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MOLECULAR AND BIOTECHNOLOGICAL APPROACHES TO THE MICROPROPAGATION AND CONSERVATION OF *LAGOCHILUS* SPECIES IN UZBEKISTAN

Dilafruz Jamalova [1,*], Gulsauir Kurbaniyazova [1], Shoxsanam Safarova [1], Shokhina Khalimova [2], Gulayim Serekeeva [3], Aziza Djurayeva [4], Muzafarova Sarviniso [5], Akmaljon Marupov [6], Fatma Turker [7], Huseyin Turker [8], Doston Turdiev [1] and Ziyoviddin Yusupov [1]

[1]Institute of Botany of the Academy of Sciences, Republic of Uzbekistan, Durmon yuli str., Tashkent, Uzbekistan
[2]Faculty of Natural Sciences and Agrobiotechnology, Bukhara State University
[3]Department of General Biology, Karakalpak State University
[4]Department of Microbiology, Virology and Immunology, Bukhara State Medical Institute
[5]Jizzakh State Pedagogical University, Jizzakh, Uzbekistan
[6]Fergana State University, Department of Ecology and Environmental Protection
[7]Department of Biology, Faculty of Science, Niğde Ömer Halisdemir University, Niğde, Türkiye
[8]Biotechnology Department, Faculty of Sciences, Niğde Ömer Halisdemir University, Niğde, 51240, Türkiye

Corresponding author: dilafruz.bel.91@mail.ru

ABSTRACT

This review synthesizes the current knowledge on the molecular, biotechnological, and conservation aspects of *Lagochilus* species distributed in Uzbekistan. The literature was systematically collected from major scientific databases, including Scopus, Web of Science, Google Scholar, and PubMed, with emphasis on publications from 2000–2025 and recent advances reported between 2020 and 2025. Studies addressing micropropagation, tissue culture, chloroplast genomics, DNA barcoding, phylogenetic analyses, and conservation biotechnology were critically evaluated. Among the species, *L. inebrians* and *L. proskorjakovii* have been the primary subjects of micropropagation studies, with several successful in vitro regeneration protocols developed, whereas other taxa, particularly *L. olgae*, remain largely unexplored from molecular and biotechnological perspectives. Chloroplast genome analyses and molecular markers provide important insights into genetic diversity, evolutionary relationships, and species identification, supporting conservation planning for these endemic and threatened taxa. This review's novelty lies in integrating molecular phylogenetics, biotechnology, and conservation biology into a comprehensive assessment of *Lagochilus* species in Uzbekistan while identifying knowledge gaps and research priorities. The review highlights the need to combine tissue culture, molecular characterization, and ecological monitoring to strengthen both *ex situ* and *in situ* conservation. These findings provide practical guidance for developing species-specific propagation protocols, preserving genetic resources, restoring natural populations, and supporting the sustainable conservation of rare and endangered *Lagochilus* species under changing environmental conditions.

Keywords: Chloroplast genomics, DNA barcoding, Phylogenetic analysis, Medicinal plants, Germplasm preservation, Genetic diversity, Rare endangered plants.

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1. INTRODUCTION

The genus *Lagochilus* Bunge ex Benth. Belongs to the subfamily Lamioideae and the tribe Leonureae of the Lamiaceae family. It comprises approximately 46 perennial herbs and subshrubs, many of which are remarkably adapted to arid and semi-arid environments. Species of *Lagochilus* display high ecological plasticity, thriving across dry foothill zones, mountain slopes, and desert ecosystems of Central Asia, where they form a characteristic component of xerophytic vegetation.

The Pamir-Alay Mountains, southwestern Tian Shan, and Turanian lowlands represent the primary centers of diversity and distribution for the genus. According to *Plants of the World Online* (POWO, Kew Science), *Lagochilus* species occupy diverse habitats ranging from gravelly or rocky slopes to sandy desert basins, reflecting strong adaptations to xerothermic conditions. The flora of Central Asia, with about 34 species recorded, is considered both

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the richest region for *Lagochilus* diversity and its probable center of origin. The evolutionary history of the genus is closely tied to major paleogeographic and climatic events, such as the uplift of the Tian Shan and the aridization of Central Asia during the Late Tertiary and Quaternary periods (Meng & Zhang 2011; Tian & Ma, 2021). These processes likely promoted vicariance, microhabitat specialization, and speciation, shaping the current distribution and diversity patterns of the genus.

Recent advances in plant molecular biology have considerably improved the understanding of phylogenetic relationships and species delimitation within taxonomically complex genera. Chloroplast genome sequencing and DNA barcoding have become powerful tools for resolving species boundaries, assessing genetic diversity, and identifying cryptic taxa that morphological characters alone cannot reliably distinguish. In medicinal plant research, these approaches are increasingly applied to support accurate species identification, germplasm management, and conservation planning. Nevertheless, molecular information for *Lagochilus* species remains extremely limited, particularly for taxa distributed in Central Asia, highlighting the need for comprehensive molecular investigations.

In Uzbekistan, *Lagochilus* is represented by 18 taxa, distributed from the Kyzylkum Desert in the lowlands to the mountain systems of the Tian Shan and Pamir-Alay in the east and southeast of the country (Akhmedov et al. 2021). Four species are officially listed in the Red Data Book of Uzbekistan (2019) due to their restricted populations and ongoing habitat degradation: *Lagochilus proskorjakovii* (Category I, Critically Endangered), *L. olgae* (Category II, Rare), *L. vvedenskyi* (Category III, Disappearing), and *L. inebrians* (Category III, Disappearing). The Kyzylkum Desert serves as a key distribution zone for many of these rare and threatened species, highlighting their adaptation to harsh arid environments with limited ecological niches (Fig. 1). However, taxonomic delimitation within the genus remains challenging due to strong morphological similarity among closely related species (Zhang et al. 2017).



Fig. 1: Some *Lagochilus* species are distributed in Uzbekistan.

Members of the genus *Lagochilus* are of considerable ethnobotanical and pharmacological importance. Many species have long been utilized in traditional medicine, particularly in Central Asia, where they are well known for their hemostatic, sedative, and antispasmodic properties (Jiao et al. 2015). *Lagochilus inebrians*, in particular, is among the best-studied medicinal species. Its leaves contain the tetracyclic alcohol lagochilin, along with tannins (up to 14%), organic acids, and essential oils (approximately 0.03%). Aqueous and alcoholic extracts of *L. inebrians* have demonstrated adaptogenic, hypotensive, analgesic, and anticonvulsant effects, while infusions and tinctures are used as hemostatic agents to promote blood clotting and relieve symptoms of neuroses, Meniere's disease, and hypertension.

Phytochemical analyses indicate that *Lagochilus* species are rich in secondary metabolites, particularly terpenoids and isoprenoids, which are known for their wide range of biological activities and serve as important taxonomic markers (Malikova & Rakhimov 1997; Mamadalieva et al. 2021; Isomov et al., 2026). To date, approximately 150 secondary metabolites have been identified across various *Lagochilus* species, including diterpenoids, triterpenoids, flavonoids, phenolic compounds, iridoid glycosides, lignans, alkaloids, and steroids, as well as diverse volatile oils, lipids, polysaccharides, carbohydrates, minerals, and vitamins (Zainutdinov et al. 1994; Akramov et al. 2020, 2021;Mamadalieva et al., 2025). Among these, diterpenoids are particularly significant due to their bioactive potential and taxonomic relevance (Zhang et al. 2015).

In parallel with molecular advances, plant biotechnology has become an essential component of modern conservation programs. In vitro propagation techniques provide an efficient approach for the rapid multiplication of threatened medicinal plants while minimizing pressure on natural populations. Tissue culture also enables the long-term preservation of valuable germplasm, production of pathogen-free planting material, and establishment of ex situ collections. Furthermore, regenerated plants can serve as a source of bioactive compounds and as starting material for future genetic improvement and restoration programs. Such approaches are particularly valuable for slow-growing or narrowly distributed endemic species facing increasing environmental pressures.

Although several studies have investigated the taxonomy, phytochemistry, and pharmacological properties of individual *Lagochilus* species, information remains fragmented and dispersed across different scientific disciplines. No previous review has comprehensively integrated molecular phylogeny, chloroplast genomics, DNA barcoding, micropropagation techniques, and conservation biotechnology specifically for the *Lagochilus* species occurring in Uzbekistan. Integrating these research areas is essential for understanding species diversity, identifying conservation

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priorities, and developing effective strategies for the sustainable utilization of endangered medicinal plants under ongoing climate change and habitat degradation.

Despite the increasing number of studies on the taxonomy, phytochemistry, and medicinal properties of *Lagochilus*, information on molecular phylogeny, chloroplast genomics, DNA barcoding, micropropagation, and conservation biotechnology remains scattered and has never been synthesized into a single comprehensive review. This lack of integrated knowledge limits the development of evidence-based conservation strategies and the sustainable utilization of these endangered medicinal plants.

Therefore, this review provides the first comprehensive synthesis of the current knowledge on molecular research, biotechnological advances, micropropagation techniques, and conservation strategies for *Lagochilus* species in Uzbekistan. In addition, the review identifies existing research gaps, highlights future research priorities, and discusses practical implications for germplasm conservation, ecological restoration, and sustainable utilization.

2. MATERIALS AND METHODS

2.1 Literature Search Strategy

This review was conducted through a comprehensive literature search of major scientific databases, including Scopus, Web of Science, Google Scholar, and PubMed. Publications published between 2000 and 2025 were considered, with particular emphasis on recent studies from 2020 to 2025 to ensure that the review reflects the latest advances in molecular biology, plant biotechnology, and conservation research.

The literature search was performed using combinations of the following keywords: "*Lagochilus*", "micropropagation", "plant tissue culture", "in vitro regeneration", "chloroplast genome", "plastome", "DNA barcoding", "matK", "trnL-F", "phylogenetic analysis", "conservation biotechnology", "medicinal plants", "genetic diversity", and "Uzbekistan".

2.2. Taxon Sampling and Data Acquisition

A total of 28 species of *Lagochilus* and one outgroup taxon (*Phlomoides rotata*) were included in the phylogenetic analyses (Table 1). Plastid DNA sequences of two markers, matK and trnL–F, were retrieved from the NCBI GenBank database. Only high-quality, complete, and unambiguous sequences were selected for analysis. Accession numbers for all taxa are provided in Table 1. To ensure taxonomic accuracy, species identifications and voucher information were verified against the original publications associated with each sequence. For *Lagochilus*, sequence data were primarily sourced from Zhang et al. (2017), whereas outgroup data were verified following Zhao et al. (2021). Sequence selection was based on the availability, quality, and taxonomic reliability of publicly accessible

Table 1: GenBank accessions of matK and trnL–F sequences included in the concatenated plastid dataset

No.	Species	matK (GenBank)	trnL–F (GenBank)	References
1	*Lagochilus xinjiangensis*	MF157017.1	MF157275.1	Zhang et al., 2017
2	*Lagochilus turkestanicus*	MF157015.1	MF157273.1	
3	*Lagochilus setulosus*	MF157013.1	MF157270.1	
4	*Lagochilus servaschanicus*	MF157010.1	MF157269.1	
5	*Lagochilus pulcher*	MF157007.1	MF157265.1	
6	*Lagochilus platycalyx*	MF157004.1	MF157263.1	
7	*Lagochilus platyacanthus*	MF157002.1	MF157260.1	
8	*Lagochilus paulsenii*	MF157001.1	MF157259.1	
9	*Lagochilus occutiflorus*	MF156999.1	MF157257.1	
10	*Lagochilus knorringianus*	MF156989.1	MF157247.1	
11	*Lagochilus intermedius*	MF156986.1	MF157244.1	
12	*Lagochilus ilicifolius*	HQ911467.1	MF157242.1	
13	*Lagochilus hirtus*	HQ911469.1	MF157241.1	
14	*Lagochilus gypsaceus*	MF156983.	MF157239.1	
15	*Lagochilus dicanthophyllus*	MF156980.1	MF157236.1	
16	*Lagochilus bungei*	MF156978.1	MF157234.1	
17	*Lagochilus androssowii*	MF156976.1	MF157232.1	
18	*Lagochilus aucheri*	MF156977.1	MF157233.1	
19	*Lagochilus leiacanthus*	MF156995.1	MF157253.1	
20	*Lagochilus lanatonodus*	MF156993.1	MF157251.1	
21	*Lagochilus macracanthus*	MF156997.1	MF157255.1	
22	*Lagochilus kaschgaricus*	MF156987.1	MF157245.1	
23	*Lagochilus cabulicus*	JN680464.1	JN680513.1	
24	*Lagochilus kschutensis*	MF156991.1	MF157249.1	
25	*Lagochilus subhispidus*	MF157014.1	MF157272.1	
26	*Lagochilus pubescens*	MF157006.1	MF157264.1	
27	*Lagochilus acutilobus*	MF156975.1	MF157231.1	
28	*Lagochilus pungens*	MF157008.1	MF157266.1	

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29	*Phlomoides rotata* (outgroup)	JF954286.1	PP955262.1	Zhao et al., 2021

These results indicate that the combined matK and trnL–F dataset provides a reliable framework for evaluating evolutionary relationships and species-level patterns within *Lagochilus*.

plastid DNA data. Only sequences with confirmed species identification, complete marker regions, and clear GenBank accession information were included. When multiple sequences were available for the same species, representative sequences with the highest completeness and quality were selected.

2.3. Sequence Alignment and Matrix Assembly

All sequences were trimmed and curated prior to alignment. Multiple sequence alignments for *matK* and *trnL–F* were generated separately using MAFFT v7 with the L-INS-i algorithm (Katoh & Standley, 2013). The resulting alignments were visually inspected, and minor insertion–deletion events (indels), ambiguous regions, and alignment inconsistencies were manually corrected using Geneious v11.1.5 (Kearse et al., 2012). Sequence lengths were standardized after removing poorly aligned terminal regions. Alignment quality was assessed based on sequence similarity and the absence of ambiguous regions before downstream phylogenetic analyses.

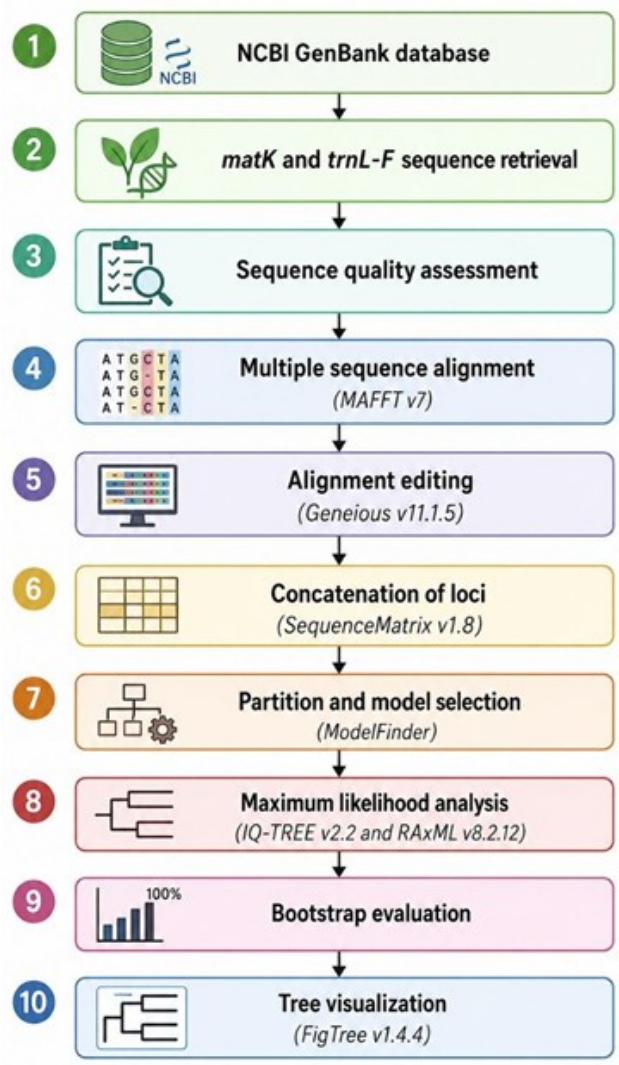


Fig. 2: Workflow of chloroplast phylogenetic analysis of *Lagochilus* species.

Following individual alignments, the two plastid loci (*matK* and *trnL–F*) were concatenated into a single combined supermatrix using SequenceMatrix v1.8 (Vaidya et al., 2011). Partition boundaries were defined according to gene regions, allowing each locus to be analyzed under an independent nucleotide substitution model. The selection of matK and trnL–F markers was based on their complementary phylogenetic properties and extensive application in plant systematics. The matK region is one of the most variable protein-coding chloroplast genes and provides strong phylogenetic signal for resolving relationships among closely related taxa. In contrast, the trnL–F intergenic spacer contains informative non-coding variation that contributes additional resolution at lower taxonomic levels. The combination of these two markers was therefore used to increase phylogenetic signal and improve resolution compared with single-locus analyses.

2.4. Phylogenetic Analyses

Phylogenetic reconstruction was conducted using the Maximum Likelihood (ML) framework. Two independent ML analyses were performed to assess the robustness and consistency of tree topologies. The overall workflow of molecular data acquisition, sequence processing, phylogenetic reconstruction, and tree visualization is presented in Fig. 2. This workflow summarizes the main analytical steps, including plastid sequence retrieval from the NCBI GenBank database, sequence alignment, concatenation of matK and trnL–F markers, maximum likelihood phylogenetic analyses, bootstrap evaluation, and phylogenetic tree visualization.

2.5. IQ-Tree Analysis

ML analysis was performed using IQ-TREE v2.2. ModelFinder was implemented to determine the best-fit nucleotide substitution model for each partition according to the Bayesian Information Criterion (BIC). The ultrafast bootstrap method (UFBoot) with 1,000 replicates was applied to estimate branch support. Bootstrap values ≥95 were considered strong support, values of 80–94 moderate support, and values below 80 weak support.

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2.6. RAxML Analysis

The analysis was conducted using RAxML v8.2.12 under the GTR+GAMMA substitution model. The robustness of inferred relationships was evaluated using 1,000 rapid bootstrap replicates. The best-scoring maximum likelihood tree was selected for comparison with the IQ-TREE topology.

2.7. Tree Rooting and Visualization

All phylogenetic trees were rooted using the outgroup species *Phlomoides rotata*, selected based on its phylogenetic placement within Lamiaceae and its use in previous plastid-based phylogenetic studies. Resulting trees were visualized, edited, and annotated using FigTree v1.4.4, with bootstrap support values displayed at the corresponding nodes.

2.8. Phylogenetic Challenges in *Lagochilus*

The genus *Lagochilus* Bunge (Lamiaceae) represents one of the taxonomically most challenging lineages of the Irano–Turanian flora. Although its species are morphologically well circumscribed at the sectional level, the evolutionary boundaries between many closely related taxa remain unclear, largely due to strong morphological convergence and the presence of cryptic species complexes. Molecular studies on *Lagochilus* have been limited, and the phylogeny of the genus is still far from being fully resolved.

Early molecular investigations of *Lagochilus* relied predominantly on the nuclear ribosomal ITS region (Ranjbar & Khosravi, 2015). Although its provided preliminary insights into species relationships, it frequently produced inconsistent or weakly supported topologies, partly due to intragenomic polymorphism, incomplete lineage sorting, and low phylogenetic informativeness at deeper nodes. These limitations prevented stable reconstruction of relationships between major species groups—particularly complexes such as *L. inebrians–L. kschutensis*, *L. schugnanicus–L. knorringianus*, and several Pamir–Alay endemics.

Plastid markers have also been used to a limited extent in previous studies. Regions such as rpl32–trnL, trnH–psbA, matK, and rbcL were sequenced for selected species, but these datasets were typically sparse and taxon sampling incomplete. As a result, plastid phylogenies lacked resolution and often failed to recover monophyletic sections. In addition, earlier studies did not combine multiple chloroplast regions into a single concatenated dataset, reducing the overall phylogenetic signal.

Recent advances in plant molecular systematics have demonstrated that concatenated plastid regions—especially combinations including matK and non-coding spacers such as trnL–F—substantially improve phylogenetic resolution in *Lamiaceae* and in Irano–Turanian desert lineages. The matK gene offers relatively high substitution rates for deeper divergences, while the trnL–F intergenic spacer provides complementary variation suitable for resolving relationships among closely related species.

However, despite the availability of numerous *Lagochilus* plastid sequences in GenBank—including complete chloroplast genomes for several species—no study to date has systematically combined plastid markers to reconstruct a robust multilocus phylogeny of the genus.

A multi-marker plastid approach is therefore essential to overcome the limitations of previous single-locus analyses. The combined use of matK + trnL–F provides a stronger, more congruent chloroplast signal, enabling the reconstruction of a well-supported phylogeny that can serve as a baseline for future taxonomic, biogeographic, and evolutionary studies.

In this study, we present the first concatenated plastid phylogeny of *Lagochilus* with expanded taxon sampling across its full geographical range. Using Maximum Likelihood analysis based on matK and trnL–F markers, we aim to (1) test the monophyly of major sections, (2) resolve relationships within species complexes, and (3) evaluate geographic structuring of clades in relation to major mountain systems of Central Asia.

3. RESULTS

3.1. Phylogenetic Relationships Inferred from the Concatenated Plastid Dataset (matK + trnL–F)

The concatenated plastid dataset (matK + trnL–F) produced a well-resolved Maximum Likelihood phylogeny of *Lagochilus*. The resulting topology consistently recovered two major lineages corresponding to sect. Inermes and sect. *Lagochilu*s. Most nodes received moderate to strong bootstrap support (BS = 62–99), indicating that the combined plastid dataset provided sufficient phylogenetic signal for resolving relationships among the analyzed taxa (Fig. 3). The taxa included in the phylogenetic analysis and their GenBank accession numbers are presented in Table 1.

Bootstrap support values were interpreted as follows: values ≥95% were considered strongly supported, values of 80–94% as moderately to strongly supported, and values below 80% as weakly supported. Nodes with lower support values were interpreted cautiously when discussing species relationships.

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3.2. Clade Corresponding to sect. Inermes

The monophyly of sect. Inermes was supported by moderate to high bootstrap values (BS = 82–94). The recovered topology indicates close genetic relationships among species distributed mainly in foothill and semi-desert regions of Central Asia. The shallow divergence observed between *L. inebrians–L. kschutensis* and *L. pubescens–L. androssowii* suggests recent diversification or incomplete lineage sorting within these species groups.

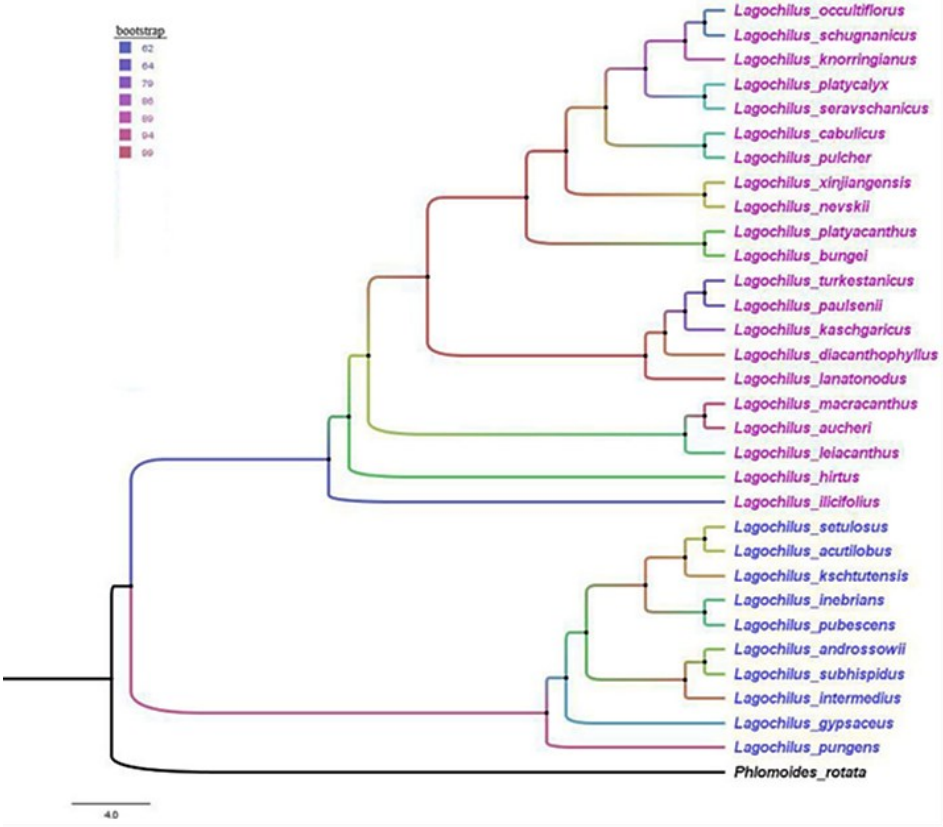


Fig. 3: Maximum Likelihood phylogenetic tree of the genus *Lagochilus* based on the concatenated plastid dataset (matK + trnL–F). Bootstrap support values are shown at the nodes and are colour-coded according to the legend ("Key"). The analysis confirms the monophyly of the two traditionally recognized sections: (1) Sect. Inermes (blue-coloured taxa), forming a well-supported monophyletic clade (BS = 82–94); (2) Sect. *Lagochilus* (pink-coloured taxa), strongly supported as monophyletic (BS = 94–99). The outgroup Phlomoides rotata is clearly separated from *Lagochilus*, validating the tree rooting and generic circumscription. Coloured branches represent geographically structured subclades within the genus, including the Xinjiang–Eastern Tien Shan group, the Pamir–Alay group, and an early-diverging Irano-Turanian lineage. The scale bar indicates relative evolutionary change.

Sect. Lagochilus was recovered as a strongly supported monophyletic lineage (BS = 94–99). The clade included 21 analyzed species and showed clear internal structuring corresponding mainly to geographic distribution patterns across the Pamir–Alay, Tien Shan, and Xinjiang regions.

Within sect. *Inermes*, several species pairs exhibited shallow genetic divergence, notably *L. inebrians–L. kschutensis* and *L. pubescens–L. androssowii*. These patterns likely reflect recent diversification events and/or incomplete lineage sorting; however, the deeper backbone of the clade remained robustly supported.

3.3. Clade Corresponding to sect. *Lagochilus*

The second major lineage corresponded to the sect. *Lagochilus* sensu stricto was recovered as monophyletic with very strong bootstrap support (BS = 94–99). This clade included *L. occultiflorus*, *L. schugnanicus*, *L. knorringianus*, *L. platycalyx*, *L. servaschanicus*, *L. cabulicus*, *L. pulcher*, *L. xinjiangensis*, *L. nevskij*, *L. platyacanthus*, *L. bungei*, *L. turkestanicus*, *L. paulsenii*, *L. kaschgaricus*, *L. dicanthophyllus*, *L. lanatonodus*, *L. macracanthus*, *L. aucheri*, *L. leiacanthus*, *L. hirtus*, and *L. ilicifolius*.

Morphologically, this clade is characterized by prominently spiny or rigid floral bracts, elongated and often sharply pointed calyx teeth, and a predominantly montane to submontane distribution across the Pamir–Alay, Tien Shan, and Xinjiang regions.

3.4. Subclade Structure within sect. *Lagochilus*

The ML topology revealed several well-supported sublineages that correspond closely to geographic distribution patterns and morphological differentiation.

3.5. Xinjiang–Eastern Tien Shan Subclade

A compact and strongly supported subclade (BS = 86–99) comprised *L. schugnanicus*, *L. knorringianus*, *L.*

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platycalyx, *L. servaschanicus*, and *L. cabulicus*. This grouping reflects both geographic proximity and shared floral morphological traits.

3.6. Pamir–Alay Subclade

The clustering of these taxa indicates genetic differentiation associated with the Pamir–Alay mountain system. A second well-supported assemblage (BS = 79–99) included *L. paulsenii*, *L. turkestanicus*, *L. kaschgaricus*, and *L. dicanthophyllus*, representing a geographically coherent Pamir–Alay lineage.

3.7. Basal Irano–Turanian Lineage

Several species with broad Irano–Turanian distributions, including *L. aucheri*, *L. hirtus*, and *L. ilicifolius*, formed an early-diverging lineage within sect. *Lagochilus*. Their basal position is consistent with both their wide geographic ranges and distinct morphological features.

Outgroup Placement

The outgroup taxon *Phlomoides rotata* was clearly separated from all *Lagochilus* accessions, confirming the appropriateness of the selected outgroup and supporting current generic circumscription within Lamiaceae.

Summary of Results

Overall, the concatenated plastid phylogeny:

1. Robustly supports the monophyly of the two traditionally recognized sections (*Inermes* and *Lagochilus*);
2. (ii) Reveals geographically structured and morphologically coherent subclades within sect. *Lagochilus*;
3. (iii) Provides a stable evolutionary framework for interpreting species boundaries, morphological evolution, and historical biogeography in the genus.

3.8. Biological Studies on *Lagochilus* Species and Suggestions for Biotechnologists

The biotechnological approach offers several significant advantages compared to conventional methods used for the conservation of endangered species. In particular, it enables the recovery of protected taxa by facilitating the establishment of artificial populations within natural ecosystems (Mustafina et al., 2025). A key advantage of *in vitro* conservation is the ability to obtain sterile plant cultures without extracting individuals from their native habitats, thereby minimizing disturbance to natural phytocenoses (Tandon & Kumaria, 2005; Aimenova et al., 2024). This aspect is especially important in studies aimed at preserving plant genetic resources through *in vitro* techniques, including the sampling of natural populations and their subsequent propagation and utilization as medicinal raw materials.

Despite the fact that the genus *Lagochilus* comprises approximately 46 species worldwide, with the majority of its diversity concentrated in the Central Asian region, only one or two species have so far been investigated from a biotechnological propagation perspective. This striking disparity strongly calls for more comprehensive research on *Lagochilus* species, particularly with respect to the development and application of biotechnological approaches for their propagation, conservation, and sustainable utilization.

3.9. Tissue Culture in *Lagochilus* Species

The genus *Lagochilus* includes 18 species, four of which (*Lagochilusvve denskyi, L. olgae*, *L. proskorjakovii* and *L. inebrians*) are in the Red Book of the Republic of Uzbekistan (Akhmedov, 2017). Reproduction in *Lagochilus* species occurs mainly through seeds, which represent the primary and most effective means of propagation. In addition to sexual reproduction, these species are also capable of vegetative reproduction by particulation, allowing them to survive and spread under favorable environmental conditions. Vegetative propagation plays an important role in population stability, especially in habitats where seed germination may be limited. Most *Lagochilus* species follow the C3 photosynthetic pathway, which is typical for plants adapted to temperate and relatively cool environments (Islamov et al., 2022). Among the species of the genus, *Lagochilus inebrians* has been the primary focus of microclonal propagation studies, and several *in vitro* protocols have been developed for this species.

Numerous scientific studies have been carried out on the microclonal propagation of *Lagochilus inebrians*, focusing on *in vitro* culture techniques, optimization of growth conditions, and improvement of regeneration efficiency.

The morphogenetic responses of *Lagochilus inebrians* explants under *in vitro* conditions were evaluated using mineral nutrient media with varying compositions of growth regulators. Based on comparative analyses, optimal culture media were identified for the micropropagation of the studied samples. The B5 basal medium supplemented with 0.1 µM BAP proved to be effective for shoot induction. For explants derived from laboratory-grown plants, BDS medium containing 5.0 µM BAP in combination with 2.0 µM NAA yielded favorable results, whereas explants obtained from greenhouse-grown plants responded best to a medium supplemented with 0.4 µM BAP and 3.2 µM NAA. In addition, B5 medium enriched with 2.3 µM IAA was found to be the most suitable for certain developmental stages. During the proliferative phase, BAP was identified as the most effective cytokinin when compared with TDZ

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and kinetin (Sultonova & Kushiev, 2022).

Another species of *this genus* propagated under *in vitro* conditions is *Lagochilus proskorjakovii*, a narrow-range endemic of the Nuratau Mountains. Its natural populations are very small and fragmented, exhibiting limited natural regeneration. Therefore, the application of biotechnological approaches, particularly microclonal propagation and tissue culture techniques, is essential for its effective propagation and conservation. Pioneering research on the *in vitro* propagation of this species, focusing on optimizing conditions for establishing sterile cultures, including sterilization methods, selection of primary explants, nutrient media, and hormonal treatments. Their studies showed that seed sterilization with 0.1% $AgNO_3$ for 15 minutes, followed by 70% ethanol for 1 minute, was essential to ensure explant viability under in vitro conditions. Other sterilization protocols resulted in high contamination or low survival rates. A series of culture media was tested to determine the most suitable medium for maintaining and proliferating the cultures. During the multiplication phase, an optimal combination of auxins and cytokinins was identified as critical for efficient shoot proliferation. Additionally, effective rooting induction conditions, enabling the complete development of plantlets *in vitro*.

Their research provides a reliable biotechnological approach for the ex situ conservation of *L. proskorjakovii*, facilitating the preservation of its genetic resources and supporting future reintroduction programs into natural habitats. These findings highlight the importance of micropropagation as a tool for the sustainable management and conservation of rare and endangered endemic species.

To date, *L. inebrians* and *L. proskorjakovii* are the only species within the genus *Lagochilus* for which successful *in vitro* propagation has been reliably reported, while the remaining members of the genus remain largely unexplored from a biotechnological perspective. This pronounced knowledge gap highlights the limited application of plant tissue culture techniques within *Lagochilus* and underscores the absence of standardized protocols for micropropagation, conservation, and genetic resource management of most species in the genus. Given the ecological, medicinal, and evolutionary significance of *Lagochilus*, the development and optimization of species-specific *in vitro* propagation systems are urgently needed. Such protocols would not only facilitate the ex situ conservation of rare and endemic taxa but also support sustainable utilization, large-scale propagation, and the long-term preservation of genetic diversity within the genus.

Lagochilus olgae is one of the endangered and conservation-dependent plant species, characterized as a rare, narrow-range endemic. Current populations of this species are unstable, small in size, and spatially fragmented. Field observations indicate a dominance of mature generative (g2) individuals, while pre-generative stages are poorly represented, suggesting limited natural regeneration capacity. This population structure reflects the species' low resilience to ongoing environmental pressures (Islamov et al., 2022).

The natural habitats of *L. olgae* are primarily confined to mountainous, rocky, and limestone slopes, where populations are increasingly threatened by grazing pressure, anthropogenic disturbances, and climate-related stressors. These factors contribute to a continuous decline in population size and habitat quality, emphasizing the urgency of developing effective conservation strategies.

In this context, the application of biotechnological approaches is highly recommended for the long-term preservation of *L. olgae*. *In vitro* micropropagation and plant tissue culture techniques offer a reliable means of producing healthy, genetically stable plantlets under controlled conditions. These methods enable ex situ conservation of genetic resources, reduce collection pressure on natural populations, and provide sufficient planting material for future reintroduction and restoration programs. The integration of biotechnological propagation with ecological monitoring represents a sustainable and science-based strategy for safeguarding *L. olgae* and ensuring its long-term survival.

In conclusion, the genus *Lagochilus* comprises several rare and endemic species, with *L. inebrians* and *L. proskorjakovii* being the only taxa for which successful *in vitro* propagation protocols have been established. The remaining species, including *L. olgae*, remain largely unexplored from a biotechnological perspective, highlighting an urgent need for species-specific micropropagation systems. The application of tissue culture techniques offers a reliable approach for ex situ conservation, the preservation of genetic resources, and the sustainable management of these threatened species. Integrating biotechnological propagation with ecological monitoring provides a science-based strategy to ensure the long-term survival and restoration of rare and endangered *Lagochilus* taxa.

4. DISCUSSION

The present study provides an integrated overview of molecular and biotechnological approaches for the conservation of *Lagochilus* species in Uzbekistan. By combining chloroplast-based phylogenetic analysis with available information on tissue culture and micropropagation, this study highlights the importance of multidisciplinary strategies for protecting rare and endemic medicinal plants. Molecular characterization provides a framework for investigating evolutionary relationships, while biotechnological approaches offer practical options

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for conserving and multiplying threatened plant genetic resources (Chokheli et al., 2020; Zhao et al., 2021; Sultonova et al., 2025).

The concatenated analysis based on the chloroplast markers matK and trnL–F resolved the major evolutionary structure of the genus. The recovery of two strongly supported lineages corresponding to sect. Inermes and sect. *Lagochilus* provides evidence for the taxonomic utility of these plastid regions in the present dataset. More broadly, plastid phylogenomic studies have demonstrated the value of chloroplast sequence data for resolving relationships and refining classifications within Lamiaceae (Zhao et al., 2021). However, the resolution obtained from matK and trnL–F should be interpreted in the context of the number of informative sites, taxon sampling, and the support values recovered in the present analysis.

The strong support obtained for sect. Inermes indicates that the sampled members form a well-supported chloroplast lineage. The shallow divergence observed between species pairs such as L. inebrians–L. kschutensis and L. pubescens–L. androssowii may reflect recent divergence or insufficient variation in the selected markers. Incomplete lineage sorting and historical gene flow are additional possible explanations, although these processes cannot be distinguished using the present chloroplast dataset alone. Consequently, nuclear markers and population-level sampling will be necessary to evaluate the evolutionary relationships among these closely related taxa.

The monophyly of sect. *Lagochilus* and the geographically structured subclades suggest that spatial isolation may have contributed to diversification within the genus. The Central Asian steppe–desert biome has experienced substantial climatic and geological changes, including shifts in aridity and the development of mountain landscapes, which have influenced regional ecosystem structure (Barbolini et al., 2020). Nevertheless, the specific contribution of the Pamir–Alay, Tien Shan, and Xinjiang regions to the diversification of the sampled *Lagochilus* lineages requires explicit biogeographic analyses. The present phylogenetic topology alone cannot establish the timing or causality of historical diversification.

Although chloroplast markers provided valuable insights, the present phylogenetic framework should be considered a foundation for future genomic studies rather than a definitive evolutionary hypothesis. Plastids are maternally inherited in many angiosperms, but biparental inheritance and paternal leakage also occur; therefore, chloroplast phylogenies do not necessarily represent the complete evolutionary history of a species. Future investigations incorporating nuclear loci, genome-wide single-nucleotide polymorphisms (SNPs), and complete chloroplast genomes would provide a more comprehensive basis for resolving recently diverged species complexes. Recent comparative chloroplast-genome research on *Lagochilus* has also identified variable genomic regions with potential applications in phylogenetic and conservation studies (Nikitina et al., 2026).

The molecular findings have direct implications for conservation planning. Accurate species identification and the assessment of genetic diversity are important for establishing representative conservation collections. However, ex situ collections do not always capture the full genetic diversity of their wild source populations. A global meta-analysis demonstrated that inadequate sampling and genetic erosion can reduce the representativeness of conserved plant material (Wei & Jiang, 2021). Accordingly, conservation programs for *Lagochilus* should prioritize representative sampling across populations, particularly where species have restricted distributions or geographically isolated populations.

The biotechnological literature indicates that tissue culture can contribute to the conservation of threatened medicinal plants. Micropropagation allows the multiplication of selected plant material under controlled conditions and can support the establishment of conservation collections (Chokheli et al., 2020; Bharathi & Prakash, 2022; Birmeta et al., 2022). For *Lagochilus*, however, the availability of species-specific protocols remains an important consideration. The evidence should be evaluated separately for each taxon rather than assuming that a protocol developed for one species will be equally effective in another.

The reported micropropagation work on L. inebrians provides a species-specific example of the application of in vitro techniques. Sultonova et al. (2025) investigated the optimization of in vitro microcloning and acclimatization under arid conditions. This work supports further investigation of propagation systems for medicinally valuable *Lagochilus* species. Nevertheless, the effectiveness of any protocol for large-scale conservation depends on reproducibility, survival during acclimatization, and the genetic fidelity of regenerated plants.

Among poorly studied taxa, L. olgae may represent a candidate for future biotechnological research if its restricted distribution, population status, and regeneration constraints are confirmed by field and conservation assessments. The development of species-specific micropropagation protocols could support ex situ conservation and future restoration activities. However, the conservation priority of this species should be justified using documented population trends, habitat information, and the relevant national or international conservation assessments.

The integration of molecular information and tissue culture approaches offers complementary advantages for germplasm conservation. Molecular data can guide the selection of genetically representative individuals, while in

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vitro techniques can facilitate their multiplication and maintenance. Such integration is consistent with broader recommendations for the conservation of rare plant genetic resources and the development of biotechnological conservation strategies (Taberlet et al., 1991; Chokheli et al., 2020; Wei & Jiang, 2021; Bharathi & Prakash, 2022). For *Lagochilus*, these approaches should be complemented by field monitoring and habitat-based conservation.

Despite these contributions, several limitations should be acknowledged. The molecular analysis was based on available GenBank sequences; consequently, the availability and quality of public sequence data influenced taxon sampling. Underrepresentation of rare species may limit the assessment of species boundaries and evolutionary relationships. In addition, the analysis relied exclusively on chloroplast markers, which represent only one component of the plant genome. Future studies incorporating nuclear genomic data and broader population sampling are therefore required to obtain a more comprehensive evolutionary perspective (Zhao et al., 2021).

From a biotechnological perspective, the principal limitation is the uneven availability of standardized propagation protocols across the genus. Differences in explant type, culture conditions, and growth-regulator requirements can influence regeneration outcomes. Research on rare plant micropropagation has emphasized the importance of optimizing culture conditions and evaluating the performance of regenerated plants (Chokheli et al., 2020; Hou et al., 2024). Further work should therefore focus on developing reproducible protocols for threatened *Lagochilus* taxa, with particular attention to survival during acclimatization and genetic stability.

Future conservation programs should combine three complementary components: (1) molecular assessment of genetic diversity and evolutionary relationships; (2) development and validation of efficient in vitro propagation systems; and (3) ecological restoration supported by habitat monitoring and population assessment. Representative sampling is particularly important because ex situ conservation collections may otherwise fail to capture the genetic diversity present in natural populations (Wei & Jiang, 2021; Khojimatov & Bussmann, 2023; Stamatakis, 2024). Such integrated strategies could improve the long-term conservation of rare *Lagochilus* species and support the sustainable management of medicinal plant resources.

Overall, this study demonstrates the complementary roles of molecular phylogenetics and biotechnology in understanding and conserving *Lagochilus* species. The integration of plastid-based evolutionary analyses with micropropagation research provides a useful framework for conservation planning in Central Asia. Further progress will depend on expanded molecular sampling, species-specific propagation protocols, and the integration of genetic information with ecological evidence.

5. CONCLUSION

The present study demonstrates that an integrated molecular and biotechnological framework is essential for the effective conservation and sustainable management of *Lagochilus* species in Central Asia. The chloroplast-based phylogenetic analysis using *matK* and *trnL–F* markers provided valuable insights into the evolutionary relationships and genetic structure of the genus, supporting the recognition of major taxonomic lineages and highlighting the importance of molecular approaches for resolving relationships among closely related species.

The review of biotechnological studies revealed that, despite the high medicinal and conservation value of *Lagochilus*, micropropagation research remains limited to only a few species, mainly *L. inebrians* and *L. proskorjakovii*. This knowledge gap emphasizes the urgent need to develop species-specific in vitro propagation protocols for other rare and endemic taxa, particularly those facing population decline and habitat fragmentation.

The integration of molecular identification, germplasm conservation, and tissue culture techniques provides a practical strategy for preserving genetic diversity while reducing pressure on natural populations. Molecular data can support the selection of genetically representative conservation materials, whereas in vitro propagation can facilitate large-scale multiplication, restoration programs, and sustainable utilization of medicinal resources.

Future research should prioritize the generation of comprehensive genomic resources, including complete chloroplast genomes, nuclear molecular markers, and population-level genetic analyses, to improve species delimitation and clarify evolutionary processes within *Lagochilus*. In parallel, optimized micropropagation protocols should be established for threatened species such as *L. olgae* and other Red Data Book taxa of Uzbekistan.

Considering the increasing impacts of habitat degradation, overharvesting, and climate change on Central Asian medicinal plants, conservation strategies for *Lagochilus* should combine ex situ preservation, habitat protection, ecological monitoring, and restoration activities. Such integrated approaches will contribute to the long-term survival of endangered species and support the sustainable use of valuable plant genetic resources.

Overall, this study provides a foundation for future molecular, biotechnological, and conservation-oriented investigations of *Lagochilus* species and highlights the necessity of interdisciplinary approaches for safeguarding rare medicinal plants under changing environmental conditions.

Declarations

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