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RESEARCH ARTICLE

The complete chloroplast genomes of five species of *Allium* subg. *Melanocrommyum* (Amaryllidaceae)

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Abstract. Subgenus *Melanocrommyum* of the genus *Allium* (Amaryllidaceae) comprises morphologically diverse and taxonomically complex species, many of which are endemic to Central Asia. In this study, we sequenced and analyzed the complete chloroplast genomes of five species, *A. alaicum*, *A. altissimum*, *A. giganteum*, *A. isakulii*, and *A. karataviense*, representing five distinct taxonomic sections. All plastomes exhibited a typical quadripartite structure with conserved gene content. Genome sizes ranged from 151,960 to 152,725 bp. Codon usage showed bias toward AGA (Arg) and UUG (Leu), and a total of 79–90 SSRs and 423 long repeats were identified. Divergence hotspot regions included *accD*, *ndhD*, and *rps4*, while *ycf2* was highly conserved but showed a high ω value, suggesting its possible adaptive evolution. Phylogenetic analysis based on protein-coding genes consistently resolved two major clades for the studied species, supporting the monophyly of the group and existing sectional classifications. This study provides valuable genomic data for *Allium*, highlights plastome evolution in *Melanocrommyum*, and identifies markers for future phylogenetic and evolutionary studies.

Keywords: *Allium*, chloroplast genome, codon usage, nucleotide diversity, phylogenetic analysis, repeat sequences, SSRs, subgenus *Melanocrommyum*

Introduction

The genus *Allium* L. is among the most diverse and taxonomically challenging genera of

monocotyledonous plants, comprising about 1000 formally recognized species, which are taxonomically subdivided into 15 subgenera and 85 sections (Friesen et al., 2006; Yusupov et al., 2022;

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Khassanov et al., 2023; Ergashov et al., 2025a). Being native predominantly to the temperate zones of the Northern Hemisphere, the genus extends from arid subtropical landscapes to boreal regions, showcasing remarkable ecological amplitudes (Wheeler et al., 2013; Rakhmatiev et al., 2025; Kurbaniyazova et al., 2026). Members of *Allium* exhibit a combination of key morphological traits, such as free or nearly free tepals, tunicated bulbs enclosed in membranous or occasionally fibrous sheaths, and distinctive inflorescence structures (Friesen et al., 2006; Ergashov et al., 2025b).

The genus is not only taxonomically rich but also economically significant. Several *Allium* species are cultivated globally as staple vegetables and spices, including onion (*A. cepa* L.), garlic (*A. sativum* L.), shallot (*A. cepa* var. *aggregatum* L.), leek (*A. ampeloprasum* L.), and chives (*A. schoenoprasum* L.), in addition to their traditional roles in herbal medicine and horticulture (Keusgen et al., 2006; Yusupov et al., 2021). As exploration continues, new species are frequently described, expanding the breadth of the genus and introducing new complexities to its classification (Khassanov et al., 2023; Eker et al., 2025).

Despite advances in molecular systematics, the precise phylogenetic relationships within *Allium*, especially at subgeneric and sectional levels, remain unresolved. Early efforts using nuclear ribosomal internal transcribed spacer (ITS) sequences provided an initial framework for genus-wide phylogenetic hypotheses (Friesen et al., 2006; Li et al., 2010). Subsequent analyses incorporating chloroplast DNA regions — such as *matK*, *rps16*, and *trnL-trnF* — offered additional resolution but left several clades poorly supported or unresolved (Li et al., 2010). These limitations underscore the need for complete chloroplast genome sequencing to enhance resolution and confidence in phylogenetic inference.

Among the subgenera of *Allium*, the subgenus *Melanocrommyum* (Webb & Berthel.) Rouy is of particular interest due to its considerable species richness and distinctive morphological features. With over 180 accepted species and subspecies, it is recognized as the second largest subgenus within *Allium* (Fritsch, 2016; Ergashov et al., 2025b). Given the unresolved phylogenetic framework within this subgenus, there is a compelling need for comprehensive genomic data. Chloroplast genome sequences, with their conserved structure and high

resolution for phylogenetic studies, provide a powerful tool for resolving evolutionary relationships and identifying regions of variability useful for future taxonomic and evolutionary research (Alieva et al., 2025; Dekhkonov et al., 2025; Nikitina et al., 2025; Tojiboeva et al., 2025; Ergashov et al., 2026).

In this study, we sequenced and analyzed the complete chloroplast genomes of five representative species from five distinct sections of *Allium* subg. *Melanocrommyum*: *Allium alaicum* Vved., *A. altissimum* Regel, *A. giganteum* Regel, *A. isakulii* R.M. Fritsch & F.O. Khass., and *A. karataviense* Regel. The primary objectives were to: (1) explore and compare the structural organization and gene content of their chloroplast genomes; (2) elucidate phylogenetic relationships among the selected taxa and their relatives using complete plastome sequences; (3) identify hypervariable regions in the plastomes that can serve as molecular markers for future phylogenetic and population-level studies in *Allium* subg. *Melanocrommyum*.

By integrating complete plastome data with existing phylogenetic frameworks, this study aims to provide refined insights into the evolutionary dynamics of *Allium* subg. *Melanocrommyum* and contribute to resolving long-standing taxonomic ambiguities in the genus *Allium*.

Materials and Methods

Taxon sampling and DNA extraction

Plant material for this study was collected from the native habitats of each taxon. The five *Allium* species newly sequenced in this work, *Allium alaicum*, *A. altissimum*, *A. giganteum*, *A. isakulii*, and *A. karataviense*, were collected from various locations across Uzbekistan, Tajikistan, and Kyrgyzstan between 2019 and 2023. Species identification was carried out by Professor F. Khassanov at the Institute of Botany, Uzbekistan. Detailed field collection data, including locality information and collection codes, are provided in Table 1. Voucher specimens for all taxa have been deposited in the herbaria of the Kunming Institute of Botany (KUN) and the Institute of Botany in Tashkent (TASH). Fresh leaf tissues were dried using silica gel in the field to preserve DNA integrity. Total genomic DNA was extracted from the dried leaf material using a modified cetyltrimethylammonium bromide (CTAB) protocol as described by Doyle (1991), with modifications optimized for high-quality chloroplast DNA extraction.

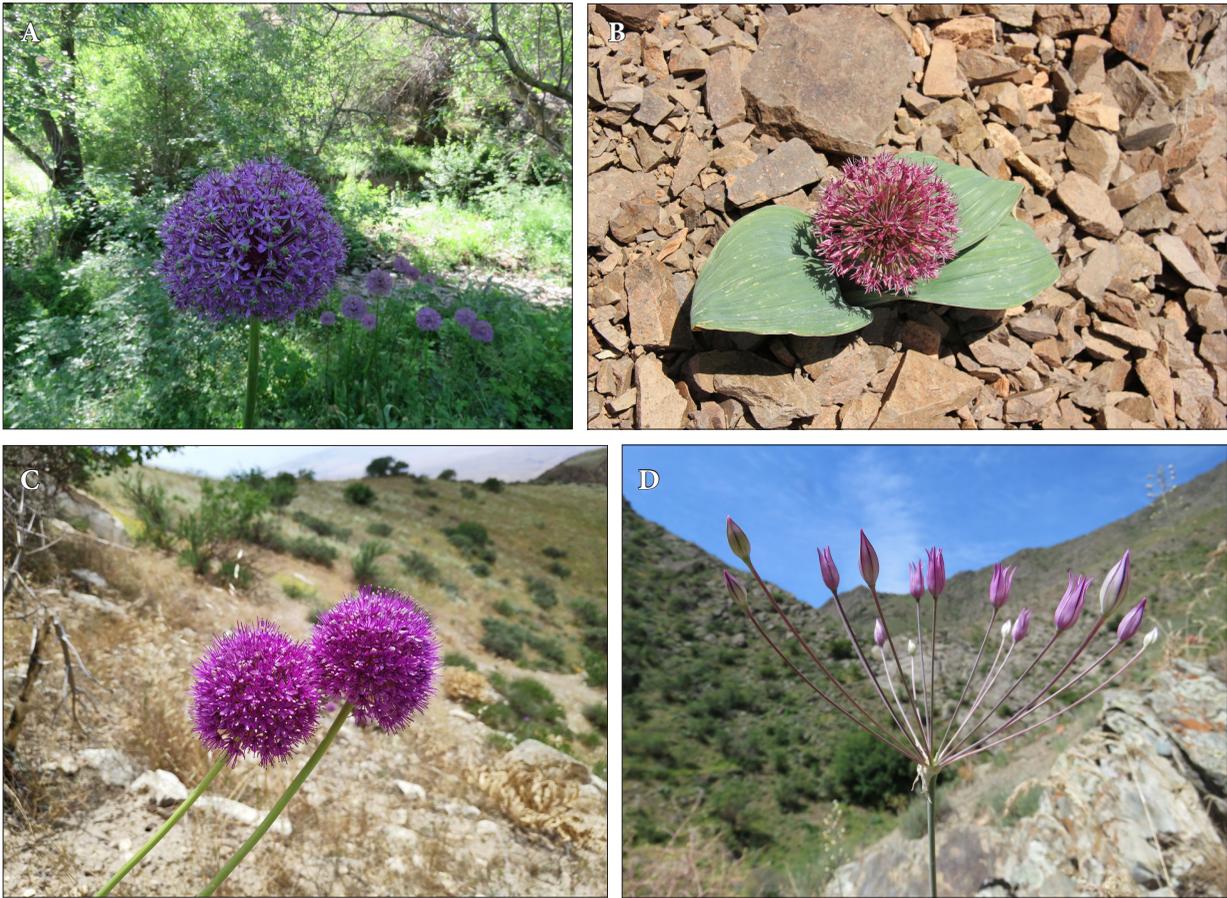


Fig. 1. Species of *Allium* subg. *Melanocrommyum* in their natural habitats. A: *Allium altissimum*, Madzherumsay tract, Nurata Reserve, Nuratau Ridge, Uzbekistan, 20 May 2017; B: *A. karataviense*, rubble-stony scree, Kurama pass, Uzbekistan, 31 May 2021; C: *A. giganteum*, Taldybulak tract, Besharcha, Babatag Ridge, Uzbekistan, 25 May 2019; D: *A. isakulii*, Madzherumsay tract, Nurata Reserve, Nuratau Ridge, Uzbekistan, 20 May 2017 (photos by N. Beshko)

Chloroplast genome sequencing, assembling, and annotation

Following quality assessment of the extracted genomic DNA, approximately 1.5 µg of high-quality DNA per sample was used for library preparation. Paired-end libraries with an average insert size of ~350 bp were constructed, and sequencing was performed using the Illumina HiSeq 4000 platform (150 bp paired-end reads) at Beijing Novogene Bioinformatics Technology Co., Ltd., Beijing, China.

Raw sequencing data were processed using the Next Generation Sequencing (NGS) QC Toolkit with default parameters to remove low-quality reads and adapter contamination (Patel, Jain, 2012). Clean reads were subsequently assembled into complete chloroplast genomes using the GetOrganelle pipeline (Jin

et al., 2020), with optimized parameters set to: -F plant_cp -w 0.6 -o -R 20 -t 8 -k 75,95,115,127.

The resulting complete plastomes were aligned using Mauve v.2.3.1 within Geneious v.10.0.2 (Darling et al., 2010) to evaluate genome collinearity. Gene annotation was also performed in Geneious, using the annotated chloroplast genome of *Allium fetisowii* Regel (NC_049100) as a reference. Start and stop codons, along with intron/exon junctions, were manually verified against previously published chloroplast genomes of *Allium* to ensure annotation accuracy (Kearse et al., 2012). Gene names and functional categories followed the standardized nomenclature of the Chloroplast Genome Database (Cui et al., 2006; <http://chloroplast.cbio.psu.edu>).

All annotated chloroplast genome sequences generated in this study were submitted to the

National Center for Biotechnology Information (NCBI). The physical maps illustrating the structure of the plastomes, including major components such as the large single-copy (LSC), small single-copy (SSC), and inverted repeat (IR) regions, were generated using OrganellarGenomeDRAW (OGDraw) v.1.3.1 (Lohse et al., 2013).

Identification of repeat sequences and simple sequence repeats

Repetitive sequences and simple sequence repeats (SSRs) within the chloroplast genomes were analyzed to better understand their structural variation and potential utility in molecular marker development. The identification of long repetitive sequences was carried out using the program REPuter (Kurtz et al., 2001), which detects four types of repeats: forward, reverse, palindromic, and complementary matches. The search parameters were set with a Hamming distance of 3, a minimum repeat length of 30 base pairs, and a sequence identity threshold of at least 90%. To further investigate microsatellite variation, SSRs were identified using the MIcroSATellite (MISA) web tool (Beier et al., 2017). The SSR search was configured to detect perfect mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide motifs, with minimum repeat thresholds of 10, 5, 4, 3, 3, and 3, respectively. These analyses enabled the comprehensive characterization of both large dispersed repeats and small tandem repeats across the five *Allium* chloroplast genomes, offering insights into genome evolution and providing a foundation for future studies in phylogenetics, species identification, and population genetics.

Genome comparison, polymorphism analysis, and codon usage analysis

To assess structural variation and sequence divergence, the complete chloroplast genome sequences generated in this study were compared with one another and with previously published genomes of other *Allium* species belonging to the subgenus *Melanocrommyum* available in GenBank (<https://www.ncbi.nlm.nih.gov/GenBank>, accessed 25 May 2025). The comparative analysis included examination of GC content and codon usage, both of which were calculated using MEGA version 11. To evaluate nucleotide diversity (P_i) and sequence polymorphism across the entire chloroplast genome and specifically within protein-coding regions, a sliding window analysis was performed using DnaSP v.6.12.03

(Rozas et al., 2017), with a window length of 800 bp and a step size of 200 bp. For detailed visualization of genome-wide variation, the program mVISTA (Frazer et al., 2004) was employed in Shuffle-LAGAN alignment mode (<http://genome.lbl.gov/vista/mvista/submit.shtml>, accessed 30 May 2025), using the chloroplast genome of *Allium fetisowii* as the reference. In addition, the Relative Synonymous Codon Usage (RSCU) values were computed using MEGA 11, to examine codon usage bias among the selected taxa. This comprehensive comparative analysis provided insights into conserved and variable regions, aiding the identification of evolutionary hotspots and species-specific genomic signatures within *Allium* subg. *Melanocrommyum*.

Gene selective pressure analysis

To evaluate the selective pressures acting on protein-coding genes within the chloroplast genomes, rates of synonymous (dS) and non-synonymous (dN) substitutions, along with their ratio ($\omega = dN/dS$), were calculated using DnaSP v.6.12.03. These analyses were performed on protein-coding genes that are commonly shared across the five newly sequenced chloroplast genomes. The dN/dS ratio serves as an indicator of the type and strength of natural selection acting on each gene, where $\omega < 1$ suggests purifying selection, $\omega = 1$ indicates neutral evolution, and $\omega > 1$ implies positive selection. This approach allowed for the detection of functional constraints or adaptive signals in chloroplast genes, contributing to a deeper understanding of the evolutionary dynamics shaping plastid genome evolution within *Allium* subg. *Melanocrommyum*.

Phylogenetic analysis

Phylogenetic relationships were inferred based on complete chloroplast genome sequences from five newly sequenced *Allium* species: *A. alaicum*, *A. altissimum*, *A. giganteum*, *A. isakulii*, and *A. karaviense*, along with 69 additional *Allium* species obtained from the NCBI GenBank database (see Supplementary Table S1 for accession details). To root the phylogenetic tree, four species from closely related genera were used as outgroups: *Gilliesia graminea* Lindl., *Milla biflora* Cav., *Pancratium maritimum* L., and *Tulbaghia violacea* Harv.

Multiple sequence alignment of all 78 complete chloroplast genome sequences was performed using MAFFT (Katoh, Standley, 2013) with default settings to ensure accurate alignment of conserved

and variable regions. Phylogenetic trees were reconstructed using three different approaches: Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI), to provide robust evolutionary insights.

MP analysis was conducted using PAUP version 4.10* (Swofford, 2003). All characters were treated with equal weight, gaps were considered as missing data, and character states were treated as unordered. A heuristic search strategy was employed, incorporating tree-bisection-reconnection (TBR) branch swapping with the Multrees option enabled, and random addition of taxa with 1000 replications.

For the likelihood-based methods, the most appropriate nucleotide substitution model was selected using jModelTest2 on XSEDE (www.phylo.org), based on the Akaike Information Criterion (AIC). The Maximum Likelihood (ML) analysis was then performed using RAxML v.8.0 (Stamatakis, 2014) under the GTR + G model, with 1000 bootstrap replicates to assess node support.

For Bayesian Inference (BI), the analysis was conducted using MrBayes v.3.2 (Ronquist et al., 2011). Two independent runs were performed, each with four Markov Chain Monte Carlo (MCMC) chains,

for 1,000,000 generations. Trees were sampled every 100 generations, and the first 25% of sampled trees were discarded as burn-in. Posterior probabilities were calculated from the remaining trees to estimate clade credibility.

The combined use of parsimony and likelihood-based approaches provided a comprehensive and well-supported phylogenetic framework for assessing relationships within the subgenus *Melanocrommyum* and across the broader genus *Allium*.

Results

Chloroplast genome features of *Allium* species

The complete chloroplast genome sequences of the five newly sequenced *Allium* species have been deposited in the NCBI GenBank database (Table 1). All five plastomes exhibit identical structural organization, gene content, and gene arrangement (Fig. 2). Each genome displays the typical quadripartite structure characteristic of angiosperm chloroplasts, consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat regions (IRa and IRb) that separate the LSC and SSC.

Table 1. Comparison of chloroplast genome features of five sequenced *Allium* species

Species name	<i>A. alaicum</i>	<i>A. altissimum</i>	<i>A. giganteum</i>	<i>A. isakulii</i>	<i>A. karataviense</i>
Region	Imom-ota, Andijon Region, Uzbekistan	Nuratau Mountains, Uzbekistan	Bobotog Mountains, Surkhandarya Region, Uzbekistan	Mogultog Mountains, Khujand Region, Tajikistan	Jalalabad Region, Kyrgyzstan
Accession number	PQ306043	PQ306040	PQ306042	PQ306035	PQ306048
Entire plastome size (bp)	152460	152464	152535	152725	151960
IR size (bp)	26815	26247	26185	26316	26366
LSC region size (bp)	81964	82752	82879	82895	81168
SSC region size (bp)	16865	17218	17283	17198	17260
Number of coding regions	267	267	267	267	267
Number of genes	134	134	134	134	134
Number of protein-coding genes	87	87	87	87	87
Number of genes duplicated in the IR	22	21	20	21	21
Number of tRNA genes	38	38	38	38	38
Number of rRNA genes	8	8	8	8	8
Number of genes with intron(s)	25	25	25	25	25
GC content of whole genome (%)	36.9	36.9	36.9	36.9	37
GC content (%) LSC/IR/SSC	34.7/42.4/30.1	34.7/42.7/30.1	34.7/42.7/30	34.7/42.7/30.1	34.7/42.6/30.2

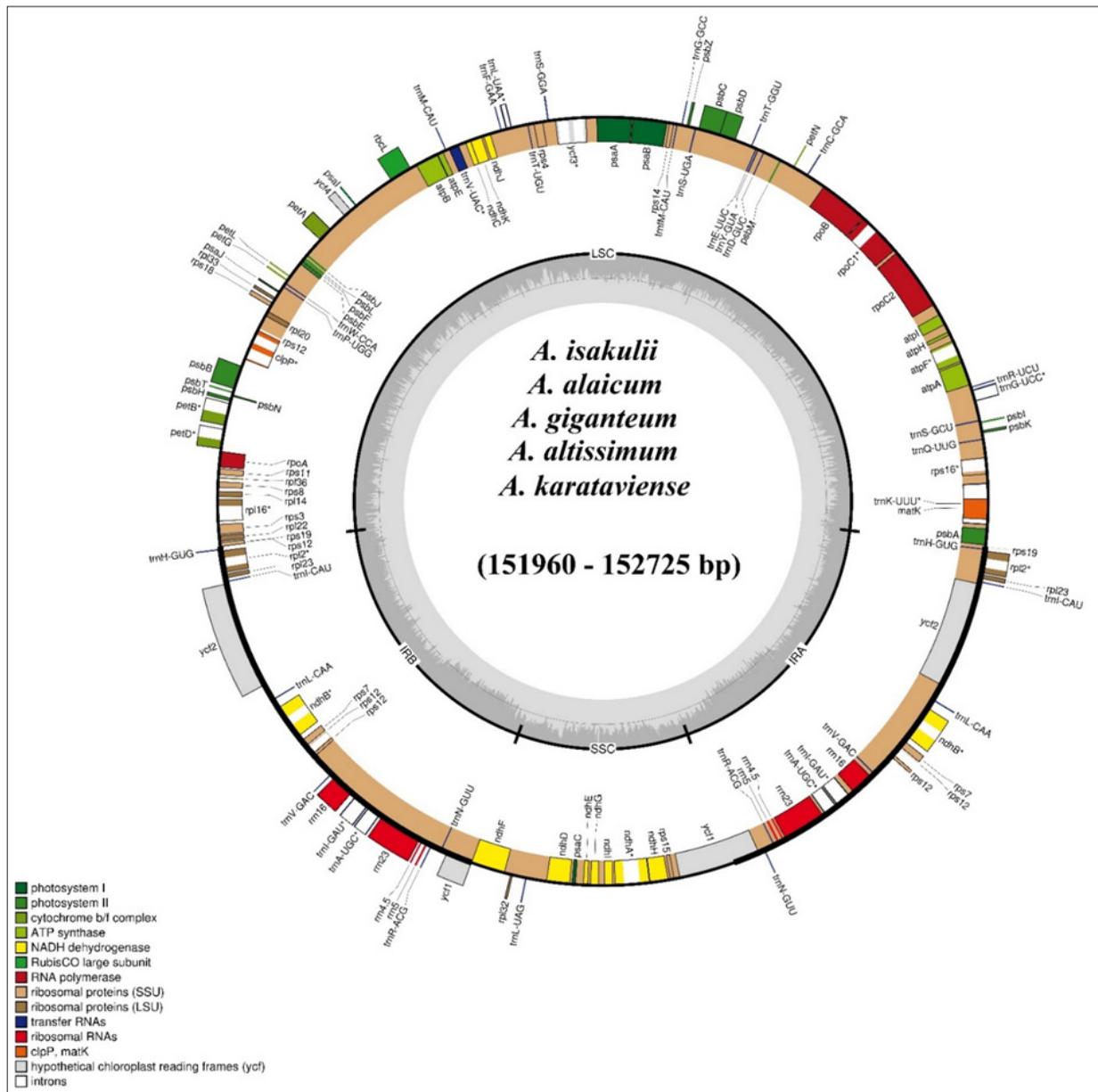


Fig. 2. Map of the newly sequenced chloroplast genome of the five species of *Allium* subg. *Melanocrommyum*. Genes inside the circle are transcribed clockwise, gene outside are transcribed counterclockwise. The dark grey inner circle indicates the GC content and the light represents AT content

Genome sizes range from 151,960 bp in *A. karataviense* to 152,725 bp in *A. isakulii*. The LSC regions vary in length from 81,168 bp (*A. karataviense*) to 82,895 bp (*A. isakulii*), while SSC regions range from 16,865 bp (*A. alaicum*) to 17,283 bp (*A. giganteum*). The length of the IR regions spans from 26,185 bp in *A. giganteum* to 26,815 bp in *A. alaicum*.

GC content varied across genome regions, with the IR regions exhibiting the highest GC content (42.4% to 42.7%), followed by the LSC regions (34.7%), and the SSC regions showing the lowest GC content (30.1% to 30.2%) (Table 1). A total of 134 genes were identified in each genome, including 87 protein-coding genes (PCGs), 38 transfer RNA (tRNA) genes, and 8 ribosomal RNA (rRNA)



Fig. 3. Comparative analysis of the LSC, IR and SSC boundary regions in the seven chloroplast genomes of *Allium* subg. *Melanocrommyum*. The JLB represents the junction of LSC and IRb, JSB indicates the junction of SSC and IRb, JSA denotes the junction of SSC and IRa, and JLA signifies the junction of LSC and IRa (Accession numbers for *Allium macleanii* and *A. fetisowii* are given in Supplementary Table S1)

genes. Notably, one copy of the *rps19* gene was absent in all five species.

Among the identified genes, 20 were duplicated within the IR regions. These included 8 PCGs (*rpl2*, *rpl23*, *ycf2*, *ycf15*, *ndhB*, *rps7*, *rps12*, *ycf1*), 8 tRNA genes (*trnH-GUG*, *trnI-CAU*, *trnL-CAA*, *trnV-GAC*, *trnI-GAU*, *trnA-UGC*, *trnR-ACG*, *trnN-GUU*), and 2 rRNA genes (*rrn4.5*, *rrn5*). The functional genes identified in the chloroplast genomes were categorized into three major groups: genes involved in self-replication, genes associated with photosynthesis, and genes with other functions (see Supplementary Table S2 for gene classifications).

Boundaries of IR regions and codon usage

Comparative analysis of the inverted repeat (IR) boundary regions across seven *Allium* species belonging to the subgenus *Melanocrommyum* revealed

minor structural variations, despite the overall conservation in gene number and order (Fig. 3). The *rps19* gene was generally positioned at the LSC–IRa junction in most genomes analyzed. However, in two species (*A. giganteum* and *A. macleanii* Baker), *rps19* was fully contained within the IRa region. In *A. fetisowii*, both *rps19* and *psbA* were found near the LSC–IRb junction. In contrast, in several other species, the second duplicate of *rps19* was absent, indicating independent loss events.

The *ycf1* gene, which spans the SSC–IRb junction, showed considerable variation in length among species. The longer *ycf1* copy (located primarily in the IRb region) ranged from 5,241 bp to 5,277 bp, while the shorter copy ranged from 1,083 bp to 1,692 bp. The portion of *ycf1* present in the SSC region varied from 3,568 bp to 4,243 bp, and its overlap with the IRb region ranged from 1,037 bp

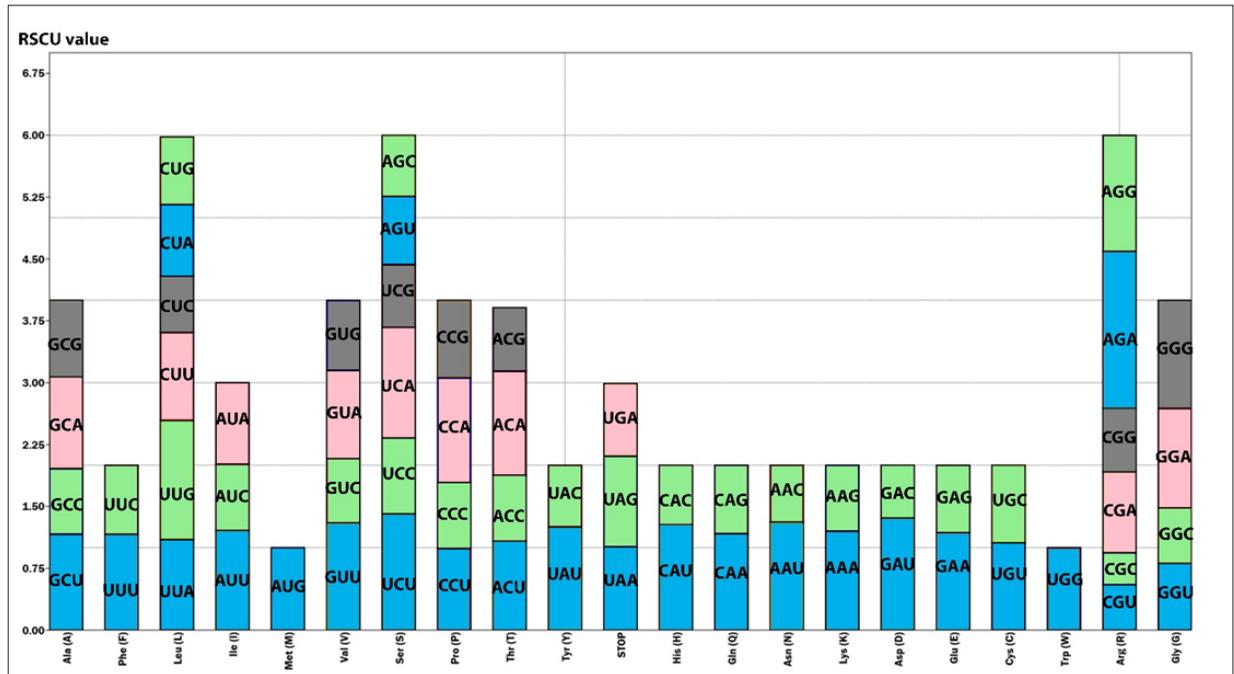


Fig. 4. Relative synonymous codon usage (RSCU) value and codon content of protein-coding genes in the chloroplast genomes of the five species of *Allium* subg. *Melanocrommyum*

to 1,690 bp. In *A. alaicum*, the *ndhF* gene was found directly at the SSC–IRa boundary, whereas in the other species, *ndhF* was located at distances ranging from 0 to 744 bp away from the junction.

These structural differences at IR junctions are important markers for understanding the evolutionary dynamics of chloroplast genomes and offer useful phylogenetic signals for resolving relationships within *Allium*.

The total length of protein-coding genes across the seven analyzed plastomes was 61,332 bp, comprising a total of 20,420 codons. Codon usage bias and amino acid frequencies were evaluated to understand translational preferences (Fig. 4). Among the 20 amino acids encoded, Leucine (Leu), Serine (Ser), and Arginine (Arg) were the most abundant, whereas Methionine (Met) and Tryptophan (Trp) were the least frequent.

The codon AGA, encoding Arginine (Arg), was the most commonly used codon with a Relative Synonymous Codon Usage (RSCU) value of 1.90, followed by UUG, encoding Leucine (Leu), with an RSCU value of 1.44. In total, 34 codons had RSCU values greater than 1, indicating a strong codon usage bias favoring these synonymous codons. The most frequently used stop codon across the species was UAG.

Repeat Sequences and SSRs Analysis

The chloroplast genomes of the five newly sequenced *Allium* species were systematically screened for simple sequence repeats (SSRs) using the Micro-Satellite (MISA) tool (Beier et al., 2017). The total number of SSRs identified varied among species, ranging from 79 in *A. isakulii* to 90 in *A. karataviense* (Fig. 5A–B). Mononucleotide repeats were the most abundant category, with counts ranging from 49 (*A. giganteum*) to 60 (*A. karataviense*). Among these, adenine (A) repeats dominated, representing the most frequent SSR motif.

Dinucleotide SSRs, particularly the AT motif, were the second most prevalent class, with counts ranging from 12 in *A. alaicum* to 15 in *A. altissimum*. Tetranucleotide repeats (e.g., AAAT) were observed in several species, including *A. giganteum* and *A. karataviense* (7 each), and *A. alaicum* (5). Pentanucleotide repeats were detected in all species except *A. karataviense*, while hexanucleotide repeats were rare and found only in *A. isakulii* (3 repeats). These SSRs represent potential molecular markers for population-level studies, species identification, and evolutionary inference.

In addition to SSRs, a total of 423 long repeat sequences were identified across the five species,

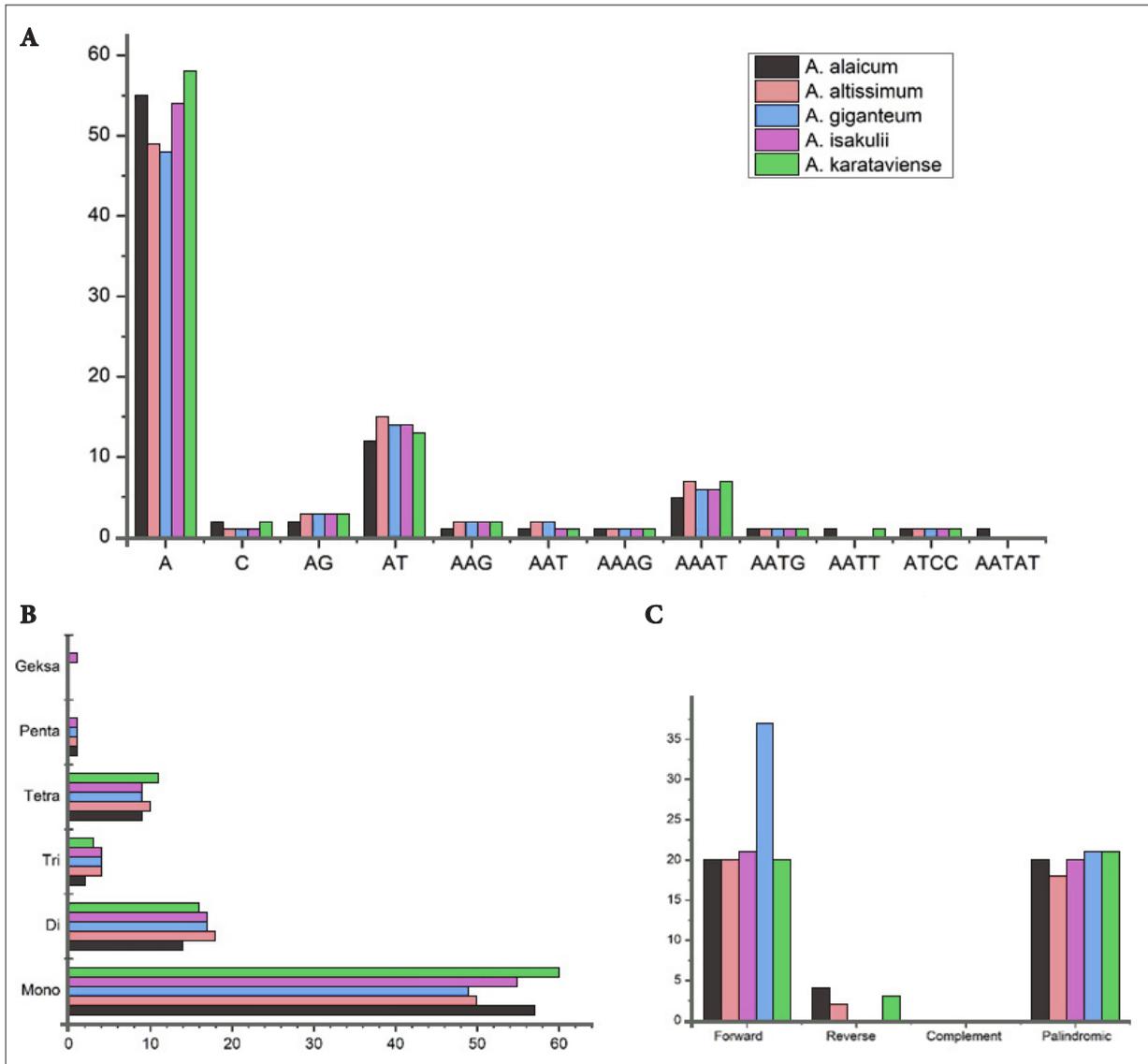


Fig. 5. Simple sequence repeats (SSRs) and number of repeats detected in the chloroplast genomes of five *Allium* species. A: type and number of SSR loci; B: number of repeat types; C: number of repeated sequences

comprising forward, reverse, palindromic, and complementary repeat types (Fig. 5C). Forward repeats were the most abundant ($n = 118$), followed by palindromic repeats ($n = 100$), and tandem repeats ($n = 158$). Among the species, *A. giganteum* exhibited the highest number of long repeats, with 37 forward and 21 palindromic repeats, whereas *A. isakulii* had the lowest, with 21 forward and 20 palindromic repeats. These dispersed repeats may play roles in structural genome rearrangement, recombination, and evolution of the plastome.

Comparative genomic divergence, hotspot regions and selection on functional genes

To explore divergence patterns across the chloroplast genomes of subgenus *Melanocrommyum*, nucleotide diversity (P_i) was calculated across the complete genome, as well as within the LSC, SSC, IR regions, and across protein-coding genes (Fig. 6A–B). The most variable regions, with P_i values of 0.02338 and 0.02175, were located between positions 113,760–114,632 bp and 58,946–59,773 bp, corresponding primarily to the SSC and LSC regions,

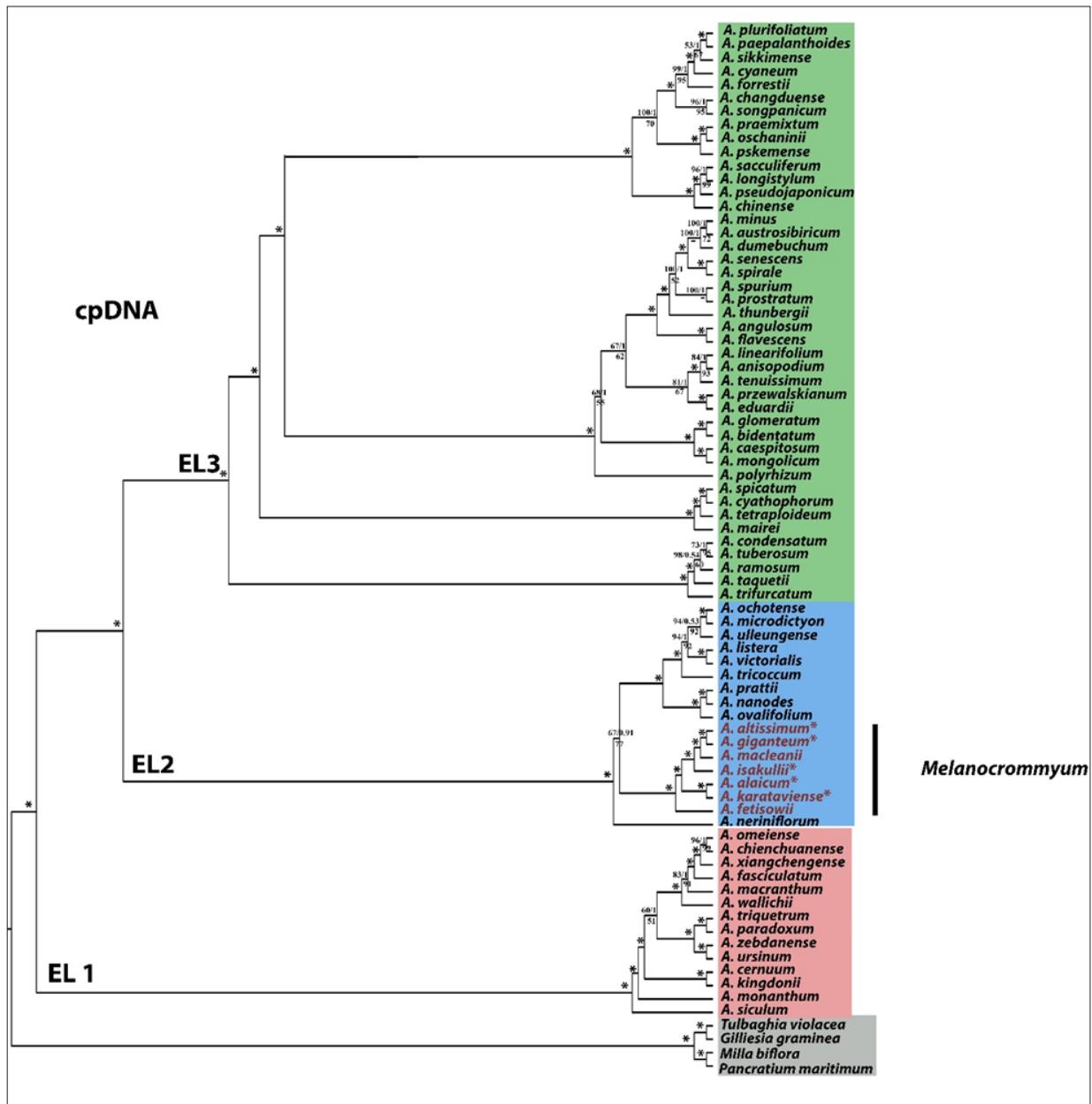


Fig. 8. Phylogenetic tree showing relationships of seven species of *Allium* subg. *Melanocrommyum* (names in red) among 67 chloroplast genomes from other taxa of the genus *Allium* and 4 outgroup species inferred using protein-coding regions. Numbers above the branches follow the order: BS (bootstrap support)/MP (bootstrap values)/PP (posterior probability); values of 100% are denoted by an asterisk. The evolutionary lines 1–3 are in red, blue, and green, respectively. The asterisk in tip labels indicates the newly sequenced species for the current study

Acmopetala R.M. Fritsch), *A. altissimum* (sect. *Procerallium* R.M. Fritsch), *A. giganteum* (sect. *Compactoprason* R.M. Fritsch), *A. isakulii* (sect. *Regeloprason* Wendelbo), and *A. karataviense* (sect. *Miniprason* R.M. Fritsch). These genomes also were

compared with two previously published plastomes of closely related *Melanocrommyum* species (*A. fetisowii*, *A. macleanii*) to evaluate patterns of structural conservation (Fig. 3).

All five newly sequenced plastomes exhibited the canonical quadripartite structure, consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeats (IRs), consistent with prior findings in the subgenus and across *Allium* (Xie et al., 2019, 2020; Munavvarov et al., 2022; Jin et al., 2022; Fu et al., 2023). Sequence identity across most regions of the chloroplast genomes was high, underscoring structural conservation; however, subtle differences were observed, some of which may represent lineage-specific characteristics of subgenus *Melanocrommyum* (Fig. 9).

Genome size variation is a well-documented feature in *Allium*, previously reported to range from 145,819 bp to 157,735 bp across members of the family *Amaryllidaceae*, subfamily *Allioideae* (Xie et al., 2019, 2020; Munavvarov et al., 2022; Fu et al., 2023). The sizes of the five newly analyzed chloroplast genomes — ranging between 151,960 bp and 152,725 bp — fall well within this established range (Fig. 2). This variation is largely attributed to multiple evolutionary mechanisms, including gene loss, intergenic region variability, and expansion or contraction of inverted repeat regions (Chen et al., 2022).

When comparing the junctions between the IR and single-copy regions (LSC/IR and SSC/IR), a high degree of similarity was observed among the examined species. However, minor differences were detected in the positioning of key genes such as *rpl22*, *rps19*, *ycf1*, and *ndhF*, consistent with patterns observed in earlier studies on *Allium* plastomes (Huo et al., 2019; Xie et al., 2020; Munavvarov et al., 2022; Fu et al., 2023). Notably, the *rpl22* gene was consistently located within the LSC region and positioned 317 to 403 bp away from the JLB (junction of LSC and IRb) boundary in all species of *Allium* subg. *Melanocrommyum*. These subtle variations may reflect fine-scale evolutionary dynamics within plastid genomes and can serve as useful markers for resolving phylogenetic relationships at the subgeneric level.

The codon usage analysis revealed strong biases across the five cp genomes. The most frequently used codons included AGA (Arginine) and UUG (Leucine), with Relative Synonymous Codon Usage (RSCU) values above 1.9 and 1.4, respectively (Fig. 4). Similar codon preference patterns have been previously observed in angiosperm cp genomes and likely reflect translational efficiency or nucleotide composition bias (Morton, 1998; Wang

et al., 2018). Among amino acids, Leucine, Serine, and Arginine were the most commonly encoded ones, while Methionine and Tryptophan appeared least frequently, reflecting their single-codon redundancy.

Nucleotide diversity (Π) across the genomes highlighted two highly variable hotspot regions located in the SSC and LSC regions (Fig. 6A), in agreement with findings by Fu et al. (2023) and Munavvarov et al. (2022), who reported elevated divergence in noncoding intergenic spacers and certain protein-coding genes. In our analysis, genes such as *accD*, *ndhD*, and *rps4* showed the highest variation, suggesting their potential as molecular markers in phylogeographic and evolutionary studies. The *ycf2* gene remained one of the most conserved loci ($\Pi = 0.00029$), corroborating its highly constrained nature in plastid evolution (Dugas et al., 2015).

The analysis of selection pressure via dN/dS (ω) ratios revealed that most genes are under strong purifying selection ($\omega < 1$), especially those related to core photosynthetic complexes such as *psa*, *psb*, and *ndh* families (Fig. 6C). However, elevated ω values for *accD* and *rps15* (> 0.6) and a notably high ω for *ycf2* (1.40) suggest relaxed or positive selection. Similar patterns were previously reported by Li et al. (2022), who linked such signatures to adaptive plastid genome evolution in harsh environments.

The SSR analysis identified 79 to 90 microsatellites across the five cp genomes, with *A. karataviense* containing the highest number (Fig. 5A, B). Mononucleotide A-repeats were the most frequent, followed by AT dinucleotides. These patterns align with previous observations in *Allium przewalskianum* and other species of *Amaryllidaceae* (Xie et al., 2020). The presence of several tetranucleotide (AAAT), pentanucleotide, and rare hexanucleotide motifs (found only in *A. isakulii*) suggests species-specific repeat dynamics that may be useful for population-level marker development.

Longer repeat types — forward, palindromic, reverse, and complement — were also abundant, with a total of 423 repeats detected (Fig. 5C). Forward repeats were the most common, especially in *A. giganteum*, which harbored the largest number of both forward (37) and palindromic repeats (21). These long repeats may contribute to plastome rearrangements, although the absence of large inversions suggests that *Allium* plastomes are largely stable (Maréchal, Brisson, 2010; Wicke, Naumann, 2018).

The mVISTA comparison of the cp genomes (Fig. 7) confirmed a high degree of sequence conservation in coding regions across the six analyzed species of *Allium* subg. *Melanocrommyum*. Divergent regions were primarily found in noncoding intergenic spacers, especially in the LSC and SSC regions, consistent with previous comparative studies in *Allium* and other monocots (Li et al., 2019; Wang et al., 2023). Such hotspots — e.g., between *accD-psaI* and *ndhF-rpl32* — could serve as candidate loci for phylogenetic and barcoding applications.

Phylogenetic reconstruction based on concatenated protein-coding genes yielded consistent topologies across Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI) analyses, with strong statistical support at all major nodes (Fig. 8). The genus *Allium* was resolved as monophyletic, divided into three well-supported evolutionary lineages (EL1, EL2, EL3). The species of *Allium* subg. *Melanocrommyum* formed a clearly defined group within EL2 and were subdivided into two clades: one comprising *A. altissimum*, *A. giganteum*, *A. macleanii*, and *A. isakulii*, and another consisting of *A. fetisowii*, *A. karataviense*, and *A. alaicum*. These clades correspond to taxonomic sections and support previous morphological and molecular hypotheses (Friesen et al., 2006; Munavarov et al., 2022, Yusupov et al., 2022).

Conclusion

This study provides new genomic insights into five species of *Allium* subg. *Melanocrommyum*. The plastomes show overall structural conservation but also exhibit subtle variations in IR boundaries, codon usage, and repeat composition that reflect lineage-specific evolution. Several highly variable genes and regions — such as *accD*, *ndhD*, and intergenic spacers in the LSC and SSC — were identified as promising markers for phylogenetic and population-level studies. Selection analyses revealed mostly strong purifying selection, with a few genes showing signals of adaptive evolution. Phylogenetic reconstruction strongly supported the monophyly of

Allium subg. *Melanocrommyum* and clarified species relationships consistent with established taxonomy. Overall, these results enhance our understanding of plastid genome evolution in this subgenus and provide valuable resources for future evolutionary and systematic research.

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SUPPLEMENTARY MATERIAL

This article includes Supplementary Material (Tables S1, S2) available as: [ukrbotj83-01-003-S1.pdf](#) (41 KB) and [ukrbotj83-01-003-S2.pdf](#) (45 KB).

ETHICS DECLARATION

There is no actual or potential conflict of interest in relation to this article.

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Повні хлоропластні геноми п'яти видів підроду *Melanocrommyum* роду *Allium* (*Amaryllidaceae*)

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Реферат. Підрід *Melanocrommyum* роду *Allium* (*Amaryllidaceae*) представлений морфологічно різноманітними і таксономічно складними видами, чимало з яких є ендемічними для Центральної Азії. У цьому дослідженні було секвеновано і проаналізовано повні хлоропластні геноми п'яти видів — *A. alaicum*, *A. altissimum*, *A. giganteum*, *A. isakulii* та *A. karataviense* — з п'яти різних таксономічних секцій. Усі пластоми демонстрували типову чотиридольну структуру з консервативним вмістом генів. Розміри геному коливалися від 151 960 до 152 725 п.н. Використання кодонів показало зміщення в бік AGA (Arg) та UUG (Leu), а загалом було ідентифіковано 79–90 простих повторів (SSRs) і 423 довгі повтори. Ділянки інтенсивної дивергенції включали *accD*, *ndhD* та *rps4*, тоді як ген *ycf2* був висококонсервативним, проте мав значний показник ω , що свідчить про можливу адаптивну еволюцію. Філогенетичний аналіз на основі генів, що кодують білки, послідовно визначив дві основні клади досліджених видів, підтверджуючи монофілію підроду та сучасну класифікацію на рівні секцій. Це дослідження надає важливі геномні дані для роду *Allium*, висвітлює еволюцію пластомів у підроді *Melanocrommyum* і визначає маркери для подальших філогенетичних та еволюційних досліджень.

Ключові слова: *Allium*, SSR, використання кодонів, нуклеотидна різноманітність, підрід *Melanocrommyum*, повторні послідовності, філогенетичний аналіз, хлоропластний геном