#### REPUBLIC OF AZERBAIJAN

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#### **ABSTRACT**

of the dissertation for the degree of Doctor of Philosophy in Biology

### SYSTEMATICS AND BIOECOLOGICAL FEATURES OF GENTIANA (GENTIANA L.) GENUS IN THE FLORA OF AZERBAIJAN

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#### INTRODUCTION

Relevance and degree of development of the topic. In modern times, the significant impoverishment of the plant gene pool leads to the disruption of the normal functioning of natural ecosystems, reduces the potential of evolution, deprives humans of the opportunity to use their useful qualities. In recent years, intensive exploitation of forest land, plowing of meadows, increase of pasture and mowing areas, and other such anthropogenic factors prove the fact that the populations of species of *Gentiana* genus, like other species, are decreasing and that many species are in danger of disappearing.<sup>1</sup>

The study of morphobiological features of plants by modern methods is considered the main source of information for the creation of their phylogenetic systems, the compilation of basic florogenetic concept and taxonomic classifications. In this regard, the systematic study of higher plants, their families, tribes and genera is a valuable floristic database. One of the higher plant groups of flowering plants is the gentian family (*Gentianaceae* Juss.) and its various genera<sup>2</sup>. It is important to conduct fundamental complex bioecological studies of species (distribution, intraspecies variability, population biology and sustainability of natural populations) in order to develop scientifically based forms and methods of protection of the gentian family, which include both decorative and medicinal species, as well as their efficient use.

Since the species of the gentian family have beautiful decorative properties, they are used as ornamental plants. Some species are planted in rock gardens and on the banks of artificial reservoirs in groups. The diversity of life forms, the wide range of tolerance of species, the adaptation to living conditions of others complicates the systematics of the family.

The information in the literature about the biological and eco-

<sup>2</sup> Ma, Y.-Ch. *Gentianopsis* - a new genus of Chinese *Gentianaceae* I Y.-Ch. Ma // Acta Phytotax. Sinica. - 1951. - Vol. 1. - № 1, - p. 7-19

<sup>&</sup>lt;sup>1</sup> Solomon, J. Red list of the endemic plants of the Caucasus: Armenia, Azerbaijan, Georgia, Iran, Russia and Turkey / J.Solomon, T.Shulkina & G.E.Schatz // Missouri Botanical Garden Press, – 2014, – 353 p.

logical characteristics of the species of gentian family growing in the Caucasus is incomplete, accurate information is rare. The territory of Azerbaijan, which is characterized by an exceptional diversity of natural landscapes, is different from other regions of the Caucasus, so it can be considered a sample area for the study of species of the *Gentianaceae*. The geographical position, the complex geological structure of limestone rocks and wide distribution in Azerbaijan create conditions for the development of endemism and the creation of biodiversity.

Our research is devoted to the systematic and bioecological analysis of species of the *Gentiana* L. common in the flora of Azerbaijan. The systematic study of the genus was carried out about 60 years ago in the "Flora of Azerbaijan", and a taxonomic list was given in A.Asgarov's book "Plant Kingdom of Azerbaijan" in 2016<sup>4</sup>. Since the status of gentian species remains controversial, there is a need for their clarification. The study of the current state of species populations in natural conditions and the clarification of their role in vegetation were also among the main tasks of researh. The results of the critical review of the herbarium materials collected during the expeditions to different botanical-geographical regions of Azerbaijan in 2016-2021, and the herbarium copies stored in the Herbarium Hund of the Institute of Botany, MSE RA show that the systematics, taxonomy and bioecological characteristics of the species of the genus distributed in Azerbaijan have not been studied for many years, the difficulty of specifying their status makes the research urgent.

**Object and subject of the research.** The object of research is the study of the systematics of the genus distributed in the flora of Azerbaijan. The subject is the study of the genus with classical and modern methods.

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<sup>&</sup>lt;sup>3</sup> Софиева, Р.М. Флора Азербайджана / Р.М.Софиева — Баку, — Т.7, —1957, — с. 85-101

<sup>&</sup>lt;sup>4</sup> Əsgərov, A.M. Azərbaycanın bitki aləmi (Ali bitkilər-*Embryophyta*) / A.M. Əsgərov, – Bakı: TEAS Press, –2016. – 312 s.

Ibadullayeva, S.J., Huseynova, I.M. An Overview of the Plant Diversity of Azerbaijan
 // - Switzerland: Biodiversity, Conservation and Sustainability in Asia, - 2021.
 - p.431-479

Goals and objectives of the study. The main goal of our research is to study the systematic composition of the *Gentiana* common in the flora of Azerbaijan, their taxonomy and nomenclature, many disputed species, the morphological and diagnostic features of difficult-to-determine vegetative and generative organs, and the patterns of geographical distribution of species.

For this purpose, the following tasks have been set:

- ➤ Clarification of species composition of *Gentiana* genus distributed in Azerbaijan, compilation of its synopsis;
  - > Compilation of a new determinant key of the genus;
- ➤ Determination of the importance of morphological-diagnostic features in the systematics of the genus;
- > Systematic analysis of species belonging to the genus *Gentiana*, investigation of their bioecological characteristics (life forms, patterns of distribution depending on altitude);
- ➤ Botanical-geographical analysis of the species included in the genus;
  - ➤ Conduction of chorological analysis of genus species;
- ➤ Determination of the economic importance and conservation measures of gentian species.

**Research methods.** Comparative-morphological, molecular-phylogenetic, biomorphological, biogeographical, bioecological and other methods were applied in the research.

#### Provisions submitted to the defense.

- The taxonomic composition of the species of the genus *Gentiana* distributed in the flora of Azerbaijan is characterized by certain changes.
- In order to determine the taxonomic composition of the species belonging to the genus, it is necessary to use molecular methods as well as classical methods.
- Searching for medicinally important species is an important part of biodiversity conservation.

**Scientific innovations of research.** As a result of our research, the following scientific innovations have been identified:

A critical review of the gentian species common in the flora of

Azerbaijan was carried out, and the taxonomic composition was determined. The taxonomy and nomenclature of *Gentiana* L. species were studied, 1 of the 14 species shown in the flora of Azerbaijan was reduced to the level of subspecies and 3 were synonymized, a new taxonomic system consisting of 9 species and one subspecies was drawn up. A new determinant key of genus species distributed in the flora of Azerbaijan has been compiled. For the first time, new distribution areas of *G.septemfida* (Absheron), *G. asclepiadea* (Ganja and Gadabay districts of the LC north) were shown in the flora of Azerbaijan. The patterns of distribution of species of the genus depending on the altitude were determined and a map scheme was drawn up based on GPS coordinates. A chorological analysis of gentians with different geographical areas was carried out, and five geographical elements were determined.

**Practical importance of the work.** A critical review of the species included in the genus *Gentiana* distributed in Azerbaijan was carried out, and the phylogenetic relationships of the species were investigated. Decorative (*G.lagodechiana*, *G.septemfida*, *G.crusiata*), medicinal (*G.septemfida*, *G.crusiata*, *G.asclepeidae*, *G.gelida*, *G.verna*, *G.lagodechiana*) and nutritionally important species were also identified. The materials on systematics, bioecology, geography and etc. regarding the genus reflected in the dissertation work can be used in the compilation of "Flora of Azerbaijan" and "Key of Azerbaijan Plants".

Approbation and application. The main results of the dissertation work were discussed at the III international scientific conference on "Ecology: Nature and Society Problems" dedicated to the 110th anniversary of Academician Hasan Aliyev (Baku, 2017); international scientific conference on the topic "Actual Problems of Modern Natural and Economic Sciences" (Ganja, 2018); Conference of Young Scientists and Students: Innovations in Biology and Agriculture to Solve Global Challenges (Baku, 2018); scientific conference on "Innovation and Traditions in Modern Botany" dedicated to Academician A.A.Grossheim's 130th anniversary (Baku, 2019); AGBI-OL Proceedings of III International Agricultural, Biological & Life science conference (Edirne, Turkey: 15-17 december 2021); scientific seminar of the Institute of Botany, MSE RA (2023)

5 articles, 5 theses related to the topic and content of the dissertation (3 articles and 4 theses in Azerbaijan, 2 articles and 1 thesis in international databases (Web of Science)) were published in peer-reviewed and indexed journals.

The name of the institution where the dissertation was carried out. The dissertation work was performed at the Department of Herbarium of the Institute of Botany of the Ministry of Science and Education of the Republic of Azerbaijan.

Separate volume of the structural sections of the dissertation. The dissertation consists of an introduction, 6 chapters, and a result, covering 147 pages. The dissertation consists of two hundred and eight thousand characters in total. The literature list is 212-titled, 41 of them are Azerbaijan, 86 Russian and 85 foreign sources. The dissertation work is enriched with 9 tables, 1 map-scheme and figures.

## MAIN CONTENT OF THE WORK CHAPTER I. LITERATURE REVIEW

The research works conducted towards studying the *Gentiana* genus in the world and within our republic were investigated, compared and analyzed, and the obtained results were broadly interpreted in this section of the dissertation.

#### CHAPTER II MATERIAL AND METHODOLOGY OF THE RESEARCH

The researches for the purpose of studying the genus *Gentiana* L. were carried out in the Herbarium Department of the Institute of Botany, MSE RA using modern and classical methods during 2016-2021 years. As research material, herbarium specimens (more than 150) collected in different environmental conditions from the territory of Azerbaijan belonging to *Gentiana* genus, numerous herbarium specimens stored in herbarium funds of Institute of Botany of the Ministry of Science and Education of Azerbaijan (BAK), Faculty of Biology of Baku State University, Komarov Botanical Institute of the Russian Academy of Sciences (LE), Institute of Botany named after N.N. Ketskhoveli of Academy of Sciences of Georgia (TBI), also numerous herbarium copies stored in different herbarium funds

of the world [GBIF] and literature materials related to research work were used.

Stationary studies were conducted along different routes in different botanical-geographical regions of Azerbaijan in all areas, from lowland to high mountain belt and alpine. The studies were conducted on the basis of comparative-morphological, geographical, areological, ecological methods, in addition to the review of literature materials covering the history of species belonging to the *Gentiana* genus.

DNA was extracted from dried young leaves of plant samples collected for molecular research using the "Dneasy Plant Mini Kit". ITS1 and ITS4 sequence sites and the American GenBank database were referenced to confirm species identification. The phylogenetic tree of the genus was constructed using the ML method.

Morphological-systematic studies were carried out based on the studies of K.Linney, M.Bieberstein, K.A.Meyer, O.Dekandol, F.Ledebur, E.Boisier, I.F.Schmalhausen, A.A.Grossheim, Y.-Ch Ma, R.M.Sofiyeva, at the same time, the taxonomy and nomenclature of the species were clarified as a result of the critical review of herbarium specimens collected in different years and morphological structure of plants was studied using MBJ-3 binocular magnifier.

Biomorphological studies of species were carried out based on systems by I.G. Serebryakov<sup>6</sup>, classification of life forms based on C. Raunkier's system<sup>7</sup>.

Since the majority of the species belonging to the genus are distributed in the subalpine and alpine zones, the Serensen-Chekonovski similarity coefficient was used to determine the taxonomic composition of the species located in these zones.

$$I_s = 2a / ((a+b)+(a+c))$$

a a- number of total species, b- number of species in the first mountain belt, c- number of species in the other mountain belt.

<sup>7</sup> Ellenberg, H.A key to Raunkiaer plant life forms with revised subdivisions / H.A.Ellenberg. – D.Mueller-Dombois, – 1968, – p. 56-73.

<sup>&</sup>lt;sup>6</sup> Серебряков, И.Г. Жизненные формы высших растений и их изучение / И.Г.Серебряков // Полевая геоботаника, − М.-Л., изд-во "Наука", − Т.1, − 1964. − с. 5-31.

The International Code of Nomenclature for algae, fungi and plants was used to clarify nomenclature issues, and various web databases were used to clarify the scientific names and synonyms of taxa.<sup>8</sup>

Bioecological characteristics of species was studied according to F.Babayev<sup>9</sup>, ecology to M.B. Fardayev<sup>10</sup>, geographic analysis of species to N.N.Portenier<sup>11</sup> and geographical elements were defined.

## CHAPTER III. IMPORTANCE OF DIAGNOSTIC TRAITS IN THE TAXONOMY OF THE GENUS GENTIANA L.

**3.1.** Morphological-diagnostic signs of species. Determining the diagnostic traits of gentian (*Gentiana* L.) species is considered one of the main issues of our research. Morphological diagnostic signs have a great role in the systematics of genera and species. The genus *Gentiana* has visually visible morphological features that are very useful for identifying diagnostic characters. Diagnostic features are of particular importance in revealing the different characteristics of species, and the differentiation of different ranks (genus, subgenus, species, subspecies, etc.) within a family and genus is based on such characteristics. Gentian are decorative plants. One of the main factors is the appearance of morphological features, such as decorative plants, both in live and herbarium.

Vegetative and generative organs are equally important in determining the diagnostic characteristics of the genus. A number of diagnostic signs determined as a result of our research on living plants in nature, as well as herbarium materials prepared from these plants, are considered the basis for determining taxa. Since identification of dried herbarium material is difficult, identification of species in nature is considered more important. The main diagnostic features of the genus *Gentiana* are the structure of the flower and the shape of the leaves.

<sup>9</sup> Babayev, F. Ə. Bitki coğrafiyası / F.Ə.Babayev – Bakı: BDU, – 2004. - 291 s.

<sup>&</sup>lt;sup>8</sup> [Elektron resurs] URL: <a href="http://www.worldfloraonline.org">http://www.worldfloraonline.org</a>

<sup>&</sup>lt;sup>10</sup> Фардеева, М.Б. Экология растений и методы фитоиндикации / М.Б.Фардеева. – Казань, – 2018, –150 с.

<sup>&</sup>lt;sup>11</sup> Портениер, Н.Н. Флора и ботаническая география Северного Кавказа / Н.Н.Портениер. – Москва, – 2012, – 293 с.

The main characters of the genus *Gentiana* were defined as subgenus, section, and interspecies taxonomic characters. These signs are considered the structure of the flower, the structure of the corolla, the shape of the stomata, the folds between the petals, etc.

The main characteristics for the subgenus are the structure of the calyx and petals. These are the traits that are specific to the genus and do not change: Flowers are four, sometimes five-membered, rarely six- or eight-membered; corolla tubular, spicate, bell-shaped, pin-like, glabrous or internally fringed, layered or unlayered between parts. Stamen ends with a corolla tube. Capsule double-layered, one-locular, multi-seeded. Genus-specific traits are specific to individual species<sup>12</sup>.

### **Vegetative organs:**

**Root** – The roots of gentians consist of rhizomes. Rhizomes have different structures in different species:

Rhizomes thickly shortened. – *G.asclepiadea*; the rhizomes thick stringlike rooted - *G.septemfida*, *G.gelida*; rhizome thick, sheathy covered with fibers of old leaves - *G.cruciata*; rhizome is thin, creeping, branching - *G.pyrenaica*, *G.verna*, *G.verna* subsp.pontica.

**Stem** - The structure, shape and branching of the stem are important in the systematics of *Gentiana* genus. Some species of the genus are tall, simple, upright - *G.asclepiadea*; some species - multiple, ascending, simple, dense leaved - *G.septemfida*, *G.gelida*; other species - numerous, densely leafy, single-flowered, simple, erect, thickened - *G.cruciata*; other species numerous, loosely branched from the base, single-flowered - *G. aquatica*; also very short, erect, four-edged, large-flowered - *G.verna subsp.pontica*; thin, simple, branching - *G.nivalis*; thin, straight or curved, simple, sometimes weakly branched - *G.pyrenaica*; low, four-edged, elongated during fruiting - has a distinct stem, like *G. verna*.

**Leaf** – Gentians have oppositely arranged leaves, but there are species with alternate and clusterlike arrangement. For the first time N.I. Kuznetsov found out that gentians have clusterlike leaves. Leaves of gentians:

Basal leaves without rosette, large, sessile, ovate, heart-shaped

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<sup>&</sup>lt;sup>12</sup> Zeynalova, A.N. *Gentiana* L. cinsi növlərinin morfoloji əlamətləri / A.N.Zeynalova // Naxçıvan Dövlət Universitetinin Xəbərləri, Naxçıvan: - 2020. №8 (109) s. 64-70

or lanceolate, lanceolate, long sharpened, 3-5-veined - G.asclepiadea; sessile, contiguous at the base tubular sheath, oblong-ovate or ovate-lanceolate, obtuse, five-veined - G. septemfida; firm, leathery, adjacent to the sheath at the base, three-veined, slightly broadly ovate below, middle and upper ones oblong, lanceolate, compressed above, obtuse or pointed – G.gelida; stem leaves - large, firm, sometimes leather-like, sessile, one-veined, basal leaves - oval, elliptic-lanceolate, compressed at the base and tip, stem leaves are numerous, ovate or lanceolate - G.cruciata; basal leaves inverted ovate oval, inverted ovate spade-shaped, narrow, the lower stem leaves narrow, the middle and upper ones elongated, the edges membranous, sharp or obtuse, with short sheaths - G.aquatica; basal leaves sessile, elliptic or ovateoblong, obtuse, basal and lower stem leaves forming a dense rosette, stem leaves 1-3, narrow, adjacent to the sheath from the base, the upper pairs are close to the calyx - G.verna subsp. pontica; basal and lower stem leaves collected in a rosette, ovate and almost circular, stem leaves ovate or ovate-lanceolate, obtuse or acute – G.nivalis; stem leaves sessile oblong-lanceolate to lanceolate, compressed above, acute, one- or three-veined - G.pyrenaica; lower leaves form a robust rosette, elliptic or elliptic-lanceolate, sharp, sometimes obtuse, three-veined, edges smooth, one or two pairs of stem leaves short and narrow, contiguous short sheath, the upper pair is located below the flower – G. verna.

### **Generative organs:**

**Flower -** numerous, located alone in the upper part of the stem, sometimes three in leaf axil – *G.asclepiadea*; A multi-flowered capitate is collected in inflorescence at the apex, sometimes densely covered by the stem leaves at the apex – *G.septemfida*, *G.gelida*; nonfringed, four, sometimes five-membered, forming a bunch and located in the axil of the leaf or in the apex of the stem - *G.cruciata*; large, purple-blue, without fringes - *G.pyrenaica*; five-membered, single, odorless – *G.aquatica*; located alone in the top part of the stem, large – *G.verna*, *G.verna subsp pontica*; bright blue, not large, located on the apex of the stem or on the branches – *G.nivalis*; four-membered, single, located at the apex of the stem or on the branches, leaves behind the flower - *G.lagodechiana*.

**Sepals** - diversity of the structure of the sepals is of great importance in the diagnosis of gentian species.

Bell-shaped, straight cut, linear-wide, lanceolate, sometimes wide, acute at apex, edges unevenly toothed, equal to or shorter than the tube - G. septemfida; elongate, bell-shaped, divided on the sides, sometimes entire, teeth narrow linear, unequal, three times smaller than the tube, with a hollow between - membranous - G.gelida; bell-shaped with four toothed, thin membranous, shiny, teeth sharp, triangular, lanceolate - G.cruciata; with a tubular end, not divided from the sides, with a sharp hollow between the teeth -G.pyrenaica; tubular, compressed at the base, oblong, lanceolate, acute, edges membranous toothed form – G.aquatica; tubular, five-edged, slightly swollen, wingless or narrowly winged, twice smaller than corolla, teeth equal, narrowly lanceolate, three times smaller than tube -G.verna; inseparable fivetoothed, sometimes four-toothed, tubular, half the size of the corolla, wingless, with black stripes at the corners, teeth narrow, lanceolate linear, smaller than a capsule, stretched pointed, several times smaller than a tube- G.nivalis; bell-shaped, twice shorter than the corolla, teeth equal to the tube, triangular lanceolate, slightly unequal, the hollow between the teeth is blunt, sometimes the sepals one-sided not attached to the corolla – G.lagodechiana; oblong bell-shaped, deeply divided on the sides, sometimes entire, teeth narrow linear, unequal, twice shorter than the tube -G. asclepiadea.

**Corolla** - The corolla of gentians is unique blue, white, whitish yellowish, and in some species s spotted. This sign varies in different species:

Twice longer than calyx, tubular pinlike, blue some cases with white spots, parts short triangular, markedly asymmetrical layer without fringe - *G.asclepiadea*; blue, 6-7 times shorter than the tube, parts sharp, long fringed layer between parts – *G.septemfida*; large tubular pin-like, yellow, 5-6 parted ovoid-triangular, pointed or somewhat pointed, 5-6 times shorter than the tube, layers between the parts shorter than the parts, asymmetrical, bipartite or toothed, sometimes entire - *G.gelida*; 3-4 times longer than calyx, blue-tubular-pinlike, 4 short, ovoid-triangular, pointed or somewhat pointed, flat-toothed or triangular-lanceolate, bipartite interlayers 2-3 times shorter

than the tooth — G.cruciata; tubular-pin-like, oblong-ovate, obtuse-parted, interlayers toothed, equal to parts or slightly shorter — G.pyre-naica; twice longer than the calyx, tubular-pinlike, bluish blue, triangular-lanceolate, acute, layers once shorter than the parts of the corolla, ovoid-oval, membranous, very unequally toothed or entire at the apex — G.aquatica; bright blue, tubular, oblong-ovate obtuse parted, twice shorter than the tube, layers between the parts triangular lanceolate, two-parted, numerous, shorter than parts bent to the side —  $G.verna\ subsp.pontica$ ; funnelled tubular, blue, extending to the middle of the parts, parts oblong or oblong inverted ovate, edges of lower parts unequal more or less fringed, obtuse or acute at apex, entire or divided — G.lagodechiana; tubular, short-ovoid triangular, acute-parted, the layers between the parts shiny, triangular, bipartite — G.nivalis.

**Fruit** - Fruit of the gentians is a multi-seeded, one-celled, pedunculated or sessile, capsule opening with two wings.

3.2. Controversial issues in the taxonomy of the genus. For the first time, the name of the genus was given by the French botanist and systematist J.P. Tournefort. In 1718, Kh. Ruppius divides the genus into two groups. Gentiana and Gentianella. After some time, the description of the genus was given by K. Linney in 1753. Gentiana L. is one of the most complex genera of the Gentianaceae family. Gentianella genus was described a little later. In 1737, C. Linnaeus in his "Genera Plantarum" united the genera Gentiana Tournef. and Centaurum minus Tournef. In 1753, he combined Gentiana and Swertia and Halenia Bork. Scientists after Linnaeus accepted his opinion and considered Gentiana genus to be a large genus. In the early 1950, interest in the study of the systematics, anatomy, and karyology of gentian increased, and many factual materials were collected. This also led to the creation of new phylogenetic systems, and the fundamental development of the taxonomic composition. At this time, the Chinese systematist Y.-Ch. Ma separated the genus Gentionopsis Ma from the genus Gentiana and took evolution as a basis in taxonomy. After that, there were many disagreements in Europe. The main principle of the systematization of gentiana should be the division of all signs into signs of level and origin.

When investigating the systematics of gentiana, we consider the above division accepted by A.A. Grossheim to be reasonable. So, we accept this reality as we identify species. As we know, in the taxonomic composition of the genus *Gentiana* given in the flora of Azerbaijan, we determined that *Gentonopsis blepharophora* and *Gentianella caucasica*, *G.lingulata* and *G. umbellata* species, which have the characteristics of the genera *Gentianella* and *Gentionopsis*, have different characteristics.

The genus *Gentianella* does not have a membrane inside the calyx. The parts of the calyx join and twist each other. There are no folds between the parts of the corolla. It is annual and perennial.

*Genianopsis* is not segmented. Corolla funnelform, without a fringe, calyx ring-shaped from the bottom. Corolla edge often fringed or ciliated. Stomata is ovoid or tubular. Capsule sessile or pedunculated. Seed scaly, sometimes winged from the apex. Annual or biennial.

Although both genera are broadly morphologically similar to the genus *Gentiana*, they differ greatly from it in terms of classical features of the species, and we consider species of all three genera to have a separate status.

In the flora of Azerbaijan, the genus was developed by R.M.Sofiyeva, and in the flora of the Caucasus by A.A.Grossheim. R.Sofiyeva showed that there are 14 species of the genus in the flora of Azerbaijan, and A.A.Grossheim showed 10 species. For the first time, the species G.septemfida Pall. caused controversy. K.Koch (Koch, 1850) initially considered this species as a subspecies. Somewhat later, A.A.Grossheim (1932) confirmed Gentiana septemfida var. lagodechiana Kusn. and independent species G.lagodechianani in the genus composition. Grossheim noted that G.lagodechiana species is not distributed in the flora of Azerbaijan, but Sofiyeva showed that this species is distributed in the territory of Guba and Zagatala. G.lagodechiana was accepted as an independent species. Currently, the controversial species included in the genus remains a debate. In 1808, the species G.asclepiadea was described by M.Biberstein. Later, in 1843, Koch described species diversity of G.schistocalyx G.asclepiadea from Ossetia. In 1850, G.schistocalyx (K.Koch) K.Khoch species was determined as an independent species. Later, Kuznetsov transferred this species to the synonym of G. asclepiadea due to the fact that its characteristics (the structure of the calyx, the arrangement of the leaves, etc.) are very similar. The results of molecular analyzes conducted in recent years confirm the correctness of this idea, and many world scientists also support this idea and currently *G.asclepiadea* is considered an independent species.

G.angulosa was considered an endemic species of the Caucasus. It was first given as an independent species by Biberstein in 1808. In 1819, first Biberstein and then other Caucasian scientists consider it as a subspecies of G. verna species. For about 60 years, this species has been known as G.angulosa. Based on recent molecular results, G.angulosa and G.pontica species are combined and transferred to the synonymy of G.verna subsp.pontica species. The species G. pontica was described by Soltok in 1901 from Pont. However, scientists studying the Caucasus, like Ledebur, Boisier, Lipsky, do not consider it an independent species in their works. Grossheim in 1932 accepts this species as it is. In 1904, Kusn considered the species G. pontica to be a subspecies of the species G. verna in his research. For more than a century, the signs seen by Kusn with the naked eye have been confirmed by numerous analyzes carried out in the new century of scientific development, and confirmed as G.verna subsp.pontica. Thus, there is a lot of similarity between these species in terms of their morphological features and mostly repeats the morphological features of G. verna. Therefore, Caucasian researchers consider subsp. pontica to be subspecies of G. verna species The species G. djimilensis was described by Koch in 1850. However, Grossheim in 1952 claims that this species is a synonym of G.pyrenaicum species based on morphological characteristics. A little later, European species G.pyrenaicum is proved to be a synonym of Caucasian species G.djimilensis.

The species *G. blepharophora* was described by Bordzilovsky from Akhalkhalaki in 1912. In 1952, Grossheim indicated that this species is a synonym of *G. sililata*. Even before Grossheim, in 1951, Ma gave a description of the genus *Gentionella*. Corolla funnel-shaped, tubular, without fringe, stomata fringed, calyx leaves not fully opened, capsule sessile or pedunculated. According to such features,

G.blepharohora was removed from the genus Gentiana and transferred to the composition of the genus Gentionopsis, which bears its characteristics.

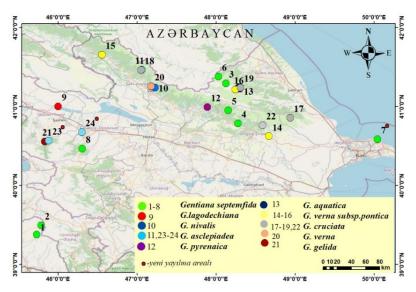
#### CHAPTER IV. SYSTEMATIC STATUS OF THE GENUS GENTIANA L.

- 4.1. General characteristics of the genus. General characteristics and systematic analysis of the genus Gentiana in the flora of Azerbaijan have not been given for more than 60 years. As a result of our research, species belonging to the genus were investigated (mapscheme 1).
- **4.2. Determinant key of the genus.** A new determinant key of the sections and species included in the genus has been compiled<sup>13</sup>.
- 4.3. Classification of the taxonomic composition of the genus. As a result of the changes in the taxonomic composition of the Gentiana genus, it was confirmed that the genus consists of 9 species and one subspecies, and a brief description and synonym of the species was given (table 1).

Table 1 Grouping of the species of *Gentiana* genus by sections

№	Seksiyalar	Növlər	Yarımnövlər	
1.	Pneumonanthe	1.G.asclepeidae L. 2. G.gelida M.B. 3.G.septemfida Pall. 4.G.lagodechiana Kusn.		
2.	Chondrophylla	5. G.pyrenaica L. 6.G.aquatica L.		
3.	Cyclostigma	7. G.nivalis L. 8. G.verna L.	G.verna subsp.pontica (Soltok.) Litard. et Maire	
4.	Aptera	9. G.cruciata L.		

<sup>&</sup>lt;sup>13</sup> Zeynalova, A.N. Azərbaycan florasının *Gentiana* L. cinsi növlərinin təyinedici cədvəli / A.N.Zeynalova, P.X.Qaraxani, T.A.Qasımova // Pedagoji Universitetin Xəbərləri Riyaziyyat və təbiət elmləri seriyası, - Bakı: - 2019. C.67, №1, səh. 236-240



Map-scheme 1. Distribution of Gentiana L. genus species in Azerbaijan

# **4.4.** Molecular-phylogenetic study of the genus Sample preparation for DNA extraction

Some of the collected plant samples were used for molecularphylogenetic studies. 100-200 mg of young leaves from each sample is placed in a 2 ml tubes. After the closed tubes were kept in liquid nitrogen for several minutes, plant material is crushed well. From dried and crushed plant samples DNA is extracted by means of "Dneasy Plant Mini Kit". 0.1 TE buffer is added to the dried DNA and stored one night in a 20°C refrigerator. The amount of extracted DNA was determined in a spectrophotometer (at 260 and 280 nm wavelengths) (Nano Drop 2000 c UV-Vis Spectrophotometer – Thermo Scientific). After determining the amount of DNA, working solutions with a concentration of 50 ng/µl from each sample DNA are prepared for PCR reaction. After collecting the reaction, the tubes are placed in the PCR machine (Gene Amp PCR System 2720, Applied Biosystems Foster City, CA) and the program is compiled in the following sequence. For the amplification of the DNA region ITS 1 (TCC GTA GGT GAA CCT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC) primers were used. After that, the primers were added to

PCR products, placed on an ABI 3730XL automated sequencer, the nucleotide sequences of the trnL intron primers were read as follows<sup>14</sup>. After the reaction is collected, the tubes are placed in the PCR machine (Gene Amp PCR System 2720, Applied Biosystems Foster City,CA) and the program is compiled in the following sequence. For the amplification of the DNA region ITS 1 (TCC GTA GGT GAA CCT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC) primers were used (table 2).

Table 2 Nucleotide sequence of ITS primers (5-3).

DNA	Primer	Nucleotide sequence 5-3	Reference	
region				
ITS	ITS 1	TCC GTA GGT GAA CCT GCG G	White et al, 1990	
	ITS 4	TCC TCC GCT TAT TGA TAT GC	White et al, 1990	

**Nucleotide sequence reading (Sequencing):** The obtained PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen, Germany) before sequencing. Then primers were added to the PCR products, placed on an ABI 3730XL automated sequencer, and nucleotide sequences were read (table 3).

Table 3 Nucleotide sequence of trnL intron primers (5-3).

DNA	Primer	Primer sequence 5-3	Reference
region			
	trnL c	CGA AAT CGG TAG ACG CTA CG	Taberlet et
trnL			al., 1991
intron	trnL d	GGG GAT AGA GGG ACT TGA AC	Taberlet et
			al., 1991

### Results of molecular- phylogenetic analysis.

The obtained nucleotide sequences were compared with those in the US GenBank data center and the following results were obtained:

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<sup>&</sup>lt;sup>14</sup> Zeynalova, A.N. Molecular analysis of the genus *Gentiana* growing in Azerbaijan / A.N.Zeynalova // International Journal of Botany Studies, İndia: - 2021. Volume 6 Issue 3, p.751-752

S1 sample matches the *Gentiana septemfida* species with Gen-Bank data.

S2 sample matches the *Gentiana pyrenaica* species with Gen-Bank data.

S3 sample matches the *Gentiana gelida* species with GenBank data.

S4 sample matches the *Gentiana nivalis* species with GenBank data.

S5 sample matches the *Gentiana blepharophora* species with GenBank data.

S6 sample matches the *Gentiana cruciata* species with GenBank data (figure 1).

Circium arvence is taken for comparison.

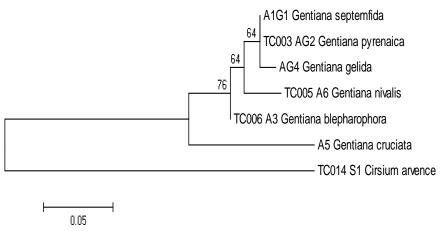


Figure 1. Phylogenetic tree of the ITS gene region in the nuclear genome of some *Gentiana* genus species distributed in Azerbaijan according to the Maximum Likelihood (ML) method

## V CHAPTER. BIOECOLOGICAL CHARACTERISTICS OF SPECIES OF THE GENUS GENTIANA L.

Bioecological research is of great importance in species formation and solving a number of controversial issues in plant systematics. In this regard, although the bioecological characteristics of the genera belonging to the *Gentianaceae* family have been studied in detail by many world scientists, the bioecology of the Azerbaijani species of the *Gentiana* genus has not been adequately studied.

**5.1. Phenology of the species.** Many seasonal changes in nature, the ecological situation provide a basis for monitoring the bioecological characteristics of species, and their phenology in general. Repeating the development phases of flowering plants is called a phenological event. Different researchers perceive the phenological phase in different ways. For example, it varies between 5-7. In herbaceous plants, the phenological phase begins with germination from seed, followed by initiation of vegetative organs, followed by budding, flowering, fruiting, and seeding. The fulfillment of phenological phases in nature depends on temperature and other environmental factors.

*Gentiana* species are divided into two groups according to their flowering dates:

- a) those flowering in summer (Gentiana aquatica, G.verna subsp. pontica, G. nivalis, G. pyrenaica, G.verna etc.)
- b) those flowering in autumn (G.gelida, G.septemflda, G. asclepiadea, G.lagodechiana, G.pyrenaica etc.).

Generally, budding occurs in gentians in June-July. The full flowering period is from August to September. The fruiting period lasts from the second ten days of August to the end of October in different species (table 4).

Table 4
Seasonal development of gentiana species in Azerbaijan

N₂	Name of the	Phenological phase			
	species	Budding	Flowering	Fruiting	Dissemination
1.	G.asclepeidae	VI-VII	VIII-IX	VIII-X	IX-XI
2.	G.gelida	VI-VII	VII-VIII	IX-X	XI
3.	G.septemfida	VII-VIII	VIII-IX	IX-X	IX-X
4.	G.lagodechiana	VII-VIII	VIII-IX	IX-X	IX-X
5.	G.pyrenaica	VI-X	VI-VIII	VII-IX	VIII-X
6.	G.aquatica	III-IV	IV-V	VII-VIII	VIII-IX
7.	G.nivalis	V-VII	VI-VIII	VII-IX	VIII-X
8.	G.verna	V-VI	VI-VII	VII-VIII	IX
9.	G.cruciata	VI-VII	VII-VIII	VIII-IX	X
10	G.verna subsp.	V-VI	VI-VIII	IX	X
	pontica				

**5.2. Species ecology and participation in plant groups.** Raunkier's system of life forms is based on traits that characterize the adaptation of plants to unfavorable conditions, that is, adaptation is based on it. Raunkier identified five life forms, based on the location of regenerative shoots on the surface of the earth. As we know, most species of Gentiana are herbs distributed in high mountains. Gentians are hemicryptophyte, they have shoots on the surface of the earth even in unfavorable times. When the weather gets cold, the aboveground parts are destroyed, and the underground organs go to the deeper layers of the soil, are protected in the remains in earth, and next year, according to favorable conditions, the above-ground stems begin to develop, and this process continues every year. Gentians are also therophytes. Thus, after the destruction of the above-ground organs in the summer, they end their unfavorable period with seeds<sup>15</sup>.

Gentians distributed in Azerbaijan are divided into three groups in relation to sunlight<sup>16</sup>:

- **1. Sun loving plants** (*Heliophytes*) The species *G.lagodechiana*, *G.pyrenaica*, *G.aquatica*, *G.septemfida* included here are sunloving, growing mainly in places with more sunlight
- **2. Shade tolerant plants** (*Heliosciophyte*) Shade tolerant plants *G.nivalis*, *G.verna*, *G.verna subsp. pontica etc.*, grow in full light, they develop better in the shade.
- **3. Shade-loving plant** (*Sciophytes*) The species *G.cruciata*, *G.asclepiadea*, *G.gelida* thrive only in the shade.

The attitude of gentians to humidity is also different. They are divided into 2 groups according to the environment they live in:

Mesophytes - these species occupy an intermediate position between xerophytes and hygrophytes. They are plants adapted to medium humidity conditions which include: G.asclepedia, G.lagodechiana,

<sup>15</sup> Zeynalova, A.N. Bioecological features of *Gentiana* species / A.N.Zeynalova // International Journal of Botany Studies, İndia: - 2022. Volume 7 Issue 1, p.369-371

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Zeynalova, A.N. Azərbaycanda yayılan Acıçiçək (Gentiana L.) növlərinin ekologiyası // Azərbaycan Milli Elmlər Akademiyası Botanika İnstitutu Azərbaycan Botanika Cəmiyyəti Akademik Aleksandr Alfonsoviç Qrossheymin 130-cu ildönümünə həsr olunmuş Gənc Alim və Tədqiqatçıların "Müasir Botanikada innovasiya və ənənələr" mövzusunda konfrans, Bakı: 20 dekabr, 2019, - səh. 42

G.cruciata, G.pyrenaica, G.nivalis. Most species are mesophytic.

*Xeromesophytes* - Species included in this intermediate group combine the characteristics of both mesophytes and xerophytes:

G.septemfida, G.aquatica, G.verna, G.gelida, G.cruciata.

According to the physical properties of the soil, gentiana species are divided into 4 groups:

- 1. Gentians growing in acidic soils -G. verna, G. pyrenaica, G.nivalis
- 2. Petrophytes (Greek "petros" stone, "phyton" plant) species distributed on stony substrates and rock crevices G. asclepiadea, G.lagodechiana, G.gelida, G.verna subsp. pontica
- 3. Argillophytes Tubular and their hybrids spread on clay soils, clay soils that hardly conduct moisture Gentiana cruciata, G. septemfida, G. asclepiadea, G. aquatica
- 4. Psammophytes those that spread on sandy substrates: G. septemfida

Gentians are mainly high-mountainous and alpine plants. The role of climate, soil, water and other factors in the development of these species is fundamental. Depending on the height gradient, we have used the scheme developed and adopted by L.I.Prilipko in 1954 to clarify distribution patterns of Gentiana species.

During our research, it became clear that 2 species distributed in the lower and middle belt, 4 in the upper mountain belt, 4 species and one subspecies in the alpine, 6 species and one subspecies in the subalpine. The subspecies (*G.verna* subsp. *pontica*) is found both in the subalpine and in the alpine.

The similarity coefficient of the low, middle and high mountain belt was determined to be  $I_s = 0.75\%$ , between the upper and the high belt -  $I_s = 0.53\%$ , in the subalpine and alpine zone -  $I_s = 0.60\%$ .

**5.2.** Phytocenological characteristics of plants of the genus Gentiana (*Gentiana* L.), common in Azerbaijan. Anthropological factors together with ecological and biotic factors contribute to the formation of vegetation on Earth. The relief of the area where we live, climate change, sudden rises and falls in temperature, heavy rainfall and other similar influences affect the structure of the soil, change it, and these changes lead to the formation of vegetation. Changes

in natural phenomena also affect the dynamics of vegetation. If we look at the vegetation of the area under study, we will see the following pattern. So, gentiana can be found both in the meadow, steppe, and in the forest type of vegetation.

**Meadow type of vegetation** - This type of vegetation is divided into subalpine and alpine. Subalpine meadows originate from the upper border of the forests in the subalpine belt. Gentiana species are found in subalpine and alpine meadows and in forest vegetation: *G.asclepiadea*, *G.gelida G.verna* subsp. *pontica*, *G.aquatica*, *G.verna*, *G.pyrenaicum* etc.

Forest vegetation type - there are many trees and shrubs. There are more than 72 species of trees and 266 species of shrubs in the forests of Azerbaijan. Among the types of vegetation, the most important is the forest. Thus, 27% of the world and 10% of Azerbaijan are surrounded by forest vegetation. Deforestation for centuries has caused their number to decrease. Subalpine forests occupy the main place in forest vegetation. The researchers studying Azerbaijan named the area between the subalpine belt and the forest as subalpine forests<sup>17</sup>. Subalpine forests are divided into two parts: transferred from the forest or mountain slopes or forests where a certain part is rich in vegetation. The species such as *G.cruciata*, *G.gelida*, *G.septemfida*, *G.asclepiadea* widespread in forest vegetation.

Rock vegetation - the primary vegetation of the rocks on the high peaks of the mountains attracts attention. In many of the rocks, rock vegetation has formed on stony steeps, as well as in parts with scree and debris. Rock vegetation has a very rich floristic composition. At first glance, blue-green algae as if covering the rocks, lichens in limestone or carbonate rocks are visible. Gradually mosses take their place. Thus, mosses cause the creation of organic compounds that have a special role in moisture protection and soil formation. Rock vegetation is classified as follows: Rock, scree, rocky debris, glacial moraines, area with cobbles. Geographical latitude, height, composition of mountain rocks play a key role in the formation of

<sup>&</sup>lt;sup>17</sup> Hacıyev, V.C. Azərbaycanın yüksək dağlıq bitkiliyinin ekosistemi/ Bakı: Təhsil – Elm, 2004, 130 s.

rock vegetation given in the classification. Many of the belts (forest, subalpine, alpine, sibnival) have rocks, and regardless of where they are, their vegetation consists of primitive and higher plants. A large number of higher plant species grow on the rocks and form a vegetation type. Along with *Dryopteris*, *Astragalus*, *Potentilla G.lagodeschiana*, *G.aquatica* are also found here.

## CHAPTER VI. PHYLOGENY AND GEOGRAPHICAL ANALYSIS OF SPECIES OF GENUS GENTIANA

6.1. Phylogenetic relationships of Gentiana L. genus species distributed in Azerbaijan. The literary materials show conflicting opinions about the Caucasian origin of gentians. However, the outstanding researcher N. Kuznetsov, who studied gentians, suggested that they belong to the Boreal origin and that Caucasian species of gentians in general migrated from the Glacial Period. In fact, confirming Kuznetsov's opinion, we accept that the Caucasus cannot be compared with large species formation centers such as the Alps and the Himalayas. It was found that some species of the genus Gentiana (for example G.asclepiadea) remained in the forest and subalpine areas from the glacial period. The species G.septemfida included in subsection Septemfida, and distributed in high mountain belt and forest was developed in the Eastern Caucasus, G. gelida in the Lesser Caucasus. During the glacial period G. verna, G. nivalis migrated from Europe to the Caucasus, G. pyrenaica, G. aquatica from Asia. A. Grossheim's research shows that Caucasian species of gentians takes its origin from the young Boreal root. For example, G.pyrenaica (G.dshimlensis) was formed at the end of the Tertiary or glacial period. Later, An.A.Feodrov, contrary to these ideas, showed that G.pyrenaica (G.dshimlensis), which is Caucasian-Asia Minor species was a mountainous Asian origin and migrated to the Arctic. A.A.Grossheim showed that the initial species formation center of perennial gentians was Central Asia. He believed that many species of the genus Gentiana migrated from Central Asia to the Caucasus and Europe during the glacial period. Modern sources consider the development of species diversity of the genus (in Central Asia, Alps) as secondary and attributed this increase to the better development of the species at

high altitudes. G. Muller puts forward the idea that the *Cyclostigma* section originated in the Aral and Caspian Seas in the Tertiary period. Comparing the species of the genus with their modern ranges, it was determined that they live in the Western Caucasus.

**6.2.** Botanical-geographical analysis of species of Gentiana L. genus distributed in Azerbaijan. The study of Gentiana L. species is of great importance in investigating many unsolved issues related to the florogenetics of the genus. As a result of the analysis of the literature by many researchers, an analysis of the geography of the species was carried out.

During the chorological analysis of *Gentiana* species with different geographical ranges distributed in the territory of Azerbaijan, five geographical elements were determined based on the system of N.N. Portenier (table 5)<sup>18</sup>.

Table 5
System of geographical elements of *Gentiana* L. species
distributed in Azerbaijan

№	Geographical elements	Number of species	Quantity in %	Species
WIDESPREAD SPECIES = 2 species				
1.	Holarctic	2	22,22%	G.aguatica, G.asclepiadea
BOREAL ELEMENT= 6 species				
2.	Circumboreal	1	11,11%	G.nivalis
3.	Caucasus	2	22,22 %	G.septemfida, G.lagodechiana
4.	Caucasus-	3	33,34 %	G.cruciata, G.pyrenaica, G.verna,
4.	Europe			
ANCIENT MEDITERRANEAN ELEMENT = 1 species				
5.	Iran-Turan	1	11,11 %	G.gelida
	TOTAL	9	100 %	

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<sup>&</sup>lt;sup>18</sup> Zeynalova, A.N. Chorological analysis of *Gentiana* L. Species distributed in Azerbaijan // AGBİOL Proceedings of III İnternational Agricultural, Biological & Life science conference, Edirne, Turkey: 15-17 december 2021, - p. 300

#### **RESULTS**

- 1. The taxonomy and nomenclature of *Gentiana* L. species were studied and a new taxonomic composition of Gentiana L., consisting of 9 species, one subspecies belonging to 4 sections was compiled in Azerbaijan.
- 2. For the sections and species of the genus *Gentiana*, a determinant table, not shown in the regional floras, was drawn up based on new diagnostic characters.
- 3. For the first time, new distribution ranges of *G.septemfida* (Absheron), *G.asclepiadea* (Ganja and Gadabey districts, north LC) were shown in the flora of Azerbaijan, the role of the studied species in the vegetation of Azerbaijan, the distribution patterns of the species depending on the altitude were studied.
- 4. For the first time, molecular-phylogenetic analysis of plant samples collected from different botanical-geographical regions of the republic was carried out and ITS1 and ITS4 (internal transcribed spacer) sequence areas were examined for species identification. A comparative analysis of the obtained nucleotide sequences with the American Gene Bank data was carried out and the status of disputed species was clarified.
- 5. During the chorological analysis of *Gentiana* species with different geographical ranges, 5 geographical elements were identified, among which the Caucasian and Caucasian-European geographical element predominates. On the basis of field studies and analysis of herbarium materials, electronic maps-schemes of the ranges of the species were drawn up.
- 6. As a result of research, it was determined that the genus contains one Caucasian endemics (*G.lagodechiana*).

### Published scientific works related to the dissertation work

- 1. Zeynalova, A.N. *Gentiana* L. genus in the flora of Azerbaijan / A.N.Zeynalova, P.Kh.Garakhani // Azerbaijan National Academy of Sciences Proceedings of the İnstitute of Botany, Baki: 2017, vol. 37, p.81-83
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- 3. Zeynalova, A.N. The Species of Genus Gentiana L. in flora of Azerbailan // Conference of Young Scientists and Students Innovations in Biology and Agriculture to Solve Global Challenges, Dedicated to the 90 th Anniversary of Academician Jalal A.Aliyev, 31 october, 2018, - p. 127
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- Zeynalova, A.N. Chorological analysis of Gentiana L. Species distributed in Azerbaijan // AGBIOL Proceedings of III International Agricultural, Biological & Life science conference, Edirne, Turkey: 15-17 december 2021, p. 300
- 10. Zeynalova, A.N. Bioecological features of *Gentiana* species / A.N. Zeynalova // International Journal of Botany Studies, İndia: 2022. Volume 7 Issue 1, p.369-371

The defense of dissertation will be held at 11<sup>00</sup> on October 27, 2023 at the meeting of the ED1.26 Dissertation Council operating at the Institute of Botany of Ministry of Science and Education of the Republic of Azerbaijan.

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