

Genetic diversity and phylogenetic analysis of *Leonurus L.* species under the conditions of Uzbekistan

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Abstract. This study investigates the genetic diversity and evolutionary relationships within the genus *Leonurus*, focusing on species found in Uzbekistan. For the first time in the region, molecular genetic tools were applied to analyze *Leonurus* species using Internal Transcribed Spacer (ITS) markers. Samples collected from the Tashkent region were sequenced, and additional ITS data from 21 representatives of the Lamiaceae family were retrieved from GenBank. The sequences were aligned using the CLUSTALW tool, refined with Snap Gene, and analyzed using the Maximum Likelihood (ML) method with the Kimura 2-parameter (K2P) model in the IQ-TREE program. The resulting phylogenetic tree revealed genetic relationships among *Leonurus* species and closely related genera like *Lagochilus* and *Paraphlomis*. High bootstrap values (99.9%-100%) confirmed the reliability of these findings. *Leonurus sp. UZB* clustered with *Leonurus glaucescens* and *Leonurus turkestanicus*, suggesting a shared evolutionary origin. Close genetic proximity was observed between *Leonurus sp. UZB* and *Leonurus cardiaca*, while *Leonurus sibiricus* was more genetically distant, likely due to ecological or geographical isolation. Additionally, the analysis highlighted significant genetic differences between *Leonurus* and the genera *Lagochilus* and *Paraphlomis*, indicating early evolutionary divergence. These findings provide valuable insights into the genetic structure and evolutionary history of *Leonurus* species, emphasizing the need to preserve genetically similar species together for long-term population stability. This research also underscores the practical importance of such studies in biodiversity conservation and adaptive management. Future work should expand genome-level analyses and explore environmental factors influencing genetic variation within *Leonurus* populations to enhance conservation efforts and sustainable use of these plant resources.

1 Introduction

The *Lamiaceae* family, one of the largest and most distinctive families of flowering plants, comprises approximately 220 genera and nearly 4,000 species worldwide. The most recent

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taxonomic revision of this family was published in 2004 [1]. Renowned for its richness in medicinal, aromatic, and essential oil-producing plants, the *Lamiaceae* family holds particular significance in the flora of Uzbekistan, where it is commonly referred to as the mint family (*Labiatae* Juss.; *Lamiaceae* Lindley). Globally, this family includes approximately 3,500 species across 200 genera. In Central Asia, 464 species belonging to 53 genera have been documented, with 41 genera and 206 species represented in Uzbekistan.

According to modern classifications of flowering plants, the *Lamiaceae* family is divided into 12 subfamilies [2], encompassing 236 genera and 7,203 species. Of these, 3,675 species from 105 genera (more than 50% of the family) belong to the subfamily *Nepetoideae* [3], which is recognized for its high essential oil content and other valuable phytochemicals.

The genus *Leonurus* L., part of the subfamily *Lamioideae*, consists of 25 species [4], four of which are reported in Central Asia. Representatives of the *Lamioideae* subfamily are distinguished by the presence of iridoid glycosides and relatively low essential oil content.

In Uzbekistan, three species of plants from the genus *Leonurus* are widely distributed, with *Leonurus turkestanicus* being of particular importance due to its medicinal properties. This species is commonly found in the Tashkent, Syrdarya, Samarkand, and Surkhandarya regions. *Leonurus turkestanicus* thrives in mountainous forests, from mid-altitudes to riverbanks, predominantly in moist habitats such as the bases of cliffs, along stream banks, and in oases, where it occasionally grows as a weed. Its raw materials are in high demand, with an annual production of approximately 10 tons. Moreover, *Leonurus turkestanicus* has been extensively used in traditional medicine due to its diverse pharmacological properties.

In addition, the valuable medicinal species *Leonurus cardiaca* and *Leonurus quinquelobatus* have been successfully introduced under the environmental conditions of Uzbekistan and are actively cultivated as sources of medicinal raw materials.

Motherwort (*Leonurus cardiaca* L.), belonging to the order Lamiales and the family Lamiaceae (formerly known as Labiatae), is commonly referred to by names such as goose foot, lion's tail, lion's heart, and wolf's foot. This perennial plant can grow up to 150 cm in height. It has branched stems that emerge from a short horizontal rhizome. The stems are coarsely hairy, four-angled, furrowed, reddish-purple in color, and hollow on the inside. The leaves, arranged oppositely, are serrated and have long petioles. The upper surface of the leaves is dark green, while the underside is lighter in color, with both sides covered in fine hairs.

The chemical composition and pharmacological activities of various *Leonurus* species have been the subject of research since the early 20th century. Research has primarily focused on *L. japonicus* (East Asia), *L. cardiaca* (Europe), *L. persicus* (Turkey, Iran), and *L. sibiricus* (Mongolia, Siberia). Additional investigations have also examined lesser-known species, such as *L. macranthus* (Northeast Asia), *L. turkestanicus* (Central and West Asia), and *L. glaucescens* (Northwest Asia). Some confusion between *L. japonicus* and *L. sibiricus* has been noted in the literature, which was clarified in the work of Pitschmann et al. [4].

The genus *Leonurus* is characterized by a wide range of specialized metabolites, including alkaloids, flavonoids, terpenoids, glycosides, and cyclic peptides [5]. The traditional use of motherwort as a uterotonic agent is primarily attributed to the alkaloids leonurine and stachydrine [6]. Additionally, *Leonurus* alkaloids exhibit other pharmacological effects, including the cardioprotective activity of leonurine [7].

Labdane diterpenoids, characteristic of the genus *Leonurus*, are notable for their structural diversity and significant abundance in these plants. These compounds exhibit various pharmacological properties, such as anti-inflammatory effects, inhibition of platelet aggregation and anti-cholinesterase activity [8].

The genus *Leonurus* (*Lamiaceae*) is widely recognized for its significant pharmacological importance, particularly in traditional Chinese medicine. Species such as *Leonurus japonicus* Houtt. are extensively used for treating cerebral apoplexy and reducing blood lipid levels.

However, frequent substitution of authentic *Leonurus* species with adulterants poses challenges in ensuring the efficacy and quality of herbal products. Traditional morphological identification methods are often inadequate for differentiating closely related or substituted species, highlighting the necessity of employing molecular markers to improve taxonomic resolution and authenticate medicinal materials. The Lamiaceae family is well-known for its medicinal components derived from terpenoids; however, *Leonurus* stands out due to its alkaloid-rich composition, with leonurine being the principal bioactive compound. Leonurine has demonstrated promising effects in reducing blood lipids and treating strokes, making it a valuable target for pharmacological and genetic studies [9]. Despite its importance, the biosynthetic pathway of leonurine remains largely unexplored. Li et al. [9] presented chromosome-level genome assemblies for *L. japonicus*, a species known for its high leonurine production, and *L. sibiricus*, characterized by minimal leonurine production. Using an integrated approach combining genomics, RNA sequencing, metabolomics, and enzyme activity assays, they constructed the leonurine biosynthesis pathway and identified key enzymes: arginine decarboxylase (ADC), uridine diphosphate glucosyltransferase (UGT), and serine carboxypeptidase-like (SCPL) acyltransferase. Evolutionary analysis revealed that the UGT-SCPL gene cluster evolved through gene duplication in the ancestor of *Leonurus*, with neofunctionalization of SCPL in *L. japonicus* driving its high leonurine accumulation.

Molecular phylogenetic studies have become an essential tool in addressing taxonomic challenges, complementing traditional morphological approaches. Taxonomic issues within several genera of the *Lamiaceae* family have been successfully resolved using molecular analyses based on plastid DNA regions (e.g., *atpB-rbcL*, *psbA-trnH*, *rpl16*, *rpl32-trnL*, *rps16*, *trnK*, *trnL-trnF*, *trnS-trnG*, *trnT-trnL*, *ycf1*) and nuclear ribosomal regions *nrITS* and *nrETS*. These techniques have significantly clarified phylogenetic relationships among taxa, facilitated the proposal of updated classification schemes, and enhanced our understanding of the evolutionary history of this family [10, 11, 12].

In Uzbekistan, molecular genetic studies of the genus *Leonurus* are in their early stages, presenting significant opportunities for exploring biodiversity and taxonomy in this region. The application of molecular tools is particularly important given the limited knowledge of *Lamiaceae* species diversity in Central Asia and their high potential for medicinal applications. Including regional populations in molecular studies will help uncover deeper insights into evolutionary relationships and biosynthetic pathways while establishing a solid foundation for the sustainable use and conservation of biodiversity.

Molecular markers, such as *nrITS* and *nrETS*, combined with plastid regions, have proven to be reliable tools for species delimitation and phylogenetic studies. These markers are highly informative, enabling the detection of both intraspecific and interspecific variation. Applying these approaches in Uzbekistan could significantly advance the taxonomy of the genus *Leonurus* and the *Lamiaceae* family as a whole, while contributing to a broader understanding of their biodiversity and evolutionary history. Genomic and phylogenetic studies have further enriched our understanding of *Leonurus*. Wang et al. [13] analyzed 59 accessions of *L. japonicus* across China, revealing an average nucleotide diversity of 0.00029 and identifying hotspot regions in the *petN-psbM* and *rpl32-trnL* (UAG) spacers suitable for genotype discrimination. Phylogenetic analysis divided the accessions into four clades, highlighting significant divergence influenced by environmental changes, such as the Hengduan Mountains uplift and global temperature drops approximately 7.36 million years ago. The study emphasized dynamic distribution shifts of *Leonurus* species from the Last Interglacial (LIG) to the present, with the Hengduan Mountains acting as a glacial refuge during climatic changes.

Huang et al. [14] reported the complete chloroplast genome of *L. sibiricus*, which comprises 151,689 base pairs with a GC content of 38.4% and includes 133 annotated genes (88 protein-coding genes, 37 tRNAs, and eight rRNAs). Phylogenetic analysis using the

maximum-likelihood method revealed a close evolutionary relationship between *L. sibiricus* and *L. japonicus*. These findings provide valuable resources for biodiversity and evolutionary research within the genus.

Earlier research demonstrated the utility of nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) regions as reliable molecular markers for species identification and phylogenetic studies. Yang et al. [15] analyzed ITS and *matK* sequences from subgenera of *Leonurus* in China, uncovering major phylogenetic clades and clarifying interspecific relationships. Their findings suggested that *L. macranthus* acts as a phylogenetic bridge between subgenera *Cardiochilium* and *Leonurus*. ITS sequences exhibited significant variation, ranging from 7.2–18.8% for ITS1 and 14.2–27% for ITS2, demonstrating their effectiveness for species authentication.

Yang et al. further advanced genomic research by sequencing the chromosome-level genomes of *L. japonicus* and *L. sibiricus*. The *L. japonicus* genome (518.19 Mb) was assembled into ten pseudochromosomes with a contig N50 of 17.62 Mb, while the *L. sibiricus* genome (472.29 Mb) was assembled into nine pseudochromosomes with a contig N50 of 13.29 Mb. These reference genomes provided foundational insights into the biosynthesis of bioactive compounds and evolutionary relationships within the genus.

Despite its recognized medicinal value, taxonomic and pharmacological studies of *Leonurus* in Uzbekistan have been limited, particularly in the application of molecular genetic tools. The findings of this study may not only contribute to a deeper understanding of *Leonurus* but also lay the groundwork for future studies on the biodiversity and taxonomy of the *Lamiaceae* family in Uzbekistan and beyond.

2 Botanical Description

2.1 *Leonurus L.* – Motherwort

***Leonurus glaucescens* Bunge** - also known as *Leonurus cardiaca* sp. *glaucescens* (Bunge) Schmalh., is a perennial or biennial plant. The entire plant has a bluish appearance due to the presence of very short, appressed hairs. Stems are typically numerous, occasionally solitary, reaching a height of 50–100 cm. They are branched and characterized by prominent ribs in the upper parts, which are covered with rough hairs. The leaves are rounded at the base and deeply divided into narrow wedge-shaped segments, which are further dissected into linear or linear-lanceolate lobes. Lower leaves may fall off during the early flowering stages. Leaves in the inflorescence region are rhomboid in shape with a wedge-shaped base and divided into three linear entire lobes. The inflorescence is elongated, composed of spaced whorls. Bracts are needle-like and covered with short appressed hairs. The calyx is narrowly conical, 7–8 mm long (occasionally up to 9 mm), slightly humped, and short-haired. The calyx teeth measure 3–3.5 mm in length, with a triangular base transitioning into a long needle-like tip that exceeds the length of the tooth base. The two lower teeth are bent and more strongly fused. The corolla is pale pink, measuring 10–12 mm in length, with the upper lip covered in long white hairs. Fruits are nutlets, measuring 2–3 mm in length. The flowering period extends from June to August.

***Leonurus kudrjaschevii* Kamelin & Tulyag.** – this perennial plant thrives on gravelly and rocky slopes, primarily in the upper mountain belt of the Kashkadarya region.

***Leonurus turkestanicus* V.I. Krecz. & Kuprian.** – is a perennial herbaceous plant belonging to the *Lamiaceae* family. It grows to a height of 50–150 cm. The stems are erect and slightly branched at the top. Leaves are simple, claw-shaped, and five-lobed, with the upper leaves being three-lobed and opposite. Flowers are bilabiate and five-lobed, situated in the axils of leaves on the stem, forming a panicle-shaped inflorescence. The corolla is ink-

pink in color. The flowering and seed maturation period occurs between June and August. The fruit comprises four triangular, hairy, brown nutlets. The plant is typically found on fine-soil, gravelly, and rocky slopes, in ravines, river valleys, and moist shady places within the middle mountain belt. It is distributed across the Urgut, Kashkadarya, and Tarkapchigay regions. It is valued as both a medicinal and honey plant. *Leonurus turkestanicus* is considered one of the promising medicinal plants. Its description, distribution, raw material preparation, quality, and chemical composition attract the attention of foresters specializing in medicinal plant cultivation, farmers, pharmaceutical industry workers, and specialists working in the Republic.

2.2 Methodology of phylogenetic analysis of *Leonurus* species

For the genetic analysis of species belonging to the genus *Leonurus*, the following methodologies were employed. Samples collected from the Republic's territory (grown in the Tashkent region) were analyzed. These samples were sequenced using specially designed primers for the ITS (Internal Transcribed Spacer) region. Additionally, ITS region sequences of 21 representatives of the Lamiaceae family were downloaded from the National Center for Biotechnology Information (NCBI, GenBank) and prepared for analysis. The collected ITS region sequences and data from GenBank were aligned using the CLUSTALW web program (<https://www.genome.jp/tools-bin/clustalw>) [16]. The alignment results were edited using the Snap Gene program (version 8.0.0, free version) and finalized. To analyze phylogenetic relationships, a tree was constructed using the Maximum Likelihood (ML) method [17]. The IQ-TREE program (version 1.6.12) was utilized for this purpose. The Kimura 2-parameter (K2P) model was applied, and 1000 iterations were performed to obtain bootstrap and aLRT statistical evaluations. The following command line was used: `iqtree -s Leonurus.aln -st DNA -m K2P -bb 1000 -alrt 1000 -o MW602123 Paraphlomis coronata` (where *Leonurus.aln* represents the aligned sequence file, DNA indicates DNA sequences were used, K2P is the Kimura 2-parameter model, and MW602123 *Paraphlomis coronata* was selected as the outgroup). The constructed phylogenetic tree was visualized using the iTOL (Interactive Tree of Life, <https://itol.embl.de>) program [18]. The tree clearly reflects the genetic proximity and differences between the species. *Paraphlomis coronata* (NCBI ID: MW602123) was chosen as the outgroup to serve as the primary reference in tree formation. This methodology proved highly effective for the genetic and evolutionary analysis of *Leonurus* species. The applied modern bioinformatics tools and methodologies ensured results with a high degree of reliability. This approach can also be widely used for the phylogenetic analysis of other species. The two figures presented illustrate the phylogenetic and genetic proximity of species within the genus *Leonurus* and related groups (Figures 1 and 2). Based on this data, evolutionary relationships, genetic distances, and clustering processes among the *Leonurus* species were analyzed.

3 Results

3.1 Phylogenetic Tree: Description

This phylogenetic tree illustrates the evolutionary relationships among species of the genus *Leonurus* and closely related genera of the *Lamiaceae* family. It highlights genetic connections, common ancestors, and evolutionary directions among the species. The tree comprehensively reflects evolutionary relationships, including species of the genus *Leonurus* as well as closely related genera *Lagochilus* and *Paraphlomis*. The branch lengths and

clustering on the diagram indicate the genetic distances between the studied species (Figure 1).

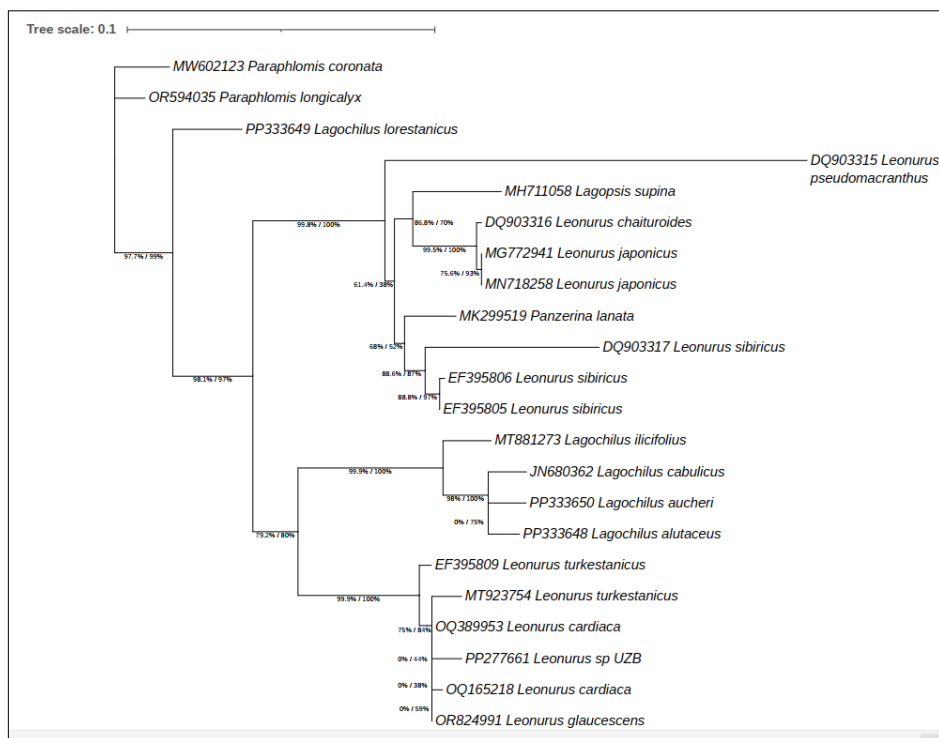


Fig. 1. Phylogenetic diagram of *Leonurus* sp. UZB (PP277661).

A distance scale of 0.1 indicates genetic distance: the shorter the branch length, the closer the relationship between species. The diagram includes Bootstrap values for each node, demonstrating the reliability of the relationships among the species. High values, for example, >90%, confirm the robustness of the results. The following monophyletic groups are shown on the diagram: *Leonurus* sp. UZB (PP277661), *Leonurus glaucescens* (OR824991), *Leonurus turkestanicus* (MT923754), *Leonurus cardiaca* (OQ165218, OQ389953), *Leonurus sibiricus* (EF395805, EF395806, DQ903317), and species belonging to the genus *Lagochilus* (*Lagochilus alutaceus*, *Lagochilus aucheri*). Among them, *Leonurus* sp. UZB (PP277661), *Leonurus glaucescens* (OR824991) and *Leonurus turkestanicus* (MT923754) form a single monophyletic group. The Bootstrap values are extremely high (99.9% - 100%), confirming the reliability of this grouping. This indicates a common ancestor. *Leonurus* sp. UZB (PP277661) and *Leonurus cardiaca* (OQ165218, OQ389953) are positioned in the closest group with a value of 98%, suggesting local adaptation of the introduced *Leonurus cardiaca* (OQ165218, OQ389953) species under Uzbekistan conditions. Meanwhile, *Leonurus sibiricus* (EF395805, EF395806, DQ903317) is genetically distant, with values of 79.2% - 80%, indicating its independent evolutionary lineage shaped by geographical or ecological isolation. *Lagochilus* species - *Lagochilus alutaceus* and *Lagochilus aucheri* - form a separate cluster, genetically distinct from the species of the genus *Leonurus*. On the diagram, *Paraphlomis coronata* (MW602123) is selected as the outgroup, as it represents a separate direction. The Bootstrap values of 99.9% - 100% confirm the reliability of the analysis results.

3.2 Genetic distance analysis and phylogenetic tree for *Leonurus* sp. UZB

The results of the genetic distance analysis for *Leonurus* sp. UZB have helped determine its genetic proximity and differences from other species. This analysis was used to construct a phylogenetic tree illustrating the relationships between *Leonurus* sp. UZB and its closely related species (Figures 2 and 3). The genetically closest species to *Leonurus* sp. UZB were identified as *Leonurus glaucescens* (genetic distance: 0.0058), *Leonurus turkestanicus* (genetic distance: 0.0197) and *Leonurus cardiaca* (genetic distance: 0.0104).

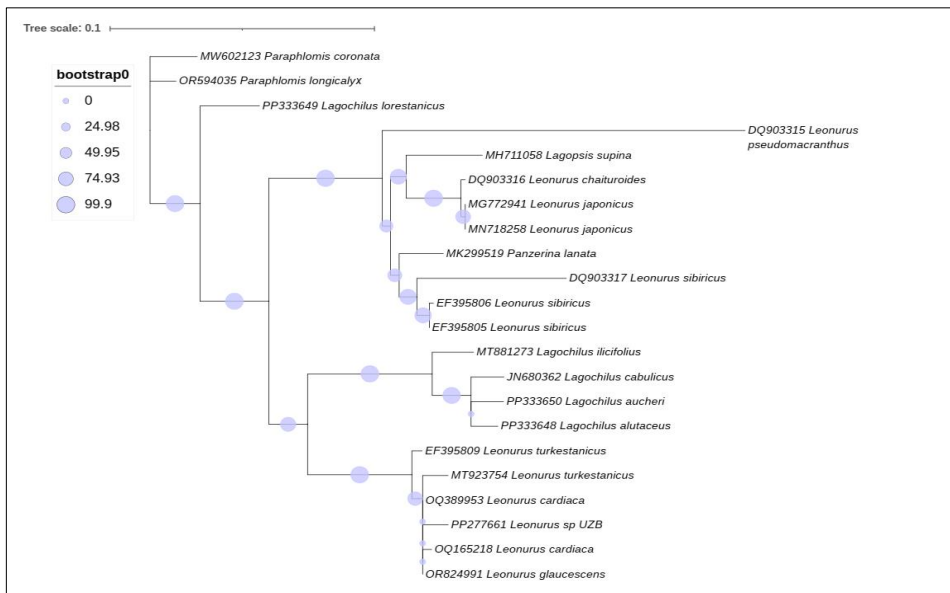


Fig. 2. Phylogenetic tree and genetic distance analysis of *Leonurus* sp. UZB.

These indicators show that the species have very similar genetic characteristics and likely originated from a common ancestor. The most genetically distant species from *Leonurus* sp. UZB are *Leonurus pseudomacranthus* (genetic distance: 0.21915) and *Leonurus sibiricus* (genetic distances: 0.1211 and 0.1192), indicating significant differences and the formation of independent evolutionary directions. Based on the results of the analysis, the following phylogenetic tree (dendrogram) was constructed. This dendrogram displays the close and distant genetic relationships of *Leonurus* sp. UZB with other species. The distance between branches on the tree reflects genetic differences between species: those located closer together originate from a single evolutionary line, while distant species demonstrate independent developmental paths.

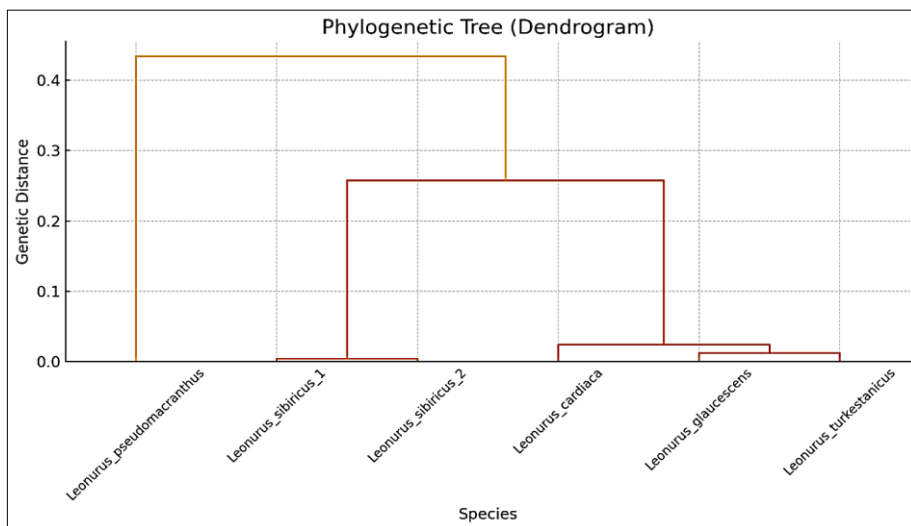


Fig. 3. Dendrogram of Genetic Distances for *Leonurus* sp. UZB.

4 Phylogenetic analysis and evolutionary relationships

This phylogenetic diagram serves as a primary resource for studying the evolutionary relationships among species of the genus *Leonurus* and other closely related genera. The diagram presents the structure of the phylogenetic tree, the percentage of genetic similarity, and evolutionary directions. To investigate genetic proximity between genera, *Lagochilus* and *Paraphlomis* were included in the analysis alongside *Leonurus*. The genetic distance between these genera was found to be significant, indicating their evolutionary divergence. For instance, the similarity level between the genera *Leonurus* and *Lagochilus* ranges from 38%-61.4% and 52%-68%, highlighting their genetic remoteness. Genetic similarity analysis between species revealed that *Leonurus cardiaca* and *Leonurus turkestanicus* exhibit a high genetic similarity of 99.9%-100%. This indicates their origin from a common ancestor and closely aligned evolutionary paths. Several genetic samples of *Leonurus sibiricus* were analyzed, showing a high level of similarity in the range of 97%-98.1%, which suggests genetic uniformity within the population.

5 Discussion

The research results offer a fresh perspective on the evolutionary processes within the genus *Leonurus*. They emphasize the intricate and multifaceted mechanisms that shape biological diversity. The genetic similarity observed between *Leonurus cardiaca* and *Leonurus turkestanicus* suggests that their evolutionary divergence occurred relatively recently. For instance, the genetic similarity between these species is recorded at 99.9%-100%, indicating their origin from a common ancestor and highlighting the impact of environmental adaptation processes. Analysis of *Leonurus sibiricus* revealed a high degree of genetic uniformity within its population, ranging from 97% to 98.1%. This suggests that the species may have evolved under isolated conditions or experienced limited genetic exchange. Such scenarios are often the result of ecological or geographical barriers. The considerable genetic distance observed between *Leonurus* and related genera, such as *Lagochilus* and *Paraphlomis*, is another

notable finding. For example, the genetic similarity between *Leonurus* and *Lagochilus* ranges from 38% to 61.4%, illustrating their divergence from a common ancestor at a much earlier stage. Phylogenetic trees serve as one of the most reliable tools for understanding evolutionary relationships. In this case, closely related species are grouped together, while genetically distant species are shown on distinct branches, reflecting their unique evolutionary paths. The findings from this research are highly relevant for biodiversity conservation programs and assessing species' adaptability. For instance, the high similarity between *Leonurus cardiaca* and *Leonurus turkestanicus* indicates the necessity of preserving them not in isolation but as a group. Such an approach can be more effective in ensuring their long-term survival. Modern genetic analysis techniques thus not only deepen our understanding of evolutionary biology but also prepare us to address future ecological challenges more effectively.

6 Conclusion

This study provided an analysis of the evolutionary relationships and genetic diversity of plants from the genus *Leonurus* growing in Uzbekistan, while also clarifying their position among closely related genera (*Lagochilus* and *Paraphlomis*). The results align with the stated objectives of the research, offering a deeper understanding of the processes underlying the formation of genetic diversity within this genus. The practical significance of this work lies in the potential application of the findings for biodiversity conservation. The identified high genetic similarity between *Leonurus cardiaca* and *Leonurus turkestanicus* highlights the importance of their joint preservation, ensuring the stability of their populations in natural habitats. Furthermore, the data on genetic differences between *Leonurus* and other genera provide valuable tools for selection programs and studying adaptive processes. Future research could focus on a more detailed examination of the genomes of various *Leonurus* populations and the analysis of environmental factors influencing their genetic structure. In particular, future studies should include genomic analysis of *Leonurus sp. UZB* to gain a more comprehensive understanding of its evolutionary characteristics. Such studies would allow for a deeper understanding of the mechanisms driving evolution and adaptation in these species, as well as the development of new approaches to their conservation. Thus, the results of this research not only expand our knowledge of the evolution of *Leonurus* species but also establish a strong foundation for applying this knowledge in biodiversity conservation and sustainable use of plant resources.

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