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ANATOMICAL STUDIES OF VEGETATIVE ORGANS IN TWO GENTIANA SPECIES (GENTIANACEAE)

The subfamily Gentianinae includes approximately 425 species, with many belonging to the extensively studied genus *Gentiana*. These species are predominantly distributed across the Eurasian continent. The Gentianaceae family is notable for its biologically active compounds and is utilized in traditional medicine for its antidiabetic, hepatoprotective, digestive, antidepressant, and antianemic properties. Purpose of the study – Almaty region, Ile district, Kazakhstan village, south-western edge of Kungey Alatau, pasture of Il river, north-eastern slope and Almaty region, Karasay district Kaskelen gorge in 2023-2024 to determine the anatomical features of vegetative organs of two collected species. Materials and Methods: Sections of the roots, stems, and leaves of *Gentiana olivieri* Griseb. and *Gentiana tianschanica* were prepared using a sliding microtome and analyzed under a light microscope. Results: The comparative anatomical analysis of the vegetative organs of *G. olivieri* and *G. tianschanica* revealed both similarities and differences. The significant anatomical feature observed was that the cells of the upper epidermis in the leaves of both species were smaller than those of the lower epidermis. Notably, the upper epidermis of the leaves lacked stomata. The vegetative organs of both species exhibited xeromesophytic characteristics in *G. olivieri* and mesophytic features in *G. tianschanica*. These anatomical features align with existing knowledge on Gentianaceae anatomy. Conclusion: The roots of *G. olivieri* displayed intraxillary phloem, a distinguishing trait of the Gentianoideae and Menyanthoideae subfamilies.

Key words: *Gentiana olivieri*, *Gentiana tianschanica*, Gentianaceae, root, stem and leaf anatomy.

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Gentiana туысындағы (Gentianaceae) екі түрінің вегетациялық мүшелерін анатомиялық зерттеу

Gentianinae тұқымдасының 425-ке жуық түрі бар, олардың көпшілігі жақсы зерттелген Gentiana тұқымдасына жатады және негізінен Еуразия континентінде таралған Gentianaceae, көптеген биологиялық белсенді қосылыстары бар, қант диабетіне қарсы, гепатопротекторлық, ас қорытуды, антидепрессант және антианемия ретінде қолданылады. халықтық медицинада. Зерттеу мақсаты – Алматы облысы, Іле ауданы, Қазақстан ауылы, Іле Алатауының оңтүстік-батыс шеті, Іле өзенінің жайылымы, солтүстік-шығыс беткейі және Алматы облысы, Қарасай ауданы Қаскелең шатқалының 2023-2024 жж. Жиналған екі түрдің вегетативтік мүшелерінің анатомиялық ерекшеліктерін анықтау. Материалдар мен әдістер*: G. olivieri* тамырының, сабағының және жапырақтарының бөлімдері. және *G. tianschanica* жылжымалы микротом арқылы алынып,

жарық микроскопында зерттелді. Нәтижелері: *G. olivieri* вегетативті мүшелерінің салыстырмалы анатомиялық талдауы. және *G. tianschanica* зерттелген үлгілер арасындағы ұқсастықтар мен айырмашылықтарды ашты. 5% маңыздылық деңгейі белгіленді. *G. olivieri* жапырақтарының анатомиялық құрылымының негізгі анықталған белгісі. және *G. tianschanica* төменгі эпидермис жасушаларынан кішірек болатын жоғарғы эпидермис жасушаларының болуы болды. Жоғарғы жапырақ эпидермисінде устьица болмайды. Екі түрдің де вегетативтік мүшелері (тамырлары, сабақтары және жапырақтары) Г.Оливьериде анатомиялық-морфологиялық құрылымның ксеромезофиттік белгілерінің және G.tianschanica-да ұйымдасуының мезофитті ерекшеліктерінің болуын көрсетеді. Түрдің анатомиялық ерекшеліктері Gentianaceae анатомиясы бойынша бар нәтижелерге ұқсас болды. Қорытынды: G. olivieri Griseb тамырында. ең алдымен Gentianoideae және Menyanthoideae топшаларын ажырататын интраксилярлы флоэма табылды.

Түйін сөздер: *Gentiana olivieri*, Gentianaceae, тамырдың, сабақтың және жапырақтардың анатомиясы.

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Анатомические исследования вегетативных органов двух видов семейства Горечавковых (Gentianaceae)

Подсемейство Gentianinae насчитывает около 425 видов, большинство из которых относится к хорошо изученному роду Gentiana и распространено в основном на Евразийском континенте Gentianaceae, имеющее множество биологически активных соединений, используется как противодиабетическое, гепатопротекторное, пищеварительное, антидепрессивное и антианемическое средство в народной медицине. Цель исследования – Алматинская область, Илийский район, село Казахстан, юго-западная окраина Кунгей Алатау, пастбище реки Или, северо-восточный склон и Алматинская область, Карасайский район, Каскеленское ущелье в 2023-2024 гг. для определения анатомических особенностей вегетативных органы двух собранных видов. Материалы и методы: Срезы корней, стеблей и листьев *G. olivieri* и *G. tianschanica* были взяты с помощью скользящего микротома и изучены под световым микроскопом. Результаты: Сравнительно-анатомический анализ вегетативных органов *G. olivieri* и *G. tianschanica* выявил сходства и различия между исследуемыми образцами. Был установлен 5-процентный уровень значимости. Основной выявленной особенностью анатомического строения листьев *G. olivieri* и *G. tianschanica* было наличие клеток верхнего эпидермиса, которые были меньше по размеру, чем клетки нижнего эпидермиса. В верхней эпидерме листа отсутствуют стоматы. Вегетативные органы (корни, стебли и листья) обоих видов указывают на наличие ксеромезофитных черт анатомо-морфологического строения у *G. olivieri* и мезофитных черт организации у *G. tianschanica*. Анатомические особенности видов были сходны с существующими результатами по анатомии Gentianaceae. Выводы: В корнях *G. olivieri* обнаружена интраксиллярная флоэма, что в первую очередь отличает подсемейства Gentianoideae и Menyanthoideae.

Ключевые слова: *Gentiana olivieri*, Gentianaceae, анатомия корня, стебля и листьев.

Introduction

Many species within the Gentianaceae family possess ornamental value, particularly those of the genus *Gentiana*. Additionally, these species are of significant pharmaceutical interest due to their unique phytochemical properties. In medicinal preparations, Gentianaceae species are valued for their high content of iridoids, which impart a characteristic bitter taste. These compounds are used in the formulation of bitter drinks traditionally prescribed for appetite loss and fever and are integral to many tonic (bitter) recipes.

Several species of *Gentiana* have been recognized for centuries, particularly in the Far East. Herbal preparations such as longdan and qinjiao, which include these plants, are employed to treat conditions such as hepatitis, constipation, rheumatism, pain, hypertension, anorexia, and inflammation [1].

Gentiana olivieri (Gentianaceae) is found from the Middle East (including Turkey, Iran, Iraq, Afghanistan) to East Asia (reaching the Tien Shan) and is an Iranian-Turanian species. This perennial herb grows on limestone, marl, or clay slopes and grassy meadows at altitudes ranging from 350 to 2300 meters. It typically reaches a height of 10-30 cm from the basal rosette. The rhizome features a taproot with a fibrous collar at the top. The plant blooms with dark blue-violet flowers from April to July. Its seeds are brown, ellipsoid, and 0.8-1 mm long, with finely reticulated achenes [2,3].

G. olivieri has been utilized as a medicinal plant in traditional folk medicine for centuries, owing to its content of secoiridoids, flavonoids, and alkaloids. Known as "Afat" in Turkey and "Ager" and "Banger" in Pakistan [4,5], this species is used in Turkish folk medicine as an antidiabetic, sedative, digestive, and antianemic agent [6,7]. Research has demonstrated its antidiabetic, antihepatotoxic, antinociceptive, anti-inflammatory, and antiulcerogenic activities [4,8-10].

In the Republic of Uzbekistan, *G. olivieri* has been employed to treat diarrhea, colds, stomach pain, and indigestion [11]. In Pakistan, the plant has been studied for its antibacterial, antifungal, antihypertensive, toxicological, and diuretic properties [12-14]. Despite numerous studies exploring new compounds using chemical methods [15-17] and tissue culture [18], there is a notable lack of data on the anatomical characteristics of *G. olivieri* Metcalfe and Chalk [19] conducted general anatomical studies on the Gentianaceae family, based on the work of Perrot and Martens [21], focusing on wood and seed architecture, pollen morphology, and ultrastructural diversity. Root architecture has been studied by Budimir et al. and Sotnikova and Lux [22] for *G. lutea* and *G. asclepiadea*, respectively. This study aims to investigate the anatomical characteristics of *G. olivieri* to facilitate further research.

A substantial body of anatomical studies exists on this family, especially concerning species with recognized medicinal properties [23]. Recent research has provided significant insights into the ontogenetic processes governing root development [24] and the structural organization of the stomatal system [25].

Purpose of the study – Almaty region, Ile district, Kazakhstan village, south-western edge of Kungey Alatau, pasture of Ile river, north-eastern slope and Almaty region, Karasay district Kaskelen gorge in 2023-2024 to determine the anatomical features of vegetative organs of two collected species.

Materials and methods

Study Area

The material for this study was collected from the wild in May 2023 and includes two species from the Gentianaceae family. *G. olivieri* (Olivier's gentian) was gathered in a geobotanical reserve, while *G. tianschanica* (Tian Shan gentian), a perennial xeromesophytic species with a Eurasian distribution, was also collected.

Flowering specimens of *G. olivieri* Griseb. were collected in the spring in the Almaty region, Ili district, approximately 10 km east of the Kapchagay highway at coordinates N 43°58′07.8″, E 077°01′10.7″ [Figure 1]. *G. tianschanica* was collected in early August in the Almaty region, Karasai district, within the Kaskelen Gorge at coordinates N 43°04′45″, E 076°58′56″ (Kazakhstan). The identification of the samples was confirmed using the key provided in the "Flora of Kazakhstan" [26]. The voucher samples were transferred for storage to the herbarium fund of the "Institute of Botany and Phytointroduction", Almaty, Kazakhstan. Herbarium № 6509.

For the anatomical study, the collected plant material was fixed in 70% ethanol. To prepare cross-sections of mature roots, stems, and leaves, the paraffin embedding method was used.

The sectioning and staining procedures followed Johansen's methods (1940) with some modifications. Plant materials, consisting of 3 mm sections of roots, stems, and leaves, were initially fixed in 70% ethanol for 48 hours. They were then processed through a series of increasing ethanol concentrations (70%, 90%, and 96%) and xylol before being embedded in paraffin. Sections were cut at a thickness of 12–18 μm using a sliding microtome. To remove the paraffin, the samples were heated at 65°C, then passed through xylol and ethyl alcohol series and soaked overnight in safranin. They were subsequently stained with Fast Green for 20 seconds. Measurements and photographs were taken with a Leica DM750 light microscope equipped with a camera [27].

For quantitative analysis, morphometric features were measured using an ocular micrometer (MOV-1-15) at an objective magnification of ×9 and an ocular magnification of ×10. Microphotographs of the anatomical sections were captured using an MC 300 microscope (Micros, Vienna, Austria) with a CAM V400/1.3 M video camera (jProbe, Tokyo, Japan). Microscopic examination of the medicinal plant materials was performed at the Laboratory of Plant Anatomy and Morphology at Al-Farabi Kazakh National University [28-30].

Figure 1 – *G. olivieri* morphology

Results and discussion

Root

The outer surface of the root is covered by a periderm (pe) consisting of thin-layered, crushed, and fragmented isodiametric cells. The bark exhibits a multilayered structure with crushed cells and large intercellular spaces. Mycorrhizae were present in the root system [31]. In regions where adventitious roots emerge, cells with 7-8 layers of irregularly shaped cortical cells were observed. The endodermis is well-defined and comprised of longitudinally elongated cells, with some cells showing complete thickening.

The central cylinder contains vascular bundles with xylem vessels that have thickened and lignified walls. The bundles lack cambium, and phloem elements are minimally represented, embedded within a mass of cellulose. The vascular bundles are collateral, diarchic, and devoid of cambium. In the interfascicular regions, there is a broad parenchymatous phloem with radially symmetric cells. Sieve tubes and companion cells are rare and are positioned adjacent to the xylem zone.

Morphometric data reveal that the diameter of the tracheids in *G. tianschanica* is 35.41 μm, whereas in *G. olivieri*, it is smaller. The tracheids of *G. tianschanica* are approximately twice as large, indicating a higher efficiency in water and mineral transport. The width of the phloem elements in the roots of *G. tianschanica* measures 14.51 μm, while in *G. olivieri*, it is slightly greater, suggesting a higher capacity for accumulating organic substances.

The length of the endodermis is consistent between the two species, but its width varies with the diameter of the section. The width of the cortex is similar in both species: 28.36 μm in *G. olivieri* and 28.15 μm in *G. tianschanica*. However, the length of the cortex is 1.5 times greater in *G. olivieri*, measuring 66.12 μm compared to 54.03 μm in *G. tianschanica*, reflecting variations depending on the root diameter analyzed. Comparative morphometric indices of the roots are summarized in Table 1.

Stem. The comparative anatomical and morphological analysis of the stems of *G. tianschanica* and *G. olivieri* revealed both similarities and differences between the two species.

In *G. olivieri* a sclerenchymatous ring is located beneath the endoderm. This ring is composed of small, multilayered cells encircling the central cylinder, which consists of parenchymatous tissue with thin primary cell walls. The anatomical and morphological study indicates that the formation of conductive tissues in *G. olivieri* is likely influenced by the humidity gradient of its habitat.

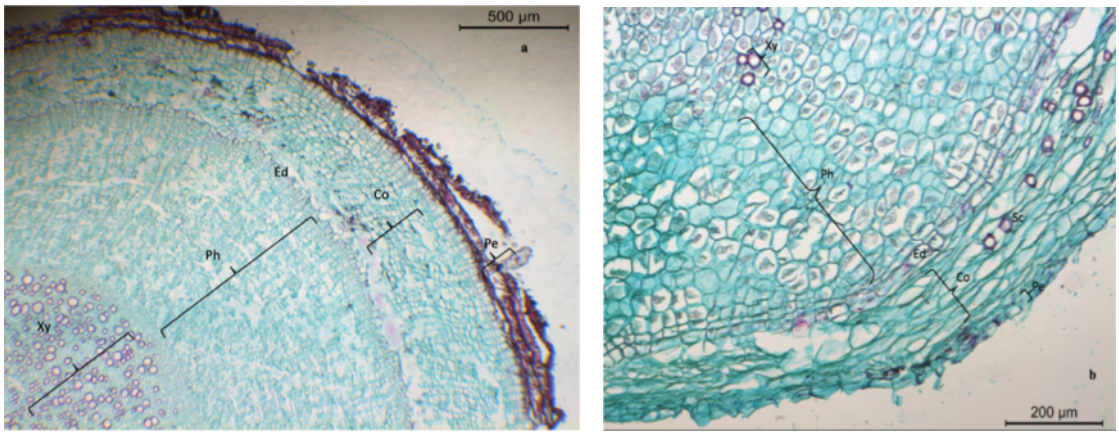

Figure 2 – Cross-section of the root of (a) G. tianschanica Rupr. (b). G. olivieri Griseb. Periderm (Pe), cortex (Co), endodermis (Ed), phloem (Ph), xylem (Xy).

Table 1 – Comparative root morphometry of *G. olivieri* and G. *tianschanica.* Significant differences are marked with an asterisk.

Root									
Pant name		Trachea	Tracheid	Floema		Endodermis		Cortex	
		Diameter (µm)	Diameter (µm)	Width (µm)	Length (µm)	Length (µm)	Width (µm)	Width (µm)	Length (µm)
G. olivieri	n	9	9	9	9	9	9	9	9
	Average	17,53 ± 1,65	10,33 ± 1,11	18,22 ± 2,93	28,31 ±6,19	35,12 ± 12,89	20,17 ± 2,13	28,36 ± 9,11	66,12 ±19,20
	Stan. error.	0,55	0,37	0,98	2,06	4,30	0,71	3,04	6,40
G. tianschanica	n	10	10	10	10	10	10	10	10
	Average	35,41 ± 6,48	13,97 ± 1,86	14,51 ± 2,67	24,53 ± 5,26	35,10 ± 7,54	35,81 ± 7,61	28,15 ± 7,78	54,03 ± 9,09
	Stan. error.	2,05	0,59	0,85	1,66	2,38	2,41	2,46	2,87

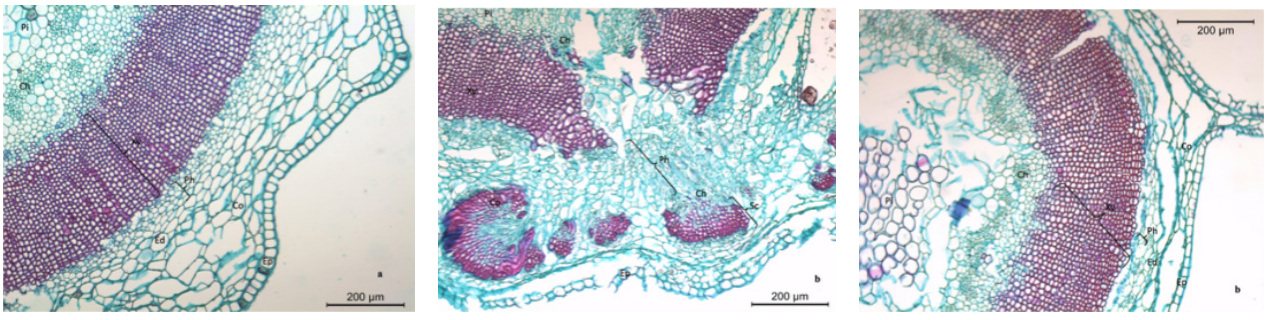

Figure 3 – Cross-section of the root: a) *G. tianschanica***,** b) *G. olivieri* Labels include periderm (Pe), cortex (Co), endodermis (Ed), phloem (Ph), and xylem (Xy).
Distinctive features of the stems are:

The peripheral part of the core is distinctly defined as a perimedullary zone, characterized by smaller, thick-walled cells.

The core is represented by pronounced parenchyma with small cells. Notable cuticulation of stem walls and a well-defined primary bark suggest

xerophytic adaptations in *G. olivieri* while *G. tianschanica* displays mesophytic characteristics. Consequently, there are distinct anatomical differences between the plants from the two study areas.

Significant differences, defined as variations of 5 μm or more, are indicated with an asterisk in Table 2.

As shown in Table 2, the most correlated indicators in the stem when comparing the two species include the diameter of the tracheids, the length of the phloem, the diameter of the core, the width and length of the endodermis, the width of the cortex, and the length and width of the epidermis.

Table 2 – Comparative morphometry of the stem of *G. olivieri* and *G. tianschanica.*

Stem												
Plant name		Trachea	Tracheid	Pith	Floema		Endodermis		Cortex		Epidermis	
		Diameter (μm)	Diameter (μm)	Diameter (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)
G. olivieri	n	9	9	9	9	9	9	9	9	9	9	9
	Average	19,85 ± 4,13*	7,44 ± 1,01	35,43 ± 6,91*	12,56 ± 3,37	23,62 ± 4,67*	15,43 ± 4,06*	33,73 ± 8,11*	40,11 ± 6,75*	71,78 ± 11,39	17,15 ±2,47 *	20,27 ± 2,53*
	Stan. error.	1,38	0,34	2,30	1,12	1,56	1,35	2,70	2,25	3,80	0,82	0,84
G. tianschanica	n	10	10	10	10	10	10	10	10	10	10	10
	Average	14,35 ± 1,40*	6,88 ± 0,73	23,55 ± 5,40*	11,07 ± 2,17	18,87 ± 1,73*	24,87 ± 4,34*	42,10 ± 4,44*	27,83 ± 4,83*	68,38 ± 13,33	21,16 ± 1,87*	27,83 ± 2,63*
	Stan. error.	0,44	0,23	1,71	0,68	0,55	1,37	1,40	1,53	4,21	0,59	0,83

Leaves: Examination of the leaf cross-section (Figure 4) reveals several types of tissue: the integumentary tissue includes the epidermis, with the upper epidermis differing from the lower. The cells of the upper epidermis are smaller, and there are few or no stomata, indicating that the leaf is hypostomatic. The mesophyll, situated between the upper and lower epidermis, contains collateral vascular bundles. The xylem is positioned on the upper side of the leaf blade, while the phloem is on the lower side. Each conducting bundle is accompanied by mechanical tissue–angular collenchyma. Similarities between the leaves are summarized in Table 3. Numerous air cavities were observed in the leaf blade. Significant differences of 5 μm or more are indicated with an asterisk in Table 3.

Distinctive Features of the Leaves are :

1. In *G. tianschanica*, the upper epidermal cells are smaller compared to those of the lower epidermis, and there are no stomata on the upper epidermis (Figure 4). The leaf is hypostomatic.

2. The mesophyll in *G. tianschanica* is looser, and there are numerous prismatic crystals of calcium oxalate. Trichomes are absent, highlighting the mesophytic nature of the leaf.

3. In *G. olivieri* the vascular bundles are smaller and surrounded by a well-defined sclerenchymatous sheath. The vascular bundle within the endodermis is clearly visible and features alternating large and small rounded cells.

Atmospheric drought, exacerbated by strong, dry winds, results in soil moisture deficits, leading to plant stress, reduced productivity, and lower yields. This stress impacts both the structure and function of plants, posing challenges for effective forest management. Significant morpho-anatomical and hydraulic changes in vegetative organs and growth patterns were observed. Drought caused notable anatomical changes in *G. olivieri* including increased thickness of the palisade and spongy mesophyll as well as the abaxial and adaxial epidermis. According to existing literature [32], a correlation analysis of ash traits indicates drought resistance. However, the relationship between morpho-anatomical traits of leaves was similarly affected by drought across all studied species, suggesting no clear distinguishing features for drought resistance [33].

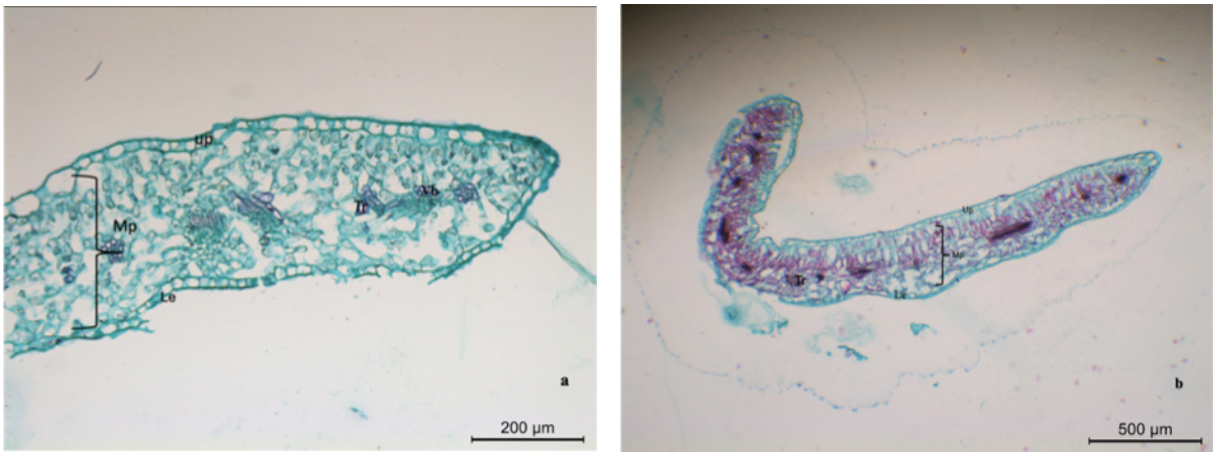

Figure 4 – Transverse section of leaves (a); *G. tianschanica* and (b); *G. olivieri*:
Upper epidermis (Up), vascular bundles (Vb), lower epidermis (Le), mesophyll (Mp), trake (Tr).

Table 3 – Comparative morphometry of leaves of *G. olivieri* Griseb and *G. tianschanica*

Leaves					
Plant name		Mesophile	Epidermis	Trachea	
		Length (µm)	Width (µm)	Length (µm)	Width (µm)
G. olivieri	n	9	9	9	9
	Average	226,16 ± 21,39*	44,34 ± 12,77*	25,02 ± 5,01*	11,22 ± 1,77
	Stan. error.	7,13	4,26	1,67	0,59
G. tianschanica	n	10	10	10	10
	Average	198,77 ± 22,75*	34,91 ± 8,15*	20,61±3,06*	10,25 ± 1,84
	Stan. error.	7,19	2,58	0,97	0,58

The anatomical features of *G. tianschanica* have not been previously studied. The diversity in the anatomical structure of high-mountain species reflects their varied adaptive strategies. Xeromorphic characteristics include reduced leaf blade size, a small-celled epidermis, abundant stomata, an isolateral or dorsoventral leaf mesophyll structure, well-developed palisade parenchyma, and, in some species, the formation of sclerenchymatous fibers and water-storage tissues. These anatomical features support plant survival in harsh cryophilic conditions. Although studies on the anatomy of this plant family are limited, recent work by Jansen and Smets has examined the wood anatomy of *Anthocleista*, *Fagraea*, *Lisianthus*, *Macrocarpaea*, *Nuxia*, *Symbolanthus*, and *Tachiadenus* within the Gentianales [34]. Radial and transverse section data provided by Carlquist and Grant also highlighted the presence of pits in tracheid openings [35]. Differences between species such as *Symbolanthus macranthus*, *Tachia occidentalis*, and 17 species of *Macrocarpaea* within the tribe Helieae were detailed in studies of stem wood anatomy.

The anatomical features of *G. olivieri* were compared using the framework from "Anatomy of Dicots" due to the limited anatomical studies on the Gentianaceae family. The anatomical characteristics of *G. olivieri* were found to align with those of the Gentianaceae family. Notably, a key anatomical difference between the subfamilies Gentianoideae and Menyanthoideae is the presence of intraradicular phloem in Gentianoideae roots, a feature observed in *G. olivieri*. Adventitious roots and rays were absent, and vessels were present both singly and in groups. The endodermis of *G. olivieri* was single-layered, longitudinally elongated, and featured Casparian strips on the radial walls, whereas some species of the genus *Swertia* have a bilayered endodermis. The papillary epidermis on the stem is a common feature of Gentianoideae. The stems of *G. olivieri*, like

those of *G. asclepiadea* and *G. septemfida*, exhibit two sclerenchymatous rings and multiple vascular bundles. In contrast, the pith of *G. pneumonanthe* was partially sclerotic, while *Lehmanniella* and *Senea* had entirely sclerotic piths. The pith of *G. olivieri* consisted of parenchymatous cells. Although crystals were observed in the cortex and pith of *Enicostemma*, they were not found in *G. olivieri*.

Conclusion

High-altitude plants have evolved significant adaptive capacities to survive and reproduce under extreme stress conditions. The anatomical study of the vegetative organs (roots, stems, and leaves) of *G. olivieri* and *G. tianschanica* indicates xeromesophytic features in *G. olivieri* and mesophytic characteristics in *G. tianschanica*.

Xeromesophytic features of *G. olivieri* include: stiff leaves with a well-developed cuticle, well-developed mechanical tissue (sclerenchyma) in the stems, vascular bundles with a prominent sclerenchymatous sheath, long roots, and a relatively smaller root diameter.

Mesophytic characteristics of *G. tianschanica* include: more friable parenchyma in roots, stems, and leaves, numerous crystalline inclusions of calcium oxalate in leaves, absence of trichomes, and a large number of air cavities in the leaf mesophyll.

This comparative anatomical study of *G. olivieri* and *G. tianschanica* enhances our understanding of the anatomy of these species in their respective habitats: *G. olivieri* in the Almaty region, Iliyskiy district, and *G. tianschanica* in the Almaty region, Karasay district, Kaskelen gorge (Kazakhstan).

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