associated with the treatment of influenza, bronchitis, and acute and chronic nephritis. It has also been described as an immunomodulatory agent (Yu et al. 2016; Qi et al. 2017). He (1994) reported the successful treatment of 100 patients using this edelweiss species, with an average of 15 doses of the plant required for a cure. Wu (2002) showed that edelweiss effectively prevented and treated bovine mastitis, as the plant extracts

Table 1. *Leontopodium* sp. patents. Source: Google Patents: https://patents.google.com/. Patent WO2007006492A1 (2007) Use of extracts and constituents of *Leontopodium* as enhancers of

cholinergic function, WO Patent, Helmut Prast, Judith Rollinger, Stefan Schwaiger, Hermann Stuppner
Patent US20130053438A1 (2013) Pharmaceutical compositions comprising lignans and their derivatives for the medical management of angiogenesis and hypovascularity, US Patent, WO Patent, Medizinische Universitaet Wien, Universitaet Innsbruck
Patent DE102013210463A1 (2014) Hair restorer containing *Leontopodium* extract, DE Patent, Henkel AG and Co., KGaA
Patent US9498430B1 (2016) A bi-phasic, non-emulsion cosmetic composition for application to skin, CN Patent, Restorsea LLC
Patent US20220040070A1 (2022) Skin lightening compostions, US Patent, Kay Mary Inc.
Patent CN111202708B (2022) Cosmetic composition for improving the skin, CN Patent, Biological Fd&c Co., Ltd

Patent	**Description**	**Current Assign ee or Inventor**
US20220040070 A1 (2022)	Compositions and methods for use that include a combination of *L. alpinum* extract and other extracts	Kay Mary Inc.
CN111202708B (2022)	Invention relates to a cosmetic composition for improving skin, comprising a plant cell complex culture, and more particularly, to a cosmetic composition comprising: a *L. alpinum* cell cul ture or an extract thereof and other extracts.	Biological Fd&c Co. Ltd.
US9498430B1 (2016)	A bi-phasic, non-emulsion cosmetic composi tion for application to skin includes a hydropho bic liquid phase (essential oils) and a hydrophilic liquid phase.	Restorsea LLC
DE102013210463 A1 (2014)	The application relates to the use of an active ingredient or mixture of active ingredients, ob tainable from plants of the genus *Leontopodium*, for revitalizing hair.	Henkel AG and Co., KGaA
US20130053438 A1 (2013)	The present invention relates to a pharmaceutical composition for stimulating angiogenesis and/or the treatment or prevention of hypovascularity and/or the prevention and/or treatment of an an giogenic disorder/disease, whereby the compo sition comprises specific compounds which may be obtained from *L. alpinum* Cass., (Edelweiss).	Medizinische Universitaet Wien, Universi taet Innsbruck

WO2007006492 A1 (2007)	Use of a plant extract from at least one *Leon topodium* species for the preparation of a medic ament for the treatment of diseases which can be modulated by the selective inhibition of the enzyme acetylcholinesterase and/or increase of acetylcholine concentration at the cholinergic synapse.	Helmut Prast, Judith Roll inger, Stefan Schwaiger, Her mann Stuppner

enhanced immunomodulation in the bovine mammary gland (Zhao et al. 2019). Additionally, studies have reported that an aqueous decoction of this species can prevent and treat diabetes, demonstrating a significant reduction in blood sugar levels and improved blood glucose levels in hyperglycemic mice (Yang et al. 2015).

Oxidative stress and reactive oxygen species (ROS) are key contributors to hair loss. *L. alpinum* var. *helvetia* shows promise in treating hair growth disorders. Hair growth occurs in three phases: anagen (growth), catagen (regression), and telogen (rest). In a study using an ethanol:water (60/40) extract, *L. alpinum* prolonged the anagen phase in ex vivo hair follicles, delaying progression to the catagen and telogen phases. The extract stimulated keratinocyte proliferation in the hair matrix and enhanced dermal papilla cell inductance, both of which are crucial for hair growth. In vivo, the application of the extract significantly increased hair density (+13,200 hairs across the scalp) and improved the anagen-to-catagen/telogen ratio, promoting active hair growth (Campiche et al. 2022).

Researchers investigated whether *L. alpinum* contains substances that enhance cholinergic neurotransmission. Intracerebroventricular injection of crude dichloromethane root extract increased extracellular acetylcholine (ACh) levels in rat brains by 79% at 1 mg ml^{-1} and inhibited acetylcholinesterase (AChE) in vitro. Fractionation revealed that sesquiterpene-rich fractions elevated ACh levels. Further testing identified compounds such as isocomene, β-isocomene, silphinene, and modhephene, with isocomene (2 μmol) demonstrating the strongest effect. A sesquiterpene acetate fraction containing silphiperfole acetate and 14-acetoxy isocomene also boosted ACh levels. Individual testing revealed prolonged effects for 14-acetoxy-isocomene and a peak effect for silphiperfole acetate. Behavioral studies showed isocomene reversed scopolamine-induced amnesia in mice, enabling them to recall and recognize an object they had previously seen, thereby improving episodic memory without enhancing overall memory. This type of object recognition relies on episodic memory, which is often affected in dementia and Alzheimer's disease. Additionally, mice injected with 42 nmol of isocomene exhibited improved working and spatial memory in a T-maze, along with reduced

neophobia, a behavior often associated with aging (Hornick et al. 2008).

Leoligin: Leoligin, a natural lignan derived from *L. nivale* ssp. *alpinum* roots, exhibits promising preventive and therapeutic potential in cardiovascular diseases, particularly atherosclerosis. Cholesterol efflux, a key elimination process, directs cholesterol from peripheral tissues to the liver for excretion. Leoligin effectively induced cholesterol efflux from THP-1 macrophages in a concentration-dependent manner, outperforming pioglitazone at 10 μM. It was particularly effective in the presence of apo A1 (apolipoprotein A1), a major component of HDL cholesterol and a potent cholesterol acceptor (Wang et al. 2016a).

The cholesteryl ester transfer protein (CETP), which plays a role in HDL

metabolism, facilitates the exchange of triglyceride and cholesteryl esters between lipoproteins. In vitro studies demonstrated that leoligin activated CETP within nanomolar ranges (100 pM–1 nM). In vivo studies of CETP-expressing transgenic mice revealed increased CETP activity and reduced LDL cholesterol levels following leoligin treatment (administered in DMSO and diluted in 0.5% methylcellulose). However, CETP's role remains controversial, as it can be either proatherogenic or antiatherogenic depending on LDL generation and degradation pathways (Duwensee et al. 2011; Aguchem et al. 2024).

Apoe−/− mice, which are prone to atherosclerosis due to impaired lipoprotein clearance, showed reduced total cholesterol and LDL cholesterol levels after leoligin treatment (1 μM–100 μM). These mice, which do not express CETP, exhibited improvements in total cholesterol/HDL and LDL/HDL ratios, particularly at lower doses. A dose-dependent effect was observed at higher concentrations, specifically with improvements in the LDL/HDL ratio, while serum triglycerides remained unchanged. Leoligin also inhibited HMG-CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol synthesis, in a concentration-dependent manner, with effects comparable to pravastatin at 5 μM and 50 μM. However, after 16 weeks, the effects diminished due to HMGCR upregulation, resulting in increased lipid and cholesterol deposition in the liver. Despite these changes, no protective effect against atherosclerotic plaques was observed. Unexplained weight loss and counter-regulation effects were noted in high-dose groups (Scharinger et al. 2016).

In vitro cultivation: Tissue cultures can be initiated using seeds or apical buds, or roots, after tissue sterilization (**Table 2**).

Seeds can be cultured on ½ Murashige and Skoog (MS) nutrient medium, while apical buds and roots can be grown for rooted in an agar-solidified (7 g l^{-1}) MS salt medium supplemented with the naphthaleneacetic acid (NAA) (0.1 mg l^{-1}), kinetin (0.05 mg l^{-1}), thiamine HCI (0.4 mg l^{-1}), mesoinositol (80 mg l^{-1}),

casein hydrolysate (100 mg l^{-1}) and sucrose (30 g l^{-1}). The resulting cultures can be subcultured by separating rosettes (**Figure 5**). Following ex vitro adaptation is also possible using potting compost (Hook 1993). In vitro tissue cultures are particularly useful for conserving the natural and endangered populations of the plant.

Fig. 5. Cultivated edelweiss tissue culture (com mercially available edelweiss assigned as *L. alpi num* - *Stella alpina* by Hortus Sementi, https://www.agrogradina.bg/cvetq-edelvais-hort). Source: A. A. Pozumentshtikov.

Reference	Pianova (Berdasova) et al. 2021 Ciocan et al. 2023 Pace et al. 2009 Cho et al. 2020 Hook 1993
Species	*L. palibinianium* *L. alpinum* *L. alpinum* *L. alpinum* *L. nivale*

Sterilization protocol	1. Chlorhexidine 3 times (for 10 min) and rinse with distilled water each time; 2. 1% AgNO3 and rinsed three times with sterile distilled water·
	1. 10% aq. solution of commercial NaClO for 20 min; 3. 0.3% commercial NaClO on a shaker for 20 min; 4. Rinse several times with sterile distilled water·
	3. Rinse several times with sterile distilled water·
	2. 10% commercial NaClO for 30 min; 2. Rinse with sterile distilled water·
	2. Rinse with distilled water; 1. 70% ethanol for 3 min; 1. 0.1% HgCl2 for 10 min
	1. 70% ethanol for 30 s;

Table 2. Sterilization protocols for in vitro cultivation·

Callus culture: Callus culture involves growing unorganized, undifferentiated plant cells (callus) on a nutrient medium under sterile conditions. Callus formation is typically induced when plant tissues, such as leaves, stems, or

roots, are wounded or treated with specific plant growth regulators like auxins and cytokinins. Several protocols have been reported for generating callus cultures (**Table 3**).

Table 3. Protocols for the induction of callus culture.

Growth regulator/component	Quantity	Source
6-Benzylaminopurine/6-BAP (cytokinin)	0.5–3 mg ml^{-1}	Cho et al. 2020
2,4-Dichlorophenoxyacetic acid/2,4-D (auxin)	0.3–1 mg ml^{-1}	
6-BAP	2 mg l^{-1}	Pace et al. 2009
2,4-D	1 mg l^{-1}	
Maintained in a medium containing:		
1-naphthaleneacetic acid/NAA (auxin)	0.1 mg l^{-1}	
6-BAP	0.4 mg l^{-1}	
MS		Kim et al. 2023
sucrose	3% (w/v)	
Gelatin	4% (w/v)	
6-BAP	0.5 mg l^{-1}	
2,4-D	0.3 mg l^{-1}	
Agar	9 g l^{-1}	Hook 1993
2,4-D	0.22 mg l^{-1}	
NAA	0.18 mg l^{-1}	
Glycine	2 mg l^{-1}	
Nicotinic acid	0.5 mg l^{-1}	
Pyridoxine HCl	0.5 mg l^{-1}	

Meosinositol	200 mg l^{-1}	
Thiamine HCl	0.5 mg l^{-1}	
Sucrose	30 g l^{-1}	

An extract from lyophilized callus culture of *L. alpinum*, obtained via heat extraction, has demonstrated strong antioxidant activity, especially under UVB exposure. At a 1% concentration, its antioxidant effect is comparable to that of N-acetyl cysteine (NAC) and surpasses vitamin C, with notable improvements in cell viability. This activity is attributed to the extract's ability to inhibit hydrogen

peroxide and ROS. Additionally, the extract reduces inflammation and wrinkles while improving skin hydration. Clinical in vivo tests confirm that regular application to the face improves the appearance of wrinkles around the eyes, boosts skin elasticity, and increases both skin density and thickness compared to placebo groups. RNA sequencing in keratinocyte cells revealed that the extract upregulated genes encoding proteins critical for skin barrier development, programmed cell death, and keratinization. At the same time, it downregulated genes associated with stress responses, including those related to metal exposure, oxidation, injury, hypoxia, and viral infections, indicating no adverse effects on the skin (Cho et al. 2020).

Another research team identified several bioactive compounds in the callus extract of *L. alpinum*, including leontopodic acid A and B, syringin, chlorogenic acid, cynarin, isochlorogenic acid A and C, isoquercitrin (Meng et al. 2023). Their study demonstrated that the callus extract protected fibroblast models from blue light-induced damage by reducing ROS levels. At concentrations of 10–15 mg ml-1, the extract promoted collagen (COL-I) production and inhibited the secretion of matrix metalloproteinase-1 (MMP-1, also known as collagenase), skin opsin (OPN3), ROS levels, and calcium influx. The mechanism of action likely involves the inhibition of OPN3-dependent calcium signal transduction pathways, thereby reducing oxidative stress caused by ROS. By suppressing these pathways, the extract prevents the secretion of MMP-1 secretion, which is critical for collagen breakdown, offering protection against blue light-induced damage.

In conclusion, it is possible to state that complementary phyloecological studies would elucidate the influence of climatic and geographical factors on the distribution and evolutionary dynamics of more sensitive organisms such as edelweiss. The integration of these approaches will not only deepen our understanding of the evolutionary mechanisms underlying these organisms but

also provide the scientific foundation needed to revise their taxonomic classifications. Such knowledge may be pivotal for conserving biodiversity and ensuring the long
term sustainability of ecosystems. This is especially important for species inhabiting microhabitats with highly restricted distributions, as they are among the most vulnerable to environmental changes or other competing more adaptable organisms. Understanding the ecological dynamics of these environments is therefore critical for developing effective conservation strategies aimed at mitigating the impacts of climate change and habitat loss. Such strategies will be vital for safeguarding vulnerable species and maintaining the integrity of ecosystems.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHORS CONTRIBUTION

A.A.P. designed and wrote the manuscript. I.A.B., G.N.B., M.L.K. and M.K.Z. contributed to adding information and improving the manuscript.

References

Abisado R. G., Benomar S., Klaus J. R., Dandekar A. A. & Chandler J. R. 2018. Bacterial quorum sensing and microbial community interactions. - mBio 9: 1–13.

Aguchem R. N., Okagu I. U., Okorigwe E. M., Uzoechina J. O., Nnemolisa S. C. & Ezeorba T. P. C. 2024. Role of CETP, PCSK-9, and CYP7-alpha in cholesterol metabolism: Potential targets for natural products in managing hypercholesterolemia. - Life Sci. 351: 122823. doi: 10.1016/j.lfs.2024.122823.

Anderberg A. A. 1994. Tribe Gnaphalieae. - In: Bremer K. (Ed.), Asteraceae: Cladistics and Classification. Timber Press, Oregon, 304–364.

Bais H. P., Weir T. L., Perry L. G., Gilroy S. & Vivanco J. M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. - Ann. Rev. Plant Biol. 57: 233–266. doi: 10.1146/annurev.arplant.57.032905.105159

Bancheva S. 2015. *Leontopodum alpinum*. - In: Peev D. (Ed-in-Chief) 2015. Red Book of the Republic of Bulgaria. Volume 1. Plants and fungi. BAS & MEW.

Barka E. A., Vatsa P., Sanchez L., Gaveau-Vaillant N., Jacquard C., Klenk H. P., Clément C., Ouhdouch Y. & van Wezel G. P. 2016. Taxonomy, physiology, and natural products of actinobacteria. - Microbiol. Mol. Biol. Rev. 80: 1–43.

Bayer R. J., Breitwieser I., Ward J. & Puttock C. 2007. Tribe Gnaphalieae. – In: Kadereit J. W. & Je□□rey C. (Eds), The families and genera of vascular plants, vol. 8, Springer Heidelberg, 246–283.

Bicchi C., Nano G.M. & Tira S. 1975. n-Paraffin components of some Gnaphalieae. - Planta Med. 28 (4): 389–391.

Blöch C., Dickoré W. B., Samuel R. & Stuessy T. F. 2010. Molecular phylogeny of the edelweiss (*Leontopodium*, Asteraceae–Gnaphalieae). - Edinb. J. Bot. 67 (2): 235–264.

Campiche R., Le Riche A., Edelkamp J., Botello A. F., Martin E., Gempeler M. & Bertolini M. 2022. An extract of *Leontopodium alpinum* inhibits catagen development ex vivo and increases hair density in vivo. - Int. J. Cosmet. Sci. 44 (3): 363–376.

Caraballo-Rodríguez A. M., Dorrestein P. C. & Pupo M. T. 2017. Molecular inter-kingdom interactions of endophytes isolated from *Lychnophora ericoides*. - Sci. Rep. 7: 5373.

Cassini M. H. 1819. Examen analytique du genre Filago d*e Linné.* - Bulletin des Sciences, par la Société Philomatique 141-144.

Charlesworth D. 2002. Plant determination and sex chromosomes. - Heredity 88: 94–101.

Chen Y. S. & Bayer R. J. 2011. *Leontopodium leontopodioides* Flora of China. Available at http://foc.eflora.cn/content. aspx?TaxonId=200024160. Chen Y. S., Zhu S. X. & Bayer R. J. 2011. Gnaphalieae. – In: Wu C. Y., Raven P. H. & Hong D. Y. (Eds), Flora of China, vol. 20–21, Beijing: Science Press and St. Louis: Missouri Botanical Garden Press, 774–818.

Chen Q., Li J., Ruan J., Qu L., Wei H., Ma X., Zhang Y & Wang T. 2018. Bioactive constituents from the whole plants of *Leontopodium leontopodioides* (Wild.) Beauv. - J. Nat. Med. 72: 202–210. doi:10.1007/s11418-017-1132-3

Ciocan A.-G., Mitoi E.-M., Helepciuc F.-E., Neguţ D., Moldovan R.-C., Petrache A.-M., Iuga C.-A., Holobiuc I.-M., Maximilian C.-R., Radu M. & Cogălniceanu G.-C. 2023. Is acute low-dose gamma irradiation an effective elicitor for secondary metabolism in *Leontopodium alpinum* (Cass.) callus

culture? - Ind. Crops Prod. 197: 116547.
Cho W. K., Kim H.-I., Kim S.-Y., Seo H. H., Song J., Kim J., Shin D. S., Jo. Y. Choi H., Jeong Hun Lee, J. H. & Moh S. H. 2020. Anti-aging effects of *Leontopodium alpinum* (Edelweiss) callus culture extract through transcriptome profiling. Genes 11 (2): 230.
Chytrý M., Danlëls F. J., Di Pietro R., Koroleva N. & Mucina L. 2015. Nomenclature adjustments and new syntaxa of the Arctic, alpine and oro Mediterranean vegetation. - Hacquetia 14 (2): 277–288.
Compant S., Clement C. & Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. - Soil Biol. Biochem. 42: 669–678.
Dobner M. J., Schwaiger S., Jenewein I. H. & Stuppner H. Antibacterial activity of *Leontopodium alpinum* (Edelweiss). 2003a. - J. Ethnopharmacol. 89 (2-3): 301–303.
Dobner M. J., Sosa S., Schwaiger S., Altinier G., Della Loggia R., Kaneider N. C. & Stuppner H. 2004. Anti-inflammatory activity of *Leontopodium alpinum* and its constituents. - Planta Med. 70 (6): 502-508. doi: 10.1055/s 2004-827148.
Dobner M. J., Ellmerer-Mülle, E. P., Schwaige, S., Batsug, O., Narantuy, S., Stütz, M. & Stuppner H. 2003b. New lignan, benzofuran and sesquiterpene derivatives from the roots of *Leontopodium alpinum*. - Helvetica Chimica Acta 86: 733–738. doi:10.1017/s0960428610000065
Duwensee K., Schwaiger S., Tancevski I., Eller K., van Eck M., Markt P.,

Linder T., Stanzl U., Ritscha A., R. Patscha J. R., Schuster D., Stuppner H., Bernhard D. & Eller P. 2011. Leoligin, the major lignan from Edelweiss, activates cholesteryl ester transfer protein. - Atherosclerosis 219 (1): 109–115.
Erhardt A. 1993. Pollination of the edelweiss, *Leontopodium alpinum*. - Biol. J. Linn. Soc. 111 (2): 229–240.
Flann C., Greuter W. & Hind D. J. N. 2010. Cassini's Compositae genera: A nomenclatural and taxonomic assessment. - Taxon 59 (4): 1206–1244. doi: 10.1002/tax.594021
Galbany-Casals M., Andrés-Sánchez S., Garcia-Jacas N., Susanna A., Rico E. & Martínez-Ortega M. M. 2010. How many of Cassini anagrams should there be? Molecular systematics and phylogenetic relationships in the "*Filago* group" (Asteraceae, Gnaphalieae), with special focus on the genus *Filago*. - Taxon. 59 (6): 1671–1689.
Ganzera M., Grei□eneder V., Schwaiger S. & Stuppner H. 2012. Chemical profiling of Edelweiss (*Leontopodium alpinum* Cass.) extracts by micellar electrokinetic capillary chromatography. - Fitoterapia 83 (8): 1680–1686.
Gao Y., Rao H., Mao L.-J. & Ma Q.-L. 2017. Chemical composition, antioxidant,

antibacterial and cytotoxic activities of essential oil of *Leontopodium leontopodioides* (Willd.) Beauverd. - Nat. Prod. Res. 33 (4): 612-615.

Grichuk V. P. 1992. Map of main types of vegetation ecosystems during maximum cooling of the last glaciationabout 20.000 to 18.000 yr B.P. and explanatory notes to the map. - In: Frenzel B., Pécsi M. & Velichko A. A. (Eds), Atlas of Paleoclimates and Paleoenvironmentsof the Northern Hemisphere, Late Pleistocene – Holocene. Gustav-Fischer-Verlag, Budapest, Stuttgart, 123–124.

Hardoim P. R., van Overbeek L. S., Berg G., Pirttilä A. M., Compant S., Campisano A., Döring M. & Sessitsch A. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. - Microbiol. Mol. Biol. Rev. 79 (3): 293–320.

He F. X. 1994. *Leontopodium leontopodioides* as main herb of treatment 100 cases of acute nephritis. - J. J. Tradit. Chin. Med. 6: 280.

He W.-Q., Zhao F.-Y., Chu, Z.-F., Chai, G.-Z., Zhao, K.-H., Tian, J.-Q., Zhang, B.-X., Zhang, F.-Y., Liao, Z.-H., Zheng, W.-L. & Lan, X.-Z. 2024. *Leontopodium nyingchiense* (Asteraceae), a new species from Xizang (Tibet), China. - PhytoKeys 249: 181–192.

Hook I. L. I. 1993. *Leontopodium alpinum* Cass. (Edelweiss): In Vitro Culture, Micropropagation, and the Production of Secondary Metabolites. - In: Bajaj Y. P. S. (Ed.), Medicinal and Aromatic Plants IV. Biotechnology in Agriculture and Forestry, vol 21. Springer, Berlin, Heidelberg. 464 p.

Hörandl E., Dobeš C., Suda J., Vít P., Ur□us T., Temsch E. M. & Ladinig U. 2011: Apomixis is not prevalent in subnival to nival plants of the European Alps. - Ann. Bot. 108 (2): 381–390.

2008. Extracts and constituents of *Leontopodium alpinum* enhance cholinergic transmission: Brain ACh increasing and memory improving properties. - Biochem. Pharmacol. 76 (2): 236–248.

Hütsch B. W., Augustin J. & Merbach W. 2002. Plant rhizodeposition - an important source for carbon turnover in soils. - J. Plant Nutr. Soil Sci. 165: 397–407.

Ischer M., Dubuis A., Keller R. & Vittoz P. 2014. A better understanding of the ecological conditions for *Leontopodium alpinum* Cassini in the Swiss Alps. - Folia Geobot. 49 (4): 541–558.

Jeon M. G., Choi K. J. & Kim J. Y. 2015. Discrimination of the genus *Leontopodium* species (Gentianales: Asteraceae) Based on RAPD. - J. For. Environ. Sci. 31 (1): 68–71.

Kim M.-J., Ko H., Kim, J.-Y., Kim H.-J., Kim H.-Y., Cho H.-E., Cho H.-D., Seo W.- S. & Kang H.-C. 2023. Improvement in yield of extracellular vesicles derived from edelweiss callus treated with LED light and enhancement of

skin anti aging indicators. - Curr. Issues Mol. Biol. 45: 10159-10178.
Khatoon S. & Ali S.I. 1988. Chromosome numbers in compositae from Pakistan. - Candollea 43: 455–462.
Kozuharova E., Panayotov M. & Spadaro V. 2018. Autecology and ex situ growth of *Leontopodium nivale* subsp. *nivale* (Asteraceae) from North Pirin marbles (SW Bulgaria). - Flora Mediterr. 28: 187–206.
Kuzmanov B. 2012. *Leontopodium* Cass. - In: Jordanov D. (Ed.), Flora Republicae Popularis Bulgaricae. Sofia, 236-258.
Lee D. H., Le J. H., Ch W. B. & Choi B. H. 2016. The establishment history of alpine *Leontopodium japonicum* (Asteraceae) resembles that of warm temperate plants on the Korean Peninsula. - Plant Syst. Evol. 302: 1483–1494.
Li X., Luo J. G., Wang X. B., Luo J., Wang J. S. & Kong L. Y. 2012. Phenolics from *Leontopodium leontopodioides* inhibiting nitric oxide production. - Fitoterapia 83: 883–887.
Luo Y., Zhou M., Zhao Q., Wang F., Gao J., Sheng H. & An L. 2020. Complete genome sequence of Sphingomonas sp. Cra20, a drought resistant and plant growth promoting rhizobacteria. – Genomics 112 (5): 3648-3657.
Ma Y. R., Wei Z., Lu Y., Li Z., Zhao Q. F. & Zhu S. X. 2022. Achene micromorphological characteristics and taxonomic significance in *Leontopodium* R. Brown ex Cass. taxa (Asteraceae: Gnaphalieae) in China. - Pak. J. Bot. 54 (4): 1461-1474. doi: http://dx.doi.org/10.30848/PJB2022-4(30)
Meng X., Guo M., Geng Z., Wang Z., Zhang H., Li S., Ling X. & Li L. 2023. Effects and mechanism of the *Leontopodium alpinum* callus culture extract on blue light damage in human foreskin fibroblasts. - Molecules 28: 2172.
Meng Y., Nie Z.-L., Sun H. & Yang Y.-P. 2012. Chromosome numbers and polyploidy in *Leontopodium* (Asteraceae: Gnaphalieae) from the Qinghai Tibet Plateau of S.W. China. - Caryologia 65, 2: 87–93.

Minz D., O□ek M. & Hadar Y. 2013. Plant Rhizosphere Microbial Communities. - In: Rosenberg E., DeLong E. F., Lory S., Stackebrandt E. & Thompson F. (Eds), The Prokaryotes, Springer, Berlin, Heidelberg, 56-84.
Mucina L., Valachovič M., Jarolímek I., Še□□er J., Kubinská A. & Pišút I. 1990. The vegetation of rock fissures, screes, and snow-beds in the Pirin Planina Mountains (Bulgaria). - Stud. Geobot. 10: 15-58.
Nie Z. L., Funk V. A., Meng Y., Deng T., Sun H. & Wen J. 2016. Recent assembly of the global herbaceous flora: evidence from the paper daisies (Asteraceae: Gnaphalieae). - New Phytol. 209 (4): 1795–1806.
Noyes R. D. 2007: Apomixis in the Asteraceae: diamonds in the rough. - Funct. Pl. Sci. Biotechnol. 1 (2): 207–222.
Oberho□er M., Hess J., Leutgeb M., Gössnitzer F., Rattei T., Wawrosch C. & Zotchev S. B. 2019. Exploring actinobacteria associated with rhizosphere

and endosphere of the native alpine medicinal plant *Leontopodium nivale* subspecies *alpinum*. - Front. Microbiol. 10: 2531.

O□ek-Lalzar M., Sela N., Goldman-Voronov M., Green S. J., Hadar Y. & Minz D. 2014. Niche and host-associated functional signatures of the root surface microbiome. - Nat. Commun. 5: 1–9.

Pace L. G., Bruno A. A. & Spanò L. 2009. In vitro plant regeneration and clonal micropropagation of *Leontopodium nivale* (Ten.) Heut ex Hand.‑Mazz. (Asteraceae). - Plant Biosyst. 143: suppl., S12-S16.

Pavlova D., Tosheva A. & Tonkov S. 2023. Systematics of higher plants. University Publishing House "St. Kliment Ohridski", Sofia, 382 pp. (In Bulgarian). Payne J. L. & Wagner A. 2019. The causes of evolvability and their evolution. - Nat. Rev. Genet. 20: 24–38.

Pianova A. S., Salokhin A. V. & Sabutski Yu 2021. In vitro propagation and conservation of *Leontopodium palibinianum* Beauverd (Asteraceae), endemic species of Primorye Territory, - Turczaninowia 24 (4): 108–113.

Qi C. L., Wang E., Jin L. Q., Yan M., Zhang X. Q., Wang H. & Ye W. C. 2017. Ent-kaurene diterpenoids and lignan from *Leontopodium leontopodioides* and their inhibitory activities against cyclooxygenases-1 and 2. - Phytochem. Lett. 21: 94–97.

Qian H., Zhang W., He Y., Li G. & Shen T. 2018. Chemical composition, antioxidant and antimicrobial activities of essential oil from *Leontopodium longifolium* Ling. - J. Essent. Oil-Bear. Plants 21 (1): 175–180.

Rieseberg L. H. 2001. Chromosomal rearrangements and speciation. - Trends Ecol. Evol. 16: 351–358. doi: 10.1016/s0169-5347(01)02187-5 Rieseberg L. H. & Willis J. H. 2007. Plant speciation. - Science 317: 910–914. Roussakova V. 2009: 6170 - Alpine and subalpine calcareous grasslands. - In: Kavrukova V., Dimova D., Dimitrov M., Tzonev R. & Belev T. (Eds), Guidelines for assessing favourable conservation status of Natura 2000 species and habitat types in Bulgaria, Sofia, 230-234.

Russell A., Sa□er S., Weiss-Schneeweiss H., Temsch E., Stuppner H., Stuessy T. F. & Samuel R. 2013. Chromosome counts and genome size of *Leontopodium* species (Asteraceae: Gnaphalieae) from south-western China. - Bot. J. Linn. 171 (3): 627–636.

Sa□er S., Tremetsberger K., Guo Y-P., Kohl G., Samuel R., Stuessy T. F. & Stuppner H. 2011. Phylogenetic relationships in the genus Leontopodium (Asteraceae: Gnaphalieae) based on AFLP data. - Bot. J. Linn. 165: 364–377.

Saleem M., Law A. D., Sahib M. R., Pervaiz Z. H. & Zhang Q. 2018. Impact of root system architecture on rhizosphere and root microbiome. - Rhizosphere 6: 47–51.

Scharinger B., Messner B., Türkcan A., Schuster D., Vuorinen A., Pitterl F.,

Heinz K., Arnhard K., Lau□er G., Grimm M., Stuppner H., Oberacher H., Eller P., Ritsch A. & Bernhard D. 2016. Leoligin, the major lignan from Edelweiss, inhibits 3-hydroxy-3-methyl-glutaryl-CoA reductase and reduces cholesterol levels in ApoE −/− mice. - J. Mol. Cell Cardiol. 99: 35–46.

Schubert I. & Lysak M. A. 2011. Interpretation of karyotype evolution should consider chromosome structural constraints. - Trends Genet. 27: 2070–216.

Schwaiger S., Adams M., Seger C., Ellmerer E., Bauer R. & Stuppner H. 2004. New constituents of *Leontopodium alpinum* and their in vitro leukotriene biosynthesis inhibitory activity. - Planta Med. 70: 978–985.

Schwaiger S., Dobner M. J., Odonchimeg B., Ellmerer-Müller E. P. & Stuppner H. 2002. Phytochemical profile of *Leontopodium alpinum* Cass in comparison to other Asian *Leontopodium* species. - Rev. Fitoter. 2: 241.

Smissen R. D., Bayer R. J., Bergh N. G., Breitwieser I., Freire S. E., Galbany Casals M., Schmidt-Lebuhn A. N. & Ward J. M. 2020. A revised subtribal classification of Gnaphalieae (Asteraceae). - Taxon 69 (4): 778–806.

Stace C. A. 1989. Plant taxonomy and biosystematics, 2nd ed. Edward Arnold, London, 138 pp.

Stebbins G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London, 216 pp.

Stille J. S., Jaeger M., Dickoré W. B., Ehlers K., Holzhauer S. I. J., Mayland Quellhorst E., Sa□er S., Schwaiger S., Stuessy T. F., Stuppner H. & Wissemann V. 2014. Chromosome numbers of the edelweiss, *Leontopodium* (Asteraceae, Compositae – Gnaphalieae). - Edinb. J. Bot. 71 (1): 23–33.

Stuppner H., Ellmerer E. P., Ongania K.-H. & Dobner M. 2002. Bisabolane derivatives from *Leontopodium alpinum*. - Helv. Chim. Acta 85: 2982–2989.

Tantray Y. R., Jan I., Wani M. S., Singhal V. K. & Gupta R. C. 2021. Chromosome numbers and meiotic behavior in some species of Asteraceae from high altitudinal regions of Kashmir Himalayas. - J. Asia-Pac. Biodivers. 14 (4): 590-606.

Tzonev R., Dimitrov M. & Roussakova V. 2009. Syntaxa according to the Braun Blanquet approach in Bulgaria. - Phytol. Balcan. 15 (2): 209–233.

Van de Peer Y., Ashman T.-L., Soltis P. S. & Soltis D. E. 2020. Polyploidy: an evolutionary and ecological force in stressful times. - The Plant Cell 33 (1): 11–26.

Vigneron J. P., Rassart M., Vértesy Z., Kertész K., Sarrazin M., Biró L. P., Ertz D. & Lousse V. 2005. Optical structure and function of the white filamentary hair covering the edelweiss bracts. - Phys. Rev. E 71 (1): 011906.

Wang L., Ladurner A., Latkolik S., Schwaiger S., Linder T., Hošek J., Palme V., Schilcher N., Ondrej Polansky O., Heiss E. H., Stangl H., Mihovilovic M. D., Stuppner S., Dirsch V. M. & Atanasov A. G. 2016a. Leoligin, the major

lignan from edelweiss (*Leontopodium nivale* subsp. *alpinum*), promotes cholesterol efflux from THP-1 macrophages. - J. Nat. Prod. 79 (6): 1651–1657.

Wang, M., Yang, P. & Falcão Salles, J. 2016b. Distribution of root-associated bacterial communities along a salt-marsh primary succession. - Front. Plant Sci. 6: 1188.

Watanabe, K., Short, P.S., Denda, T., Konishi, N., Ito, M. & Kosuge, K. 1999. Chromosome numbers and karyotypes in the Australian Gnaphalieae and Plucheeae (Asteraceae). - Aust. Syst. Bot. 12: 781–802.

Wu Y. X. 2002. Pharmacological study on the common edelweiss (*Leontopodium leontopodioides*) in the prevention and treatment of bovine mastitis. Master Thesis, Zhejiang University, China.

Xu X-M, Wei Z, Sun J-Z, Zhao Q-F, Lu Y, Wang Z-L & Zhu S-X. 2023. Phylogeny of *Leontopodium* (Asteraceae) in China—with a reference to plastid genome and nuclear ribosomal DNA. - Front. Plant Sci. 14: 1163065.

Yang J. B., Yuan Z. Y. & Guo P. 2015. Screening of effective hypoglycemic constituents of *Leontopodium leontopodioides*. - J. Chin. J. Exp. Tradit. Med. Formulae. 18: 104–106.

Yu Q. W., Hu J., Wang H., Chen X., Zhao F., Gao P., Yang Q. B., Sun D. D., Zhang L. Y. & Yan M. 2016. Antagonistic effects of extracts from *Artemisia rupetris* L. and *Leontopodium leontopodioides* to CC chemokine receptor 2b (CCR2b). - Chin. J. Nat. Med. 14: 363–369.

Zhao Y., You X.-M., Jiang H., Zou G.-X. & Wang B. 2019. Spectrum–effect relationships between high-performance liquid chromatography fingerprints and anti-inflammatory activities of *Leontopodium leontopodioides* (Willd.) Beauv. - J. Chromatogr. B 1104: 11–17.

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