

Original research

Characterisation of the complete chloroplast genome of the genus *Lappula* (Boraginaceae)

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Abstract

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The evolutionary relationships and genomic variability within the genus *Lappula* Moench remain poorly understood due to limited genomic data and taxonomic complexity. In this study, we conducted a comparative analysis of the complete chloroplast genomes from eleven *Lappula* species to investigate plastome structure, sequence divergence, and phylogenetic relationships. All species exhibited the typical quadripartite structure of angiosperm plastome, with conserved gene content and order. Subtle but informative variations were detected at the inverted repeat boundaries, particularly involving the *ndhF* and *ycfI* genes, reflecting lineage-specific expansion and contraction of inverted repeats. Nucleotide diversity analysis revealed several hypervariable regions, especially within intergenic spacers such as *trnT-UGU-ndhJ* and *ycf4-cemA*, which may serve as potential molecular markers for species delimitation. Phylogenomic reconstruction using complete plastome sequences resolved two major clades within *Lappula* and supported the relationships among the sampled species. These findings highlight the utility of whole plastome data for resolving interspecific relationships and underscore the need for expanded taxon sampling to refine the phylogeny and classification of *Lappula* within the tribe Rochelieae.

Keywords: boundary variation, genome annotation, non-coding regions, phylogenetic relationships, plastome structure, sequence divergence.

INTRODUCTION

The genus *Lappula* Moench (Boraginaceae) comprises 50–70 herbaceous species that exhibit significant morphological and ecological variation (Huang

et al., 2013; Ebadi & Nikzat, 2021). It is found in Eurasia, including Central Asia, and parts of North America and North Africa (Liu et al., 2025). Species of this genus are adapted to xeric habitats, particularly to the arid and semi-arid regions of Central

Asia, where their annual life cycles confer ecological advantages under seasonal drought and extreme temperatures (Ergashov et al., 2025a; Tojibaev et al., 2022; Yusupov et al., 2022; Khassanov et al., 2023; Rakhmataliev et al., 2025, Kurbaniyazova et al. 2025). Despite its broad distribution, the centre of species diversity lies in the Irano-Turanian floristic region, especially in Central Asia (Liu et al., 2025).

The taxonomy of *Lappula* has been historically complicated due to convergent nutlet morphology and a lack of reliable floral diagnostic traits (Ebadi & Nikzat, 2021). Traditional classifications primarily relied on fruit morphology, but recent molecular phylogenetic studies have revealed that *Lappula*, as currently defined, is polyphyletic (Khoshokhan-Mozaffar et al., 2018; Huang et al., 2013). These studies show that *Lappula* species are dispersed across different evolutionary lineages within the tribe Rochelieae DC. However, many of these findings are based on limited molecular data, such as internal transcribed spacer (ITS) and selected plastid regions, and key species from Central Asia remain underrepresented in these studies.

One such species is *Lappula nuratavica* Nabiev & Zakirov, a geographically restricted endemic species with significant morphological variation, including distinctive traits in nutlet micromorphology that are central to species delimitation (Ebadi & Nikzat, 2021; Liu et al., 2021). However, these morphological traits are often homoplastic, making them insufficient for resolving phylogenetic relationships. As a result, molecular tools like the chloroplast genome are essential for providing clearer insights into its evolutionary history (Tojiboeva et al. 2025; Ergashov et al. 2026).

The complete chloroplast genome has become a powerful tool for plant phylogenetics due to its conserved structure, uniparental inheritance, and relatively low recombination rates (Dekhkonoov et al., 2025; Nikitina et al., 2025; Ergashov et al., 2025b). This genomic resource provides valuable insights into evolutionary relationships, ecological adaptations, and structural variations across plant species. The complete chloroplast genome of *Lappula myosotis* has been previously sequenced, serving as a useful comparative reference for other species in the genus (Yan et al., 2023).

In this study, we expand upon this work by sequencing the chloroplast genome of *Lappula nuratavica* and comparing it with those of 10 other *Lappula*

species using publicly available plastomes. In the study, we aim to sequence and analyse the complete chloroplast genome of *Lappula nuratavica*, an endemic species from the Nuratau Mountains of Uzbekistan. Specifically, we seek to (1) explore *Lappula nuratavica*'s phylogenetic relationships within the genus *Lappula*, (2) investigate structural variations in the chloroplast genome, and (3) assess nucleotide diversity across the species in comparison with other *Lappula* species.

MATERIALS AND METHODS

Study species

Lappula nuratavica Nabiev & Zakirov (Fig. 1) is a geographically restricted species endemic to the Nuratau Mountains in Uzbekistan. This species was selected for the study due to its unique ecological adaptations and limited distribution. Fresh leaf sample of *Lappula nuratavica* was collected from its natural habitat in the Jizzakh region of Uzbekistan (40.508056 °N, 66.723889 °E) in May 2024. The species was identified by N. Beshko at the National Herbarium of Uzbekistan, and a representative voucher specimen was deposited at the TASH Herbarium (TASH 001328). Immediately after collection, the leaf samples were dried using silica gel and stored at room temperature until DNA extraction.

DNA extraction and sequencing

Total genomic DNA was isolated from the leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method, as described by Doyle & Doyle (1987). The DNA extraction process adhered to the standard protocol, ensuring high-quality genomic DNA suitable for sequencing.

For genomic sequencing, high-throughput sequencing was performed on the Illumina NovaSeq 6000 platform. Paired-end sequencing with a read length of 150 base pairs (bp) was performed to achieve sufficient depth for chloroplast genome assembly. The raw sequencing data were processed to remove low-quality reads and adapter sequences using Trimmomatic version 0.39 (Bolger et al., 2014). After quality control, clean reads were used for de novo genome assembly.



Fig. 1. Two individuals of *Lappula nuratavica* in their natural habitats: (A) flowering and fruiting individual on rocky-gravelly slope, 1850 m above sea level, 5 June 2022, Khayatsoy and Madjerumsay watershed, Nurata Reserve, Nuratau ridge, Jizzakh region, Uzbekistan; (B) flowering individual on rocks about 1850 m above sea level, 15 May 2024, Khayatsoy and Madjerumsay watershed, Nurata Reserve, Nuratau ridge, Jizzakh region, Uzbekistan. Photographs by N. Beshko.

Chloroplast genome assembly

The clean reads were mapped and assembled de novo using the GetOrganelle pipeline version 1.7.5 (Jin et al., 2020), specifically designed for the assembly of organellar genome. The assembled chloroplast genome was annotated in Geneious Prime version

2025.1.2 (Tillich et al., 2017), using the chloroplast genome of *Lappula myosotis* (NC_060614) as the reference. After initial annotation, the results were manually checked in Geneious Prime to ensure the accuracy of gene predictions and genomic features. The final annotated chloroplast genome map was visualised using CPGview (Liu et al., 2023).

Comparative analysis of chloroplast genome structure

To compare the chloroplast genome structure across species, the boundaries of the large single-copy, small single-copy, and inverted repeat (IR) regions were identified for 11 *Lappula* species. These boundaries were compared to detect any shifts at the junctions between the large single-copy and IRb, the small single-copy and IRb, the small single-copy and IRa, and IRa and large single-copy. IRa and IRb represent the two copies of the inverted repeat, which are sequence-identical but located in opposite orientations and at different junctions with the large single-copy and small single-copy regions. The boundary shifts were visualised using the IRscope tool (Amiryousefi et al., 2018), and manual alignments were performed in Geneious Prime version 2025.1.2 to refine the analysis.

Nucleotide diversity analysis

To assess nucleotide variability across the chloroplast genomes, complete genome sequences of the selected *Lappula* species were aligned using MAFFT version 7.471 (Katoh & Standley, 2013). Multiple sequence alignment of these genomes allowed the identification of regions with varying levels of genetic divergence. Nucleotide diversity (π) was calculated using DnaSP version 6.12.03 (Rozas et al., 2017), providing insights into the genetic variation present across the species. A sliding window analysis was performed with a window length of 800 base pairs (bp) and a step size of 200 bp to identify regions of high nucleotide diversity. These hypervariable regions, identified as potential markers for phylogenetic and barcoding studies, were plotted to visualise patterns of genetic diversity.

Phylogenetic analysis

To infer phylogenetic relationships among *Lappula* species, a maximum-likelihood analysis was conducted using complete chloroplast genome sequences. Sequence alignment was performed using MAFFT version 7.520 (Katoh & Standley, 2013), and phylogenetic trees were constructed using RAxML version 8.2.12 (Stamatakis, 2014). The analysis was

performed under the GTRGAMMA substitution model, with 1°000 bootstrap replicates to assess the reliability of the inferred relationships. Some species from *Mertensia* Roth, *Trigonotis* Steven, *Eritrichium* Schrad. ex Gaudin, *Tournefortia* L. and *Brachybotrys* Maxim. ex Oliv. were used as outgroup species to root the phylogenetic tree (Appendix).

RESULTS

The complete chloroplast genomes of *Lappula* species exhibited a typical quadripartite structure comprising a large single-copy region, a small single-copy region, and two inverted repeat regions (IRa and IRb), with total genome sizes ranging from 145 958 to 147 653 base pairs (bp). The complete chloroplast genome sequence of *Lappula nuratavica* was found to be 147°509 bp in length. The genome architecture and gene order were largely conserved across all examined species. The *Lappula nuratavica* genome contained 130 genes, including 84 protein-coding genes, 37 tRNAs, and 4 rRNAs. Genes were unevenly distributed across the regions, with the majority located in the large single-copy. The inner ring of the genome map revealed a higher guanine-cytosine (GC) content concentrated in the IR regions (Fig. 2).

The comparative analysis of inverted repeat boundary regions across 11 *Lappula* species reveals a generally conserved chloroplast genome structure with subtle but meaningful variations in gene length and boundary positioning. At the large single-copy and IRb junction, all species exhibit a consistent configuration where the *rpl22* gene terminates within the large single-copy region, followed by a short 5 bp overlap of the *rpl2* gene at the junction between the IRb region and the large single-copy region. This structural consistency across all taxa indicates a highly conserved JLB boundary within the genus (Fig. 3).

The IRb and small single-copy junction shows greater variation, particularly in the extent to which the *ndhF* gene extends across the small single-copy boundary into the IRb region. The shortest *ndhF* overlap is observed in *Lappula monocarpa* (1 bp), while *Lappula balchaschensis* and *Lappula duplici-carpa* exhibit the longest overlap at 147 bp. Some species generally do not show any overlap (*Lappula*

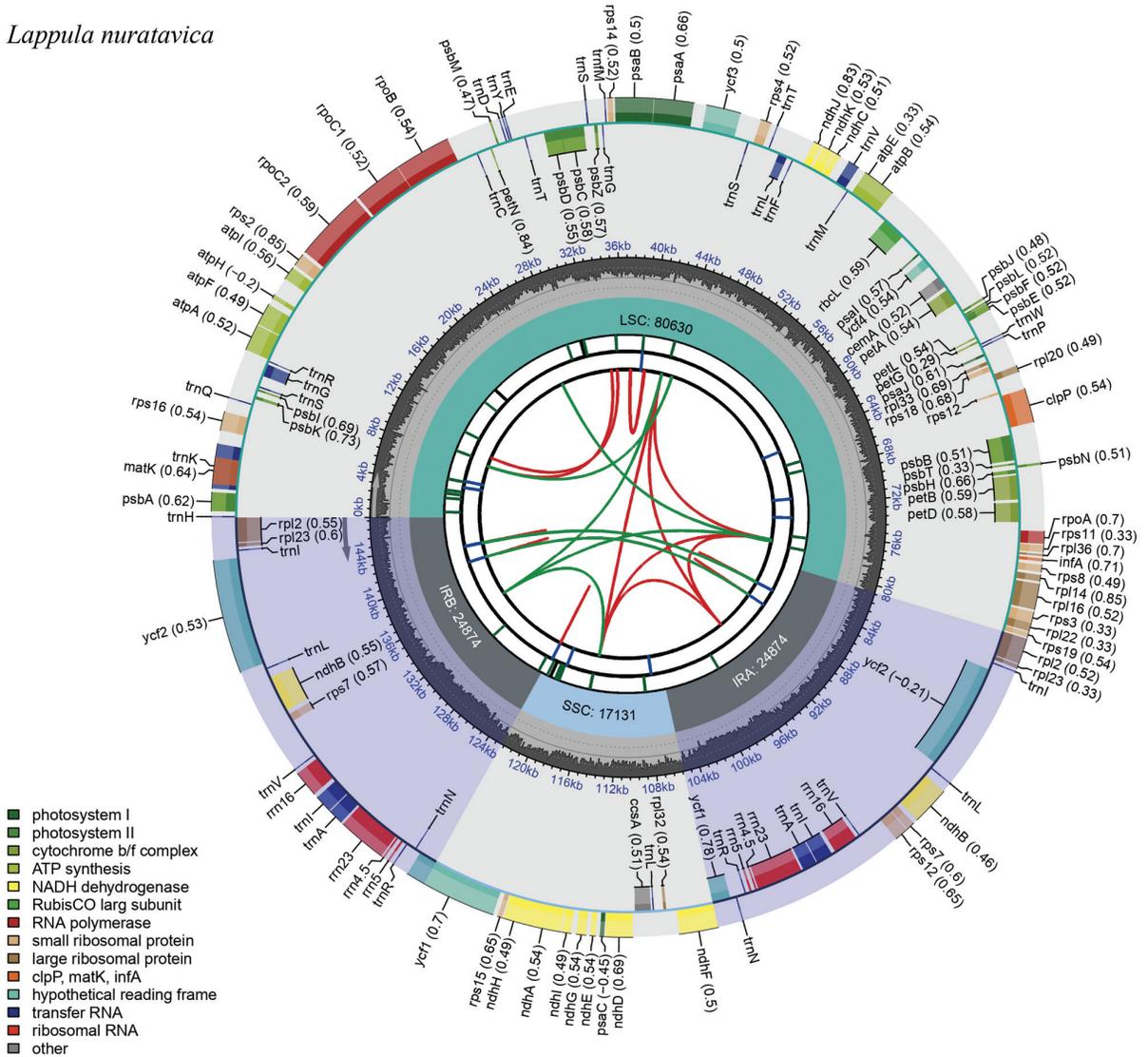
Lappula nuratavica

Fig. 2. Map of the chloroplast genome of the species of the genus *Lappula*. Genes inside the circle are transcribed clockwise, genes outside are transcribed counterclockwise. The dark grey inner circle indicates the guanine-cytosine (GC) content and the light represents adenine-thymine (AT).

lasiocarpa, *Lappula macrantha*, *Lappula myosotis*, *Lappula patula*, *Lappula stricta*). The position of the adjacent *ycf1* gene also varies, with *ndhF* overlapping at IRb and the small single-copy boundary. Notably, the *ycf1* genes of *Lappula lasiocarpa*, *Lappula macrantha*, *Lappula monocarpa*, *Lappula patula*, *Lappula stricta* and *Lappula nuratavica* overlap IRb and small single-copy boundary. At the small single-copy and IRa junction, all species exhibit the *ycf1* gene spanning into IRa with varying lengths. Most species possess longer *ycf1* extensions in IRa, ranging from 5330 to 5373 bp. Finally, the IRa, a large

single-copy junction, is the most conserved among the four examined regions. In all species, the *rpl2* gene terminates within IRa and is separated from the *trnH-GUG* gene in the large single-copy by a consistent 5 bp intergenic space. While most species show a *rpl2* gene length of 1469 bp, *Lappula nuratavica* stands out with a slightly longer *rpl2* segment of 1473 bp, suggesting a modest expansion at the IRa end unique to this taxon.

The nucleotide diversity (π) values ranged from 0 to approximately 0.0065, indicating generally low but variable genetic divergence among the species

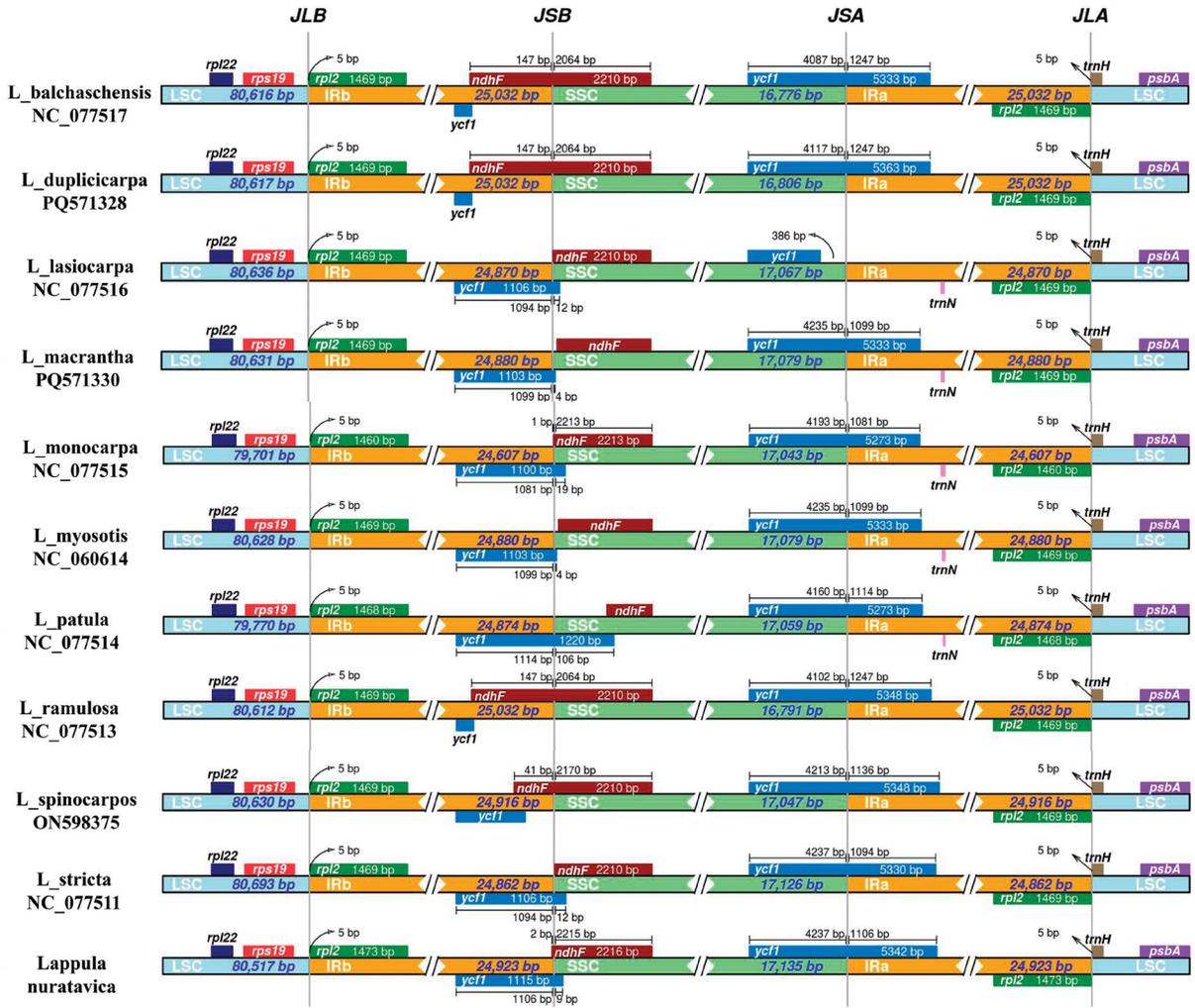


Fig. 3. Comparative analysis of the large single-copy, inverted repeat and small single-copy boundary regions in the 11 chloroplast genomes of the genus *Lappula*. The JLB represents the junction of large single-copy and IRb, JSB indicates the junction of small single-copy and IRb, JSA denotes the junction of small single-copy and IRa, and JLA signifies the junction of large single-copy and IRa.

(Fig. 4). Several hypervariable regions were detected, particularly in the regions spanning nucleotide positions 48 000–49 000 (Intergenic Spacer region between *trnT-UGU* gene and *ndhJ* gene) and 58 000–59 000 (IGS region between *ycf4* and *cemA*), which exhibited peak π values above 0.004. These peaks suggest localised hotspots of sequence divergence, likely corresponding to non-coding and coding intergenic regions. Conversely, several regions, including 82 000–96 000 and 134 000–148 000, showed very low nucleotide diversity, often close to zero, indicating high sequence conservation in these portions of the genome (Fig. 4).

The phylogenetic analysis of *Lappula* species based on complete chloroplast genome sequences produced a well-resolved tree with some species from *Mertensia*, *Trigonotis*, *Eritrichium*, *Tournefortia* and *Brachybotrys* designated as outgroups (Fig. 5). The tree reveals two major clades within *Lappula*. The first clade includes two accessions each of *Lappula spinocarpos*, *Lappula myosotis*, *Lappula ramulosa*, and *Lappula stricta*, and two accessions of *Lappula duplicaripa*, all supported by moderate to high bootstrap values, suggesting a close evolutionary relationship among these species. *Lappula nuratavica* also groups closely within this clade. The sec-

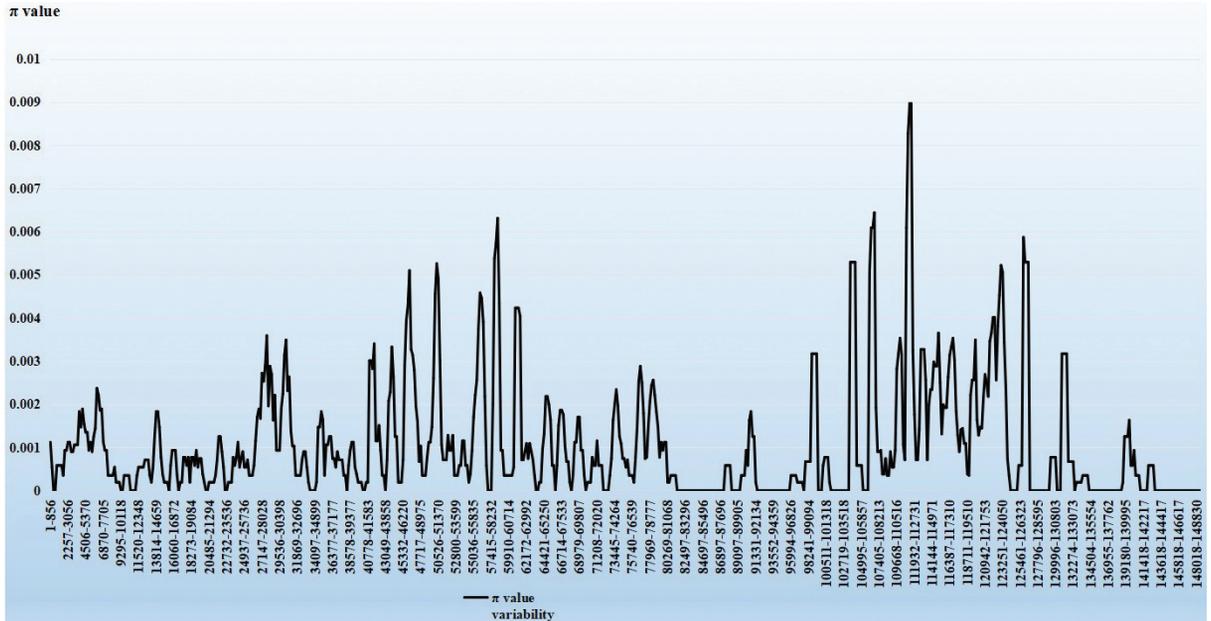


Fig. 4. The nucleotide diversity (π value) of the genomes of the genus *Lappula*, presented in a sliding window (window length: 800 bp; step size: 200 bp).

ond major clade comprises *Lappula patula*, *Lappula balchaschensis*, one accession of *Lappula duplicicarpa*, two accessions of *Lappula macrantha*, *Lappula monocarpa*, and *Lappula lasiocarpa*. Within this clade, *Lappula monocarpa*, *Lappula lasiocarpa*, and one accession from *Lappula macrantha* form a highly supported subclade, indicating strong genetic similarity. Notably, two accessions of *Lappula duplicicarpa* appear in different clades, which may suggest intraspecific variation or potential misidentification (Fig. 5).

DISCUSSION

The comparative analysis of complete chloroplast genomes across eleven *Lappula* species reveals a high degree of structural conservation typical of angiosperm plastomes (Zheng et al., 2025; Dekhkonov et al., 2025; Alieva et al., 2025). All species exhibit the canonical quadripartite structure, comprising a large single-copy region, a small single-copy (SSC) region, and a pair of inverted repeat (IR) regions, consistent with recent plastome studies, such as those of *Lappula myosotis* (Yan et al., 2023). The overall gene content and order are conserved across species, with minor yet informative variations observed in the IR length and junction positioning.

Genes such as *ycf1* and *ndhF*, which are often located at the small single-copy and IR boundaries, demonstrate variability in pseudogene length and position. This phenomenon is a common feature across flowering plants, attributed to IR expansion and contraction dynamics (Abdullah et al., 2025). These subtle but informative variations suggest lineage-specific evolution, with the potential for further taxonomic refinement within the genus.

The sliding window analysis of nucleotide diversity (π) reveals that plastomes of *Lappula* species exhibit generally low variation, characteristic of conserved chloroplast DNA (Guzmán-Díaz et al., 2022; Amenu et al., 2022). However, distinct hypervariable regions were identified, particularly within intergenic spacers such as *trnT-UGU-ndhJ* and *ycf4-cemA*. These regions exhibit higher nucleotide diversity, suggesting they may play a role in differentiating species within *Lappula* and may serve as useful markers for phylogenetic studies (Du et al., 2025). Such patterns of hypervariable intergenic regions have been observed in other plant groups, including Sapindaceae (Lin et al., 2018) and the genus *Acer* (Ma et al., 2019), further supporting the potential of these regions to enhance phylogenetic resolution.

These variable loci are particularly valuable for *Lappula*, where morphological convergence, espe-

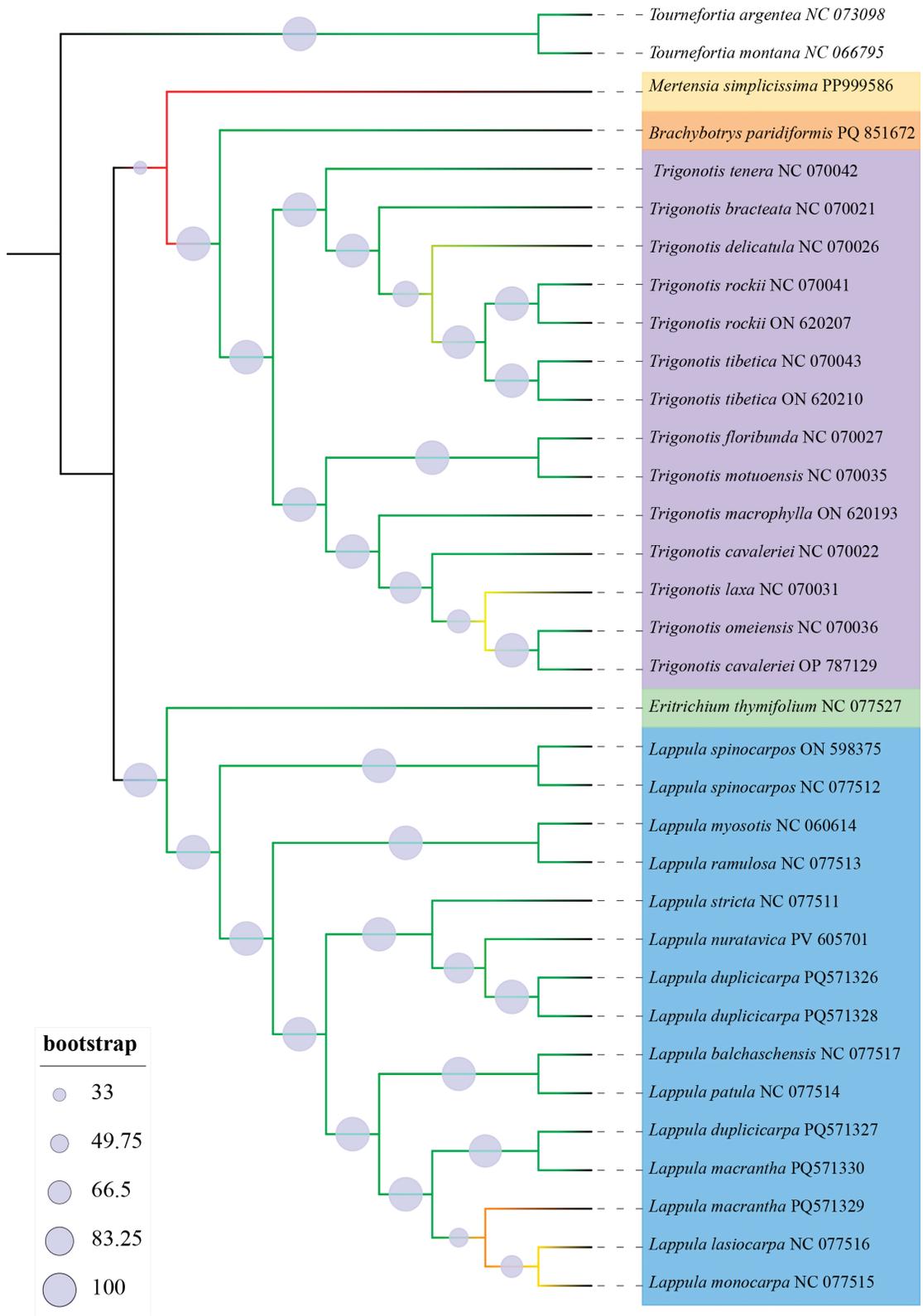


Fig. 5. Phylogenetic tree of the *Lappula* and its close species based on the complete chloroplast genomes using maximum likelihood. Maximum support values are indicated at the nodes.

cially in nutlet morphology, often obscures species boundaries (Ebadi & Nikzat, 2021). The molecular markers derived from these hotspots can complement traditional morphological traits and improve the resolution of both interspecific and intraspecific relationships.

Phylogenomic reconstruction based on complete chloroplast genomes yields a well-resolved, strongly supported maximum-likelihood tree, revealing two primary clades among the sampled *Lappula* species. Despite robust bootstrap support for these clades, one accession from *Lappula duplicicarpa* (PQ571327) and one accession from *Lappula macrantha* did not cluster as sister taxa, suggesting potential intraspecific divergence, taxonomic uncertainty, or cryptic diversity.

However, it is critical to acknowledge that our analysis does not provide conclusive evidence for the monophyly of *Lappula*. While the two primary clades are well-supported, the absence of key species, such as *Lappula sinaica*, *Lappula lipschitzii*, *Lappula marginata*, and members of the genera *Eri-trichium* and *Rochelia*, limits our ability to definitively assess the genus's overall monophyly. For example, *Lappula sinaica* has recently been reclassified into the monotypic genus *Pseudolappula* based on phylogenetic, morphological, and palynological evidence (Liu et al., 2021), and *Lappula occultata* has similarly been moved to *Pseudolappula occultata*. In contrast, *Lappula lipschitzii* remains within *Lappula*, forming a monophyletic clade in ITS (= internal transcribed spacer) and *trnL-F*-based phylogenies (Liu et al., 2021).

Thus, while the present analysis provides valuable insights into the relationships among the sampled *Lappula* taxa, it does not allow for a conclusive determination of the genus's monophyly. The exclusion of divergent taxa, such as *Lappula sinaica*, *Lappula marginata*, and *Lappula lipschitzii*, which have been central to the polyphyly hypothesis, was a necessary limitation due to the unavailability of their complete chloroplast genomes.

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Author contributions. Conceptualisation and literature analysis were performed by I.E., S.I., A.T., and E.O. The first draft of the manuscript was prepared by I.E., F.G., A.P., Sh.M., and N.N., who critically analysed and gave suggestions to finalise the manuscript. Supervision was conducted by Z.Y. All authors read and approved the final version of the article.

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APPENDIX

Accession numbers for the data on species of the genus *Lappula* and species used for outgroups, obtained from the National Centre of Biotechnology Information (NCBI).

No	Species name	NCBI number	Voucher ID
1	<i>Tournefortia argentea</i>	NC073098	
2	<i>Tournefortia montana</i>	NC066795	
3	<i>Mertensia simplicissima</i>	PP999586	
4	<i>Trigonotis tenera</i>	NC070042	
5	<i>Trigonotis bracteata</i>	070021	
6	<i>Trigonotis delicatula</i>	NC070026	
7	<i>Trigonotis rockii</i>	NC070041	
8	<i>Trigonotis rockii</i>	ON620207	
9	<i>Trigonotis tibetica</i>	NC070043	
10	<i>Trigonotis tibetica</i>	ON620210	
11	<i>Trigonotis floribunda</i>	NC070027	
12	<i>Trigonotis motuoensis</i>	NC070035	
13	<i>Trigonotis macrophylla</i>	ON620193	
14	<i>Trigonotis cavaleriei</i>	NC070022	
15	<i>Trigonotis laxa</i>	NC070031	
16	<i>Trigonotis omeiensis</i>	NC070036	
17	<i>Trigonotis cavaleriei</i>	OP787129	
18	<i>Eritrichium thymifolium</i>	NC077527	Ge130605
19	<i>Lappula spinocarpos</i>	ON598375	Q-094
20	<i>Lappula spinocarpos</i>	NC077512	Q-137
21	<i>Lappula myosotis</i>	NC060614	
22	<i>Lappula ramulosa</i>	NC077513	Ge130319
23	<i>Lappula stricta</i>	NC077511	Ge130033
24	<i>Lappula nuratavica</i>	PV605701	TASH001328
25	<i>Lappula duplicicarpa</i>	PQ571327	BNU2021XJ043
26	<i>Lappula duplicicarpa</i>	PQ571328	2023EH899
27	<i>Lappula balchaschensis</i>	NC077517	XJ-78
28	<i>Lappula patula</i>	NC077514	TW-0024
29	<i>Lappula duplicicarpa</i>	PQ571327	BNU2021XJ043
30	<i>Lappula macrantha</i>	PQ571329	BNU2020XJ147
31	<i>Lappula macrantha</i>	PQ571330	2023EH158
32	<i>Lappula lasiocarpa</i>	NC077516	XJ-79
33	<i>Lappula monocarpa</i>	NC077515	Ge130443

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