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Comparative Anatomical and Ecological Study of *Gentiana Algida* and *Gentiana Macrophylla* in Mongolia

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ABSTRACT

This study aims to investigate the anatomical, morphological, and ecological adaptations of two medicinal species, *Gentiana algida* and *Gentiana macrophylla*, growing in different ecological regions of Mongolia. These species are widely used in traditional medicine and are increasingly affected by environmental stress associated with climate change. Comparative analyses of stem, leaf, and seed structures were conducted using light microscopy, scanning electron microscopy (SEM), and standard anatomical techniques. Detailed observations revealed species-specific differences in vascular bundle arrangement, mesophyll organization, and seed coat surface ornamentation. *G. algida* exhibited structural traits associated with cold and high-altitude environments, whereas *G. macrophylla* showed anatomical features linked to temperate habitats. The findings provide new insights into adaptive anatomical characteristics relevant to taxonomy, ecological resilience, conservation, and sustainable utilization of *Gentiana* species.

Keywords: *Gentiana algida*, *Gentiana macrophylla*, leaf anatomy, stem morphology, seed morphology, scanning electron microscopy.

INTRODUCTION

The genus *Gentiana* L., comprising nearly 400 species distributed across a wide range of ecological regions, represents one of the most significant groups of medicinal plants used in traditional Asian medical systems (Zhang *et al.*, 2020). In environmentally extreme regions such as the Mongolian steppe, the survival and distribution of *Gentiana* species are closely linked to their anatomical and morphological traits, which enable adaptation to fluctuating climatic and edaphic conditions (Liu *et al.*, 2019). Increasing evidence suggests that *Gentiana* populations are under growing threat from climate change and human-induced disturbances, underscoring the importance of targeted conservation measures to safeguard biodiversity (Wang *et al.*, 2021).

Effective conservation planning and the development of sustainable cultivation approaches require a comprehensive understanding of structural adaptations and reproductive characteristics, particularly seed morphology and germination

behavior (Chen *et al.*, 2022). In addition, investigations of stem and leaf anatomical features offer critical insight into physiological adaptation mechanisms. They may help explain the long-standing medicinal value attributed to these species in traditional healing practices (Kim & Park, 2023).

Phytochemical studies have revealed a diverse array of bioactive constituents in *Gentiana* species that contributing to their pharmacological prominence (Hou *et al.*, 2020; Jia *et al.*, 2016). These compounds have been reported to exhibit anti-inflammatory, antioxidant, hepatoprotective, and metabolic regulatory activities (Cao *et al.*, 2021; Choi *et al.*, 2019). Moreover, analyses of volatile components have further demonstrated substantial chemical variability within the genus, reinforcing its medicinal significance (Georgieva *et al.*, 2005).

Currently, twenty-nine *Gentiana* species have been recorded from the Mongolian Plateau, with nine species traditionally employed in the treatment of digestive, dermatological, musculoskeletal, and respiratory ailments. Phytochemical screening of these

taxa has identified more than 600 compounds, including iridoids, triterpenoids, flavonoids, lignans, coumarins, xanthenes, alkaloids, fatty acids, amino acids, organic acids, and polysaccharides. Among these, gentiopicroside and swertiamarin have received the greatest research attention due to their pronounced biological activity (Hongzhen Yu & Batzaya G., 2025). Despite promising in vitro and in vivo results indicating broad therapeutic potential and low toxicity, clinical validation remains limited.

According to the IUCN Red List (2001), several *Gentiana* species are categorized as rare or threatened. *Gentiana algida* Pall. and *Gentiana macrophylla* Pall. are widely utilized in traditional medicine; however, ongoing habitat degradation and climatic shifts have led to declining natural populations, underscoring the urgency of developing cultivation and conservation strategies. Fundamental anatomical and morphological investigations are therefore essential to characterize these species and to establish baseline data for assessing their ecological adaptations.

Previous anatomical studies of *Gentiana* species have documented substantial variation in stem cross-sectional features and vascular bundle arrangements, reflecting adaptive responses to environmental stress (Chen *et al.*, 2018). Comparative analyses of leaf anatomy have further elucidated the development of specialized tissues associated with photosynthetic performance and stress tolerance (Li & Zhao, 2020). Notably, leaf structural traits often correlate with habitat conditions and water availability, highlighting their relevance for cultivation planning.

Seed coat structure and surface ornamentation, as revealed by scanning electron microscopy (SEM), provide valuable diagnostic characters for species identification and taxonomic clarification, thereby supporting conservation efforts for medicinal plants of limited distribution (Wang *et al.*, 2021). Integrating anatomical observations with ecological and phytochemical data may also improve understanding of how bioactive compounds are distributed within plant tissues, enhancing the pharmaceutical relevance of *Gentiana* species (Zhang *et al.*, 2020). Accordingly, the present study aimed to: (1) investigate the cellular and tissue organization of stems and leaves; (2) assess seed morphology, size, mass, and surface characteristics; (3) compare anatomical and morphological differences between *G. algida* and *G. macrophylla* in relation to environmental adaptation; and (4) provide a scientific basis for the conservation, cultivation, and sustainable management of these rare and valuable medicinal plants.

MATERIAL AND METHODS

Field Survey and specimen identification Field surveys were carried out from 24 July to 9 August 2023 in selected soums of Tuv, Arkhangai, and Khentii

provinces, which are situated within the Khangai and Khentii regions of the Mongolian phytogeographical zone. Specimens of two *Gentiana* species were collected at sites located near 47°08' N latitude and 100°56' E longitude (Figure 1). During the survey period, both seeds and whole plant materials were gathered for subsequent morphological and anatomical analyses.

Taxonomic identification of the collected specimens was performed by botanists N. Nyambayar and Ts. Battseren at the Botanical Garden and Research Institute, Mongolia. Voucher specimens were deposited in the Herbarium Fund of the Botanical Garden of the Mongolian Academy of Sciences (UBA) in October 2023 under accession numbers UBA0013196 and UBA0012854. The herbarium collections are publicly available for reference and verification.

The Botanical Garden of the Mongolian Academy of Sciences functions as a national institution under the authority of the Ministry of Environment and Tourism of Mongolia and is officially mandated to collect, conserve, and cultivate rare and very rare plant species using ex situ and in vitro approaches for scientific research. All fieldwork, including the sampling of *Gentiana algida* and *Gentiana macrophylla*, was conducted in compliance with national regulations and institutional ethical guidelines. These species are not classified as endangered or protected under the IUCN Red List or listed in the CITES Appendices; therefore, no special permits were required for sample collection.

Anatomical studies: Anatomical analyses of plant cross-sections were performed using conventional microtechnique approaches, including tissue sectioning and histological staining, to assess cellular organization and vascular structure (Esau, 1980). To ensure adequate preservation of tissue morphology and optimal visualization, both paraffin embedding and fresh freehand sectioning methods were employed following established protocols (Pausheva, 1974). Differential staining procedures, including Safranin–Fast Green and Toluidine Blue, were applied to distinguish major tissue types, allowing clear identification of vascular elements, sclerenchymatous tissues, and epidermal features (Gahan, 1984).

To complement light microscopic observations, scanning electron microscopy (SEM) and fluorescence microscopy were utilized to obtain high-resolution images of internal tissue arrangement and surface microstructures (Ruzin, 1999).

Ecological studies: The combined use of anatomical techniques within an ecological framework enables a more integrated evaluation of plant structural responses to environmental factors (Cutler *et al.*, 2007). Key climatic parameters, including recent changes in temperature and precipitation across the study areas, are summarized in Table 1.

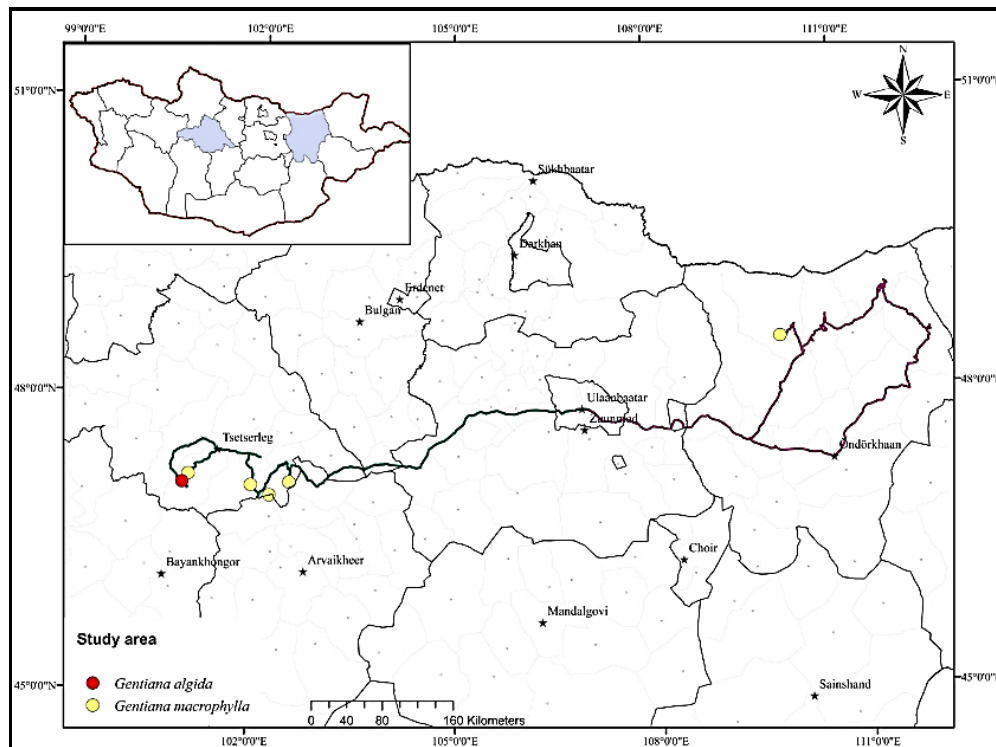


Figure 1. Field survey route

Table. 1. Key climatic parameters

Region / Province	Altitude (meters above sea level)*	Mean annual temperature change (°C, last 30 years)	Annual precipitation change (%)	Dominant climatic stress factors	Potential impact on <i>Gentiana</i> species
Tuv Province	1200–1500	+1.9 °C	–8 to –12 %	Temperature increase, drought	Reduced soil moisture, shortened growing season
Arkhangai Province	1600–2200	+2.1 °C	–10 to –15 %	Cold–warm fluctuation, drought	Stress on seed germination and seedling establishment
Khentii Province	1400–1800	+1.7 °C	–5 to –10 %	Reduced snowfall, dry springs	Altered phenology and habitat contraction

Seed measurements were carried out in accordance with standard analytical procedures. Seeds were counted in groups of hundreds or thousands and stored in paper envelopes prior to analysis. The mass of one thousand seeds was calculated based on three to five replicate measurements. Seed dimensions, including length and width, were determined using a digital calliper, with measurements taken from 50 to 100 seeds across three to five replicates. Seed length was defined as the distance from the hilum to the distal apex, whereas width was measured along the transverse axis. Seed thickness was assessed by measuring the transverse diameter from either the dorsal or ventral surface.

Seed coat surface characteristics were examined using a scanning electron microscope at magnifications of $\times 130$ and $\times 500$. SEM imaging enabled a detailed

assessment of seed surface architecture and micro-topographical features.

RESULT

The stem of *Gentiana macrophylla* displayed an oval outline in transverse section and was composed of a thin epidermal layer consisting of one to two rows of cells, followed by a prominently developed sclerenchyma zone, compact parenchymatous tissue with relatively small cells, and more than thirty circular vascular bundles arranged in a regular pattern (Figure 2). The epidermal layer serves a protective role, whereas the underlying sclerenchyma contributes to mechanical reinforcement, supporting stem rigidity and resistance to external mechanical stress

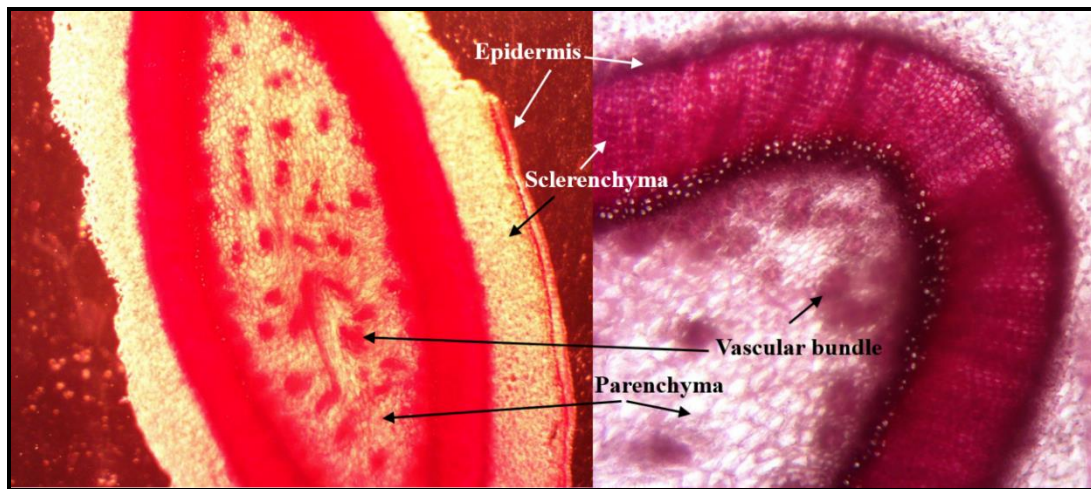


Figure 2. Stem anatomy of *Gentiana macrophylla*. Cross-sectional anatomy of the stem, highlighting the epidermis, sclerenchyma, parenchyma, and vascular bundles.

Parenchymatous cells were uniformly distributed across the stem and are likely involved in the storage and internal transport of nutrients, thereby contributing to the species' capacity to adapt to a range of ecological conditions. The presence of numerous circular vascular bundles arranged in an orderly manner throughout the stem suggests an efficient system for conducting water and nutrients from the root system to aerial tissues. Collectively, these anatomical features indicate that *Gentiana macrophylla* possesses structural traits that

enhance survival in high-altitude and temperate habitats. Leaves of *G. macrophylla* exhibited a typical dorsiventral organization, with the mesophyll differentiated into two layers of chlorenchyma and relatively weakly developed phloem tissue. The epidermal cells were characterized by thin cell walls and a linear arrangement, while the vascular bundles displayed a concentric configuration and were enclosed by surrounding parenchyma cells (Figure 3).

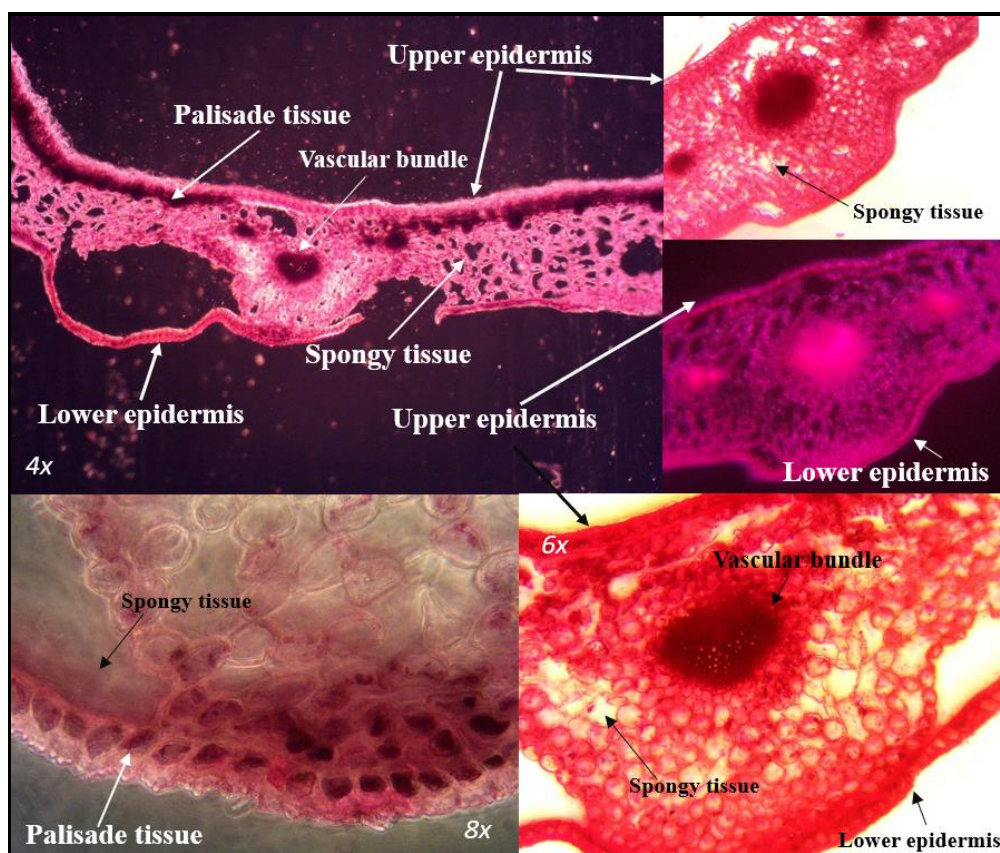


Figure 3. Leaf anatomy of *Gentiana macrophylla*. Cross-sectional view of the leaf showing the upper and lower epidermis, palisade tissue, spongy tissue, and vascular bundles at different magnifications.

In transverse section, leaves of *Gentiana macrophylla* exhibited clearly differentiated adaxial and abaxial epidermal layers that function as protective barriers against environmental stressors. The palisade mesophyll was composed of two layers of chlorenchymatous cells densely enriched with chloroplasts, supporting enhanced photosynthetic capacity. Below this region, the spongy mesophyll consisted of loosely arranged cells with well-developed intercellular spaces, promoting efficient gas exchange and transpiration. The vascular bundles were embedded within parenchymatous tissue and serve as conduits for the translocation of water, mineral nutrients, and assimilates throughout the leaf.

Together, these structural attributes suggest that *G. macrophylla* possesses a leaf architecture well suited for effective light interception and physiological performance under variable environmental conditions.

By contrast, the stem of *Gentiana algida* displayed a cylindrical outline in cross-section and was characterized by a thin epidermal layer composed of one to two cell layers, followed by a prominently developed sclerenchymatous zone and compact parenchyma consisting of uniformly small cells. A single ring of small vascular bundles was arranged concentrically within the parenchymatous tissue, and the stem featured a distinct hollow central cavity (Figure 4).

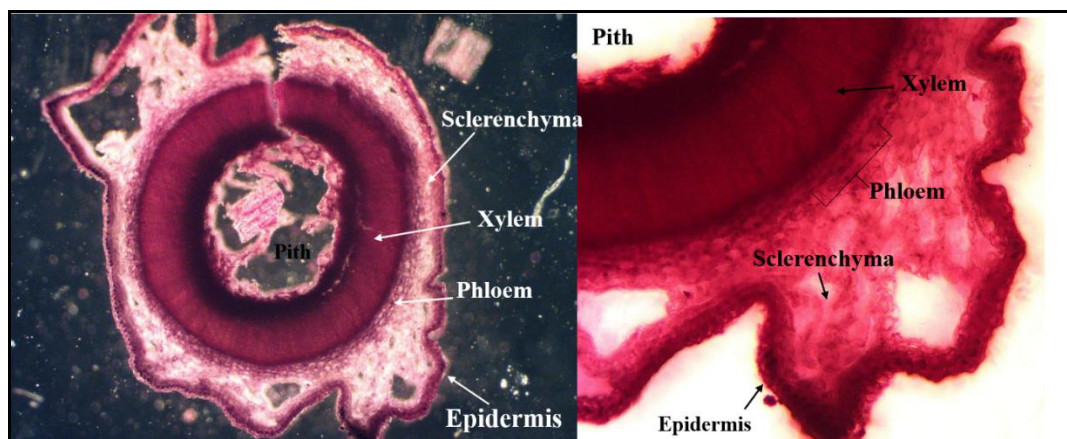


Figure 4. Stem anatomy of *Gentiana algida*. Cross-sectional anatomy of the stem illustrating the epidermis, sclerenchyma, parenchyma, and vascular bundles.

In *Gentiana algida*, the epidermal layer functions as the primary protective barrier, whereas the subjacent sclerenchyma provides mechanical reinforcement and enhances resistance to environmental stress. Parenchymatous tissue was arranged around a prominent central lumen, which represents a distinctive anatomical characteristic of this species. The concentric distribution of vascular bundles supports the effective transport of water and nutrients throughout the stem. Collectively, these features indicate that *G. algida* exhibits specialized structural adaptations that facilitate resource conduction and structural resilience in cold, high-altitude environments.

Leaves of *G. algida* also displayed a dorsiventral anatomical organization and were composed of two to three layers of chlorenchymatous tissue, accompanied by relatively sparse pubescence. The epidermal cells were characterized by thin cell walls arranged in a linear pattern, while the vascular bundles were radially distributed and enclosed by surrounding parenchyma cells (Figure 5).

As illustrated in Figure 5, transverse sections of *Gentiana algida* leaves revealed clearly differentiated adaxial and abaxial epidermal layers that function in protection against external environmental factors. Immediately beneath the upper epidermis, a compact palisade mesophyll layer was present, indicating its primary role in photosynthetic activity. The underlying

spongy mesophyll was composed of loosely arranged cells with abundant intercellular spaces, which facilitate gaseous exchange within the leaf tissue. Vascular bundles, consisting of xylem and phloem elements and embedded in parenchymatous tissue, are responsible for the internal conduction of water and nutrients throughout the leaf.

Seed morphometric analysis of *Gentiana macrophylla* was based on measurements of 100 seeds. The mean seed length was 1.17 ± 0.73 mm, while the average width measured 0.62 ± 0.40 mm. The seeds were orange in coloration, and their surfaces exhibited elongated, groove-like ornamentation reminiscent of riverbed patterns. The mass of one thousand seeds ranged between 0.150 and 0.153 g. Observations using scanning electron microscopy (SEM) demonstrated that the seed surface displayed a pronounced reticulate pattern. This surface architecture consisted of interconnected ridges and depressions forming a mesh-like network, with the seed coat (testa) characterized by polygonal epidermal cells with prominently raised boundaries. The overall micro-topography was defined by gentle elevations and shallow depressions.

Such surface ornamentation is likely associated with ecological functions, including seed dispersal efficiency, water uptake, or attachment to potential dispersal vectors. The anticlinal cell walls were notably thickened and elevated, whereas the periclinal walls

exhibited varying degrees of concavity. Reticulate surface patterns of this type are widely reported among *Gentiana* species and may therefore possess taxonomic relevance. Consequently, the distinctive

micromorphological features observed in the SEM images may serve as useful diagnostic characters for species identification and classification within the genus (Figure 6).

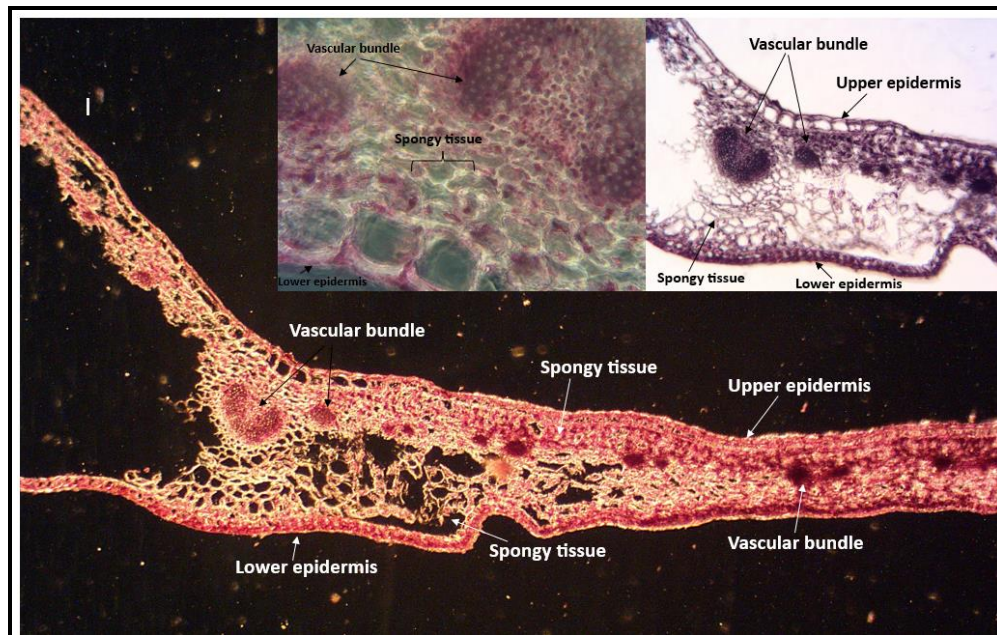


Figure 5 Leaf anatomy of *Gentiana algida*. Cross-sectional view of the leaf showing the epidermis, palisade tissue, spongy tissue, and vascular bundles at different magnifications.

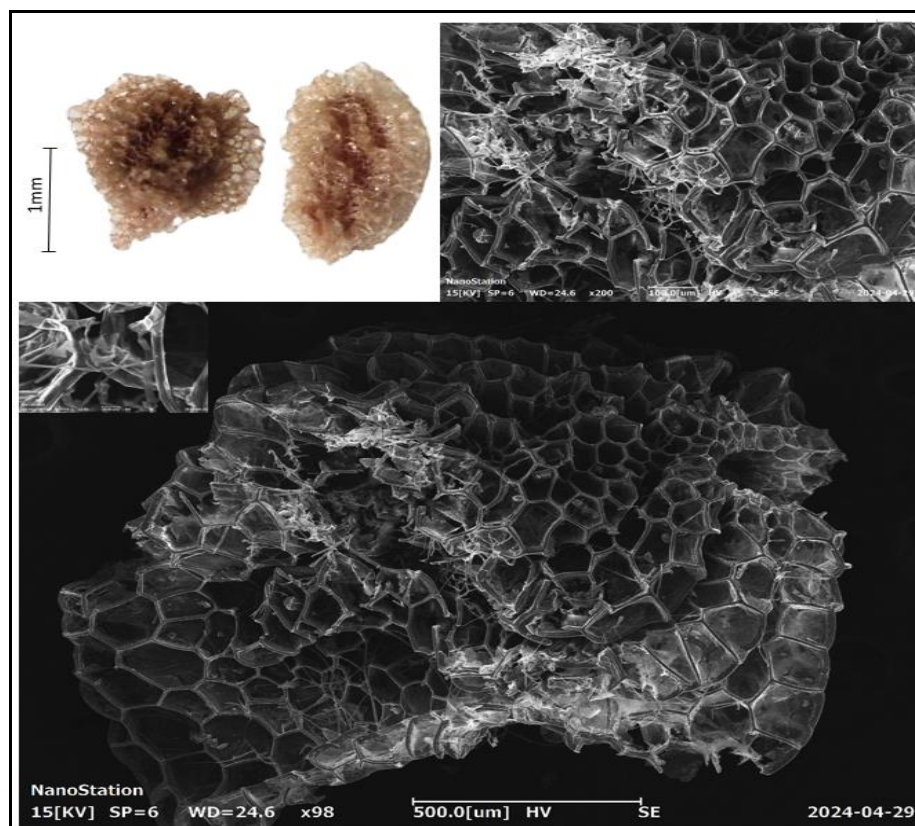


Figure 6. Seed surface morphology of *Gentiana macrophylla*. Macroscopic and SEM images highlighting seed coat patterns and microstructures at magnifications of $\times 130$ and $\times 500$.

Measurements of 100 seeds of *Gentiana algida* revealed an average length of 1.89 ± 0.94 mm and a

width of 1.71 ± 1.17 mm. The seeds were yellowish white in color and exhibited a frothy surface texture.

The mass of one thousand seeds ranged from 0.193 to 0.203 g. SEM observations showed that the seed surface of *Gentiana algida* exhibited a consistent reticulate pattern without trichomes. The surface appeared textured, with intricate ridges and grooves that may play a role in seed dispersal or water absorption. The seed coat displayed a net-like reticulate structure composed of polygonal cells arranged in a honeycomb-like pattern, with clearly defined boundaries between adjacent cells. The primary level of seed coat ornamentation was characterized by a reticulate framework formed by prominently raised ridges, whereas secondary ornamentation was expressed as shallow undulations or depressions within the cell lumina. Tertiary ornamentation consisted of fine micro-textural features that became apparent only at higher magnifications. According to standard seed coat terminology, this surface configuration corresponds to a reticulate type, more specifically a macro-reticulate pattern, as the polygonal network is clearly visible at lower magnifications and displays a regular to semi-regular arrangement.

Within palynological and seed morphological classifications, reticulate ornamentation represents one of the principal categories of seed surface structure. This type is commonly distinguished alongside other surface patterns such as psilate (smooth), rugose, striate, foveolate, papillate, and verrucae ornamentation.

DISCUSSION

This study establishes baseline anatomical and morphological information for *Gentiana algida* and *Gentiana macrophylla*, aiming to characterize their structural features and to evaluate potential adaptive responses to environmental conditions. The combined use of conventional anatomical approaches and advanced imaging techniques enhances the documentation of plant genetic resources and provides a scientific basis for their sustainable utilization while preserving species-specific structural traits.

Comparative analysis of stem and leaf anatomy revealed clear interspecific differences, indicating that tissue organization is a key factor influencing adaptation to local environmental pressures (Chen *et al.*, 2018). In particular, contrasting vascular bundle configurations concentric in one species and radial in the other suggest structural divergence that may be associated with differences in physiological performance and internal resource distribution (Li & Zhao, 2020).

Differences in seed morphology further reflect adaptive differentiation between the two species. The relatively larger seeds of *G. algida* compared with *G. macrophylla* may affect dispersal capacity and germination success under the fluctuating and often extreme climatic conditions characteristic of high-altitude regions (Wang *et al.*, 2021). These observations highlight the importance of integrating

anatomical, ecological, and reproductive characteristics to gain a more comprehensive understanding of adaptive strategies in *Gentiana* species within their natural habitats (Zhang *et al.*, 2020).

Earlier photographic studies documenting seeds of rare and medicinal plants in Mongolia reported morphometric traits of *G. algida* seeds (Tserenbaljid, 2013). In contrast, the present investigation employed scanning electron microscopy to examine seed surface micromorphology in greater detail, revealing a consistently reticulate ornamentation pattern. In *G. macrophylla*, the seed coat displayed a foamy surface texture with a uniform crystalline reticulate structure. To our knowledge, this represents the first detailed SEM-based description of seed surface micromorphology for this rare species.

Future research should prioritize the identification of genetic factors underlying anatomical and morphological variation in *Gentiana* species to better clarify their adaptive capacity and resilience under ongoing climate change (Chen *et al.*, 2018). Moreover, exploring the relationship between leaf anatomical traits and photosynthetic efficiency may provide deeper insight into how these species optimize energy acquisition in resource-limited environments (Li & Zhao, 2020). Expanding comparative analyses to include additional *Gentiana* taxa from diverse ecological regions would further strengthen conservation planning and improve understanding of evolutionary differentiation within the genus (Wang *et al.*, 2021).

Among the two studied species, *Gentiana algida* demonstrated greater anatomical and morphological adaptation to climate change, particularly to cold and high-altitude environmental conditions, as reflected by its stem structure, vascular bundle arrangement, and seed characteristics.

The integration of field-based sampling with laboratory analyses, including scanning electron microscopy, enabled a comprehensive assessment of anatomical and morphological traits that would not have been achievable through a single methodological approach. This combined framework provides a robust reference for future botanical, ecological, and conservation-oriented studies of *Gentiana* species.

CONCLUSION

The integration of field-based sampling with laboratory analyses, including scanning electron microscopy, enabled a comprehensive assessment of anatomical and morphological traits of two important medicinal plant species of Mongolia that would not have been achievable through a single methodological approach. This combined framework provides a robust reference for future botanical and ecological investigations of *Gentiana* species.

AUTHORS CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Conceptualization, D.G., B.T., and A.B.; Formal analysis, D.G.; Funding acquisition, A.B., and B.T.; Investigation, D.G., L.B., and B.T.; Methodology, D.G., B.T., A.B., H.Y., T.L., X.U., W.W., and B.G.; Project administration, B.T., A.B., U.M., and B.G.; Resources, D.G., and B.T.; Software, D.G. and L.B.; Supervision, B.T., and L.B.; Validation, D.G., L.B., and B.T.; Visualization, D.G. and L.B.; Writing—original draft, D.G.; Writing review and editing, L.B. All authors have read and agreed to the published version of the manuscript.

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