



GENOME NOTE

Comparative Chloroplast Genomics and Phylogenetic Relationships of *Poa angustifolia* and *Poa lipskyi* (Poaceae)

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Shukhrat Abdullaev¹, Temur Asatulloev², Ziyoviddin Yusupov¹,
Naimaxon Zaynobidinova³, Mengmeng Sun⁴, Komiljon Sh. Tojibaev²,
Davron Dekhkonov¹

¹Namangan State University, Namangan, Uzbekistan

²Institute of Botany, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

³Namangan State Pedagogical institute, Namangan, Uzbekistan

⁴Northeast Asia Research Institute of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Jingyue Economic Development District, Changchun, China

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Abstract

Poa L. (Poaceae), comprising over 500 species, is taxonomically challenging due to morphological plasticity, hybridization, and polyploidy. To clarify plastome architecture and phylogenetic relationships within the genus, we sequenced and annotated complete chloroplast genomes of *Poa angustifolia* (accessions P10, P14) and *Poa lipskyi* (P17) from Uzbekistan. The three plastomes (135,310–135,609 bp) shared the typical quadripartite structure and an identical complement of 130 genes across self-replication, photosynthesis, and other functional categories. Codon usage and amino acid composition were highly conserved, with a consistent bias toward A/T-ending codons and leucine, isoleucine, glycine, and serine as the dominant residues. Sliding-window analysis identified two nucleotide-diversity hotspots, in the *rpl32-trnL-UAG* and *trnC-GCA-rpoB* regions, and SSR profiling showed predominant mononucleotide A/T repeats alongside a few taxon-specific pentanucleotide motifs. Maximum-likelihood phylogenomic analysis placed the *P. angustifolia* accessions in a strongly supported clade sister to *P. lipskyi*, consistent with shared membership in sect. *Poa*. These results indicate that *Poa* plastomes are structurally conserved yet retain informative variable regions useful for phylogenetics and barcoding.

Keywords

Phylogeny, *Poa*, plastome



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This article is included in the **Genomics and Genetics** gateway.

Corresponding author: Davron Dekhkonov (davron-b@bk.ru)

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Introduction

The genus *Poa* L. (Poaceae), known as bluegrasses, consists of over 500 species, forming one of the most diverse and complicated genera in the grass family.¹ Members of the genus *Poa* are dispersed in temperate, alpine, and subarctic ecosystems, playing an important part in grassland habitats, pasture lands, and lawns. In spite of the significance of *Poa* both ecologically and economically, its taxonomy and phylogeny are extremely complicated because of the presence of many morphotypes, hybridization, and polyploidy.^{2,3}

The taxonomic delineation of *Poa* species has largely relied on phenotypic traits such as the morphology of spikelets, lemma, and life habits. However, phenotypic traits are highly polyphyletic and thus are unreliable as a tool to determine phylogenies.⁴ For this reason, there has been an increase in the use of molecular methods to study the evolution of relationships within the *Poa* genus based on cpDNA.⁵ Chloroplast DNA is widely employed in plant phylogenetic studies due to its stable nature, uni-parental inheritance, and slow rate of molecular evolution.^{6,7} Plastome-based studies of the genus *Poa* have been instrumental in the understanding of the relationships among different species. Complete plastome sequences have made comparative studies possible, where similarities and differences can be seen at different levels including SNPs, indels, and SSRs.⁸

Previous comparative chloroplast genomics research in *Poa* has revealed that although the general architecture of the chloroplast genome is extremely conserved, functional selection pressures maintain genome stability while enabling diversification.⁸ Within the variable components, SSRs are ubiquitous within the chloroplast genomes and functionally significant for genome rearrangement and recombination processes.⁹ Notably, SSRs are primarily abundant in noncoding IGS regions compared to intronic and coding regions.⁸

Nevertheless, in spite of such advances, genomic analysis of numerous *Poa* species is still poorly studied, and the investigation of chloroplast genomes in a large number of *Poa* species is still not well studied. Such problems do not allow us to have the whole picture of the evolutionary history of these organisms.

Materials and Methods

Plant Material

Leaf material used for DNA extraction was obtained from herbarium specimens collected during 2020–2022 from Uzbekistan. Species were identified by Shukhrat Abdullaev. Herbarium materials were deposited to TASH National Herbarium of Uzbekistan under TASH00247 (*P. angustifolia*-P10), TASH00377 (*P. angustifolia*-P14) and TASH00327 (*P. lipskyi*). All species were collected from Uzbekistan.

DNA Extraction, Library Preparation, and Sequencing

DNA extraction, library construction, and sequencing were carried out by Novogene (Beijing, China). Sequencing libraries were prepared using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina®, following the manufacturer's protocol. The prepared libraries were sequenced on the Illumina HiSeq 4000 platform to produce paired-end reads with a read length of 150 bp.

Quality Control of Sequencing Data

Raw sequencing data were processed using fastp v1.1.0¹⁰ to remove adapter sequences, filter out low-quality reads, and eliminate short fragments. After quality filtering, more than 99% of the reads were retained.

Chloroplast Genome Assembly

High-quality clean reads were assembled de novo using GetOrganelle¹¹ within the CGAS pipeline (v1.0.1),¹² with parameters optimized for embryophyte chloroplast genomes.

Genome Annotation

Chloroplast genome annotation was performed using PGA¹³ with a reference-guided approach. Protein-coding genes, transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs) were identified and manually curated when necessary. Annotations were standardized across all taxa, and the finalized genomes were exported in both FASTA and GenBank formats. Additional manual verification and refinement were conducted in Geneious v9.0.2.¹⁴ Chloroplast genome structure and gene maps were visualized using OGDRAW,¹⁵ an online tool for graphical representation of plastome organization.

Comparative Plastome Analysis

Comparative plastome analyses were conducted using the CGAS framework.¹² Key genomic features, including genome size, GC content, gene content, and the structure of large single-copy (LSC), small single-copy (SSC), and inverted repeat

(IR) regions, were examined across all species. In addition, codon usage bias (RSCU), amino acid composition and simple sequence repeats (SSRs) were analyzed. Nucleotide diversity (π) was also calculated using DnaSP¹⁶ to identify highly variable regions within the plastomes.

Chloroplast Microsatellites or simple sequence repeats (SSRs) were detected in the perl script MISA.¹⁷ The basic repeat setting of SSRs was determined: ten for mononucleotide, five for dinucleotide, four for trinucleotide and three for tetranucleotide pentanucleotide hexanucleotide.

Phylogenetic Analysis

We have downloaded eight chloroplast genomes of eight species of *Poa* and 2 species of *Elymus* (as outgroups) from NCBI. Phylogenetic relationships were inferred using complete chloroplast genome sequences. Multiple sequence alignment was carried out with MAFFT.¹⁸ Maximum likelihood (ML) analysis was performed in RaxML,¹⁹ where the best-fit nucleotide substitution model was automatically selected, and branch support was evaluated using bootstrap replicates. The resulting phylogenetic tree was visualized and annotated using FigTREE (<https://tree.bio.ed.ac.uk/software/figtree/>).

Results

The full sizes of chloroplast genomes for all tested *Poa* species revealed some differences in their lengths (Figure 1). Two samples of *Poa angustifolia* had small differences in the length of its plastome sequence, which amounted to 135,310 bp

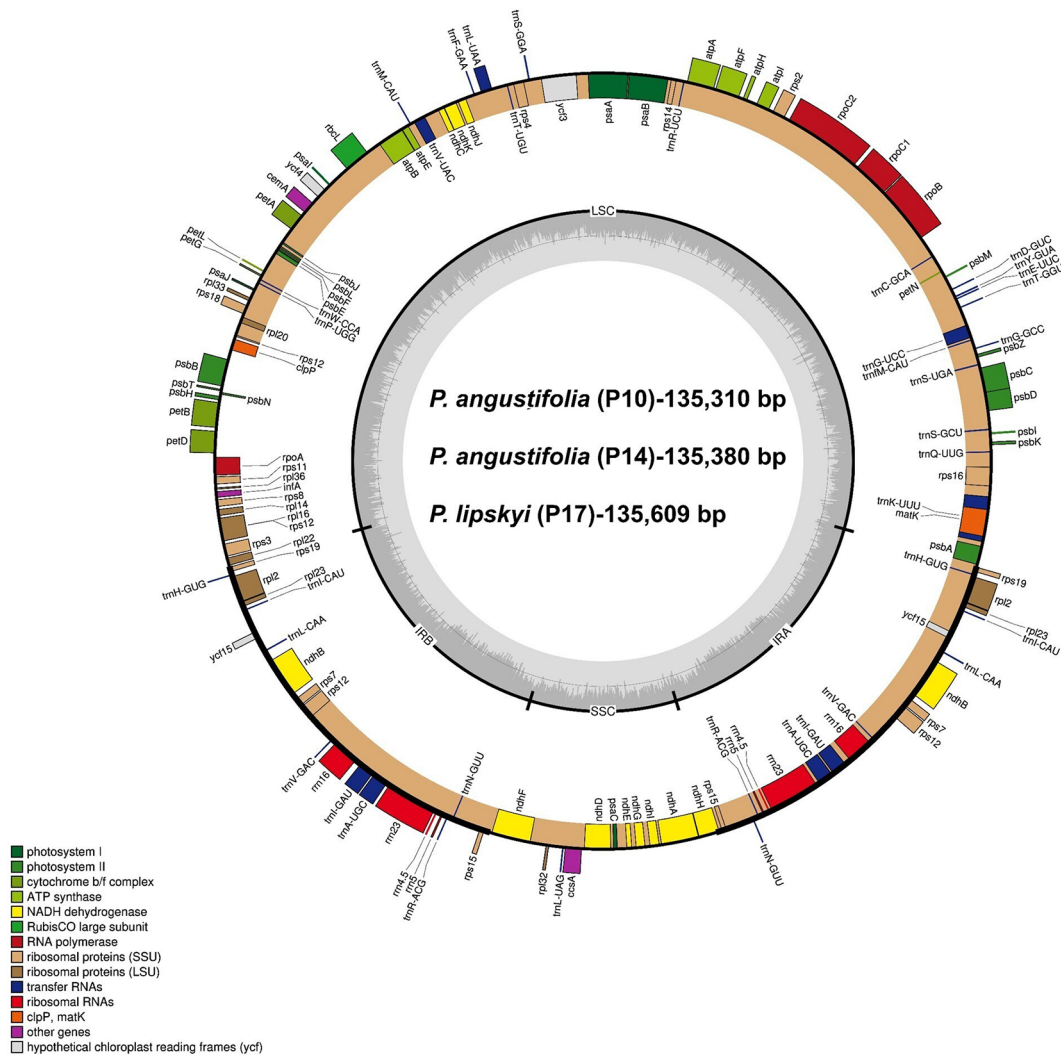


Figure 1. Circular map of the complete plastid genome of *Poa*. Genes shown outside the circle are transcribed clockwise, while those inside are transcribed counterclockwise.

(P10) and 135,380 bp (P14). The chloroplast genome of *Poa lipskyi* was found to be relatively bigger than those of other *Poa* species, with a length of 135,609 bp (P17).

It was established that the genome of the *Poa* chloroplasts had 130 genes that were divided into several functional categories according to their functions: self-replication, photosynthesis, and other functions (Table 1). Genes related to self-replication were the most numerous among them, and this group included ribosomal protein genes, RNA polymerase subunit genes, ribosomal RNA (rRNA) genes, and transfer RNA (tRNA) genes. Among the ribosomal protein large subunit genes, there were 11 genes such as *rpl14*, *rpl16*, *rpl2*, and *rpl20*. At the same time, among genes coding for the small ribosomal subunit, there were 15 genes (e.g., *rps11*, *rps12*, *rps14*, *rps15*, *rps16*, *rps18*, *rps19*, *rps2*, *rps3*, *rps4*, *rps7*, *rps8*). Moreover, there were four genes for

Table 1. Gene content of the chloroplast genome of sequenced *Poa* species.

Category for genes	Group of genes	Name of genes	Total
Self-replication	Large subunit of ribosome	<i>rpl14</i> , <i>rpl16</i> [*] , <i>rpl2</i> ^{**a} , <i>rpl20</i> , <i>rpl22</i> , <i>rpl23</i> ^a , <i>rpl32</i> , <i>rpl33</i> , <i>rpl36</i>	11
	Small subunit of ribosome	<i>rps11</i> , <i>rps12</i> ^{**} , <i>rps14</i> , <i>rps15</i> ^a , <i>rps16</i> [*] , <i>rps18</i> , <i>rps19</i> ^a , <i>rps2</i> , <i>rps3</i> , <i>rps4</i> , <i>rps7</i> ^a , <i>rps8</i>	15
	DNA dependent RNA polymerase	<i>rpoA</i> , <i>rpoB</i> , <i>rpoC1</i> , <i>rpoC2</i>	4
	rRNA genes	<i>rrn16</i> ^a , <i>rrn23</i> ^a , <i>rrn4.5</i> ^a , <i>rrn5</i> ^a	8
	tRNA genes	<i>trnA</i> -UGC ^a , <i>trnC</i> -GCA, <i>trnD</i> -GUC, <i>trnE</i> -UUC, <i>trnF</i> -GAA, <i>trnG</i> -GCC, <i>trnG</i> -UCC [*] , <i>trnH</i> -GUG ^a , <i>trnI</i> -CAU ^a , <i>trnI</i> -GAU ^{**a} , <i>trnK</i> -UUU [*] , <i>trnL</i> -CAA ^a , <i>trnL</i> -UAA [*] , <i>trnL</i> -UAG, <i>trnM</i> -CAU, <i>trnN</i> -GUU ^a , <i>trnP</i> -UGG, <i>trnQ</i> -UUG, <i>trnR</i> -ACG ^a , <i>trnR</i> -UCU, <i>trnS</i> -GCU, <i>trnS</i> -GGA, <i>trnS</i> -UGA, <i>trnT</i> -GGU, <i>trnT</i> -UGU, <i>trnV</i> -GAC ^a , <i>trnV</i> -UAC [*] , <i>trnW</i> -CCA, <i>trnY</i> -GUA, <i>trnJ</i> M-CAU	38
Photosynthesis	Photosystem I	<i>psaA</i> , <i>psaB</i> , <i>psaC</i> , <i>psaI</i> , <i>psaJ</i>	5
	Photosystem II	<i>psbA</i> , <i>psbB</i> , <i>psbC</i> , <i>psbD</i> , <i>psbE</i> , <i>psbF</i> , <i>psbH</i> , <i>psbI</i> , <i>psbJ</i> , <i>psbK</i> , <i>psbL</i> , <i>psbM</i> , <i>psbN</i> (<i>psbF1</i>), <i>psbT</i> , <i>psbZ</i> (<i>lhbA</i>)	15
	NADPH dehydrogenase	<i>ndhA</i> [*] , <i>ndhB</i> ^{**a} , <i>ndhC</i> , <i>ndhD</i> , <i>ndhE</i> , <i>ndhF</i> , <i>ndhG</i> , <i>ndhH</i> , <i>ndhI</i> , <i>ndhJ</i> , <i>ndhK</i>	12
	Cytochrome b/f complex	<i>petA</i> , <i>petB</i> [*] , <i>petD</i> [*] , <i>petG</i> , <i>petL</i> , <i>petN</i>	6
	Subunits of ATP synthase	<i>atpA</i> , <i>atpB</i> , <i>atpE</i> , <i>atpF</i> [*] , <i>atpH</i> , <i>atpI</i>	6
	Large subunit of Rubisco	<i>rbcl</i>	1
	Photosynthesis assembly genes	<i>ycf3</i> (<i>pafl</i>) ^{**} , <i>ycf4</i> (<i>paflI</i>)	2
Other genes	Protease	<i>clpP</i>	1
	Maturase	<i>matK</i>	1
	Envelop membrane protein	<i>cemA</i>	1
	C-type cytochrome synthesis gene	<i>ccsA</i>	1
	Translation initiation factor	<i>infA</i>	1
	Conserved open reading frames	<i>ycf15</i> ^a	2
		Total number of genes	130

Note: * and ** indicate genes containing one and two introns, respectively; ^a indicates duplicated genes in inverted repeat (IR) regions. The *rps12* gene is a trans-spliced gene and is not marked as duplicated despite appearing in multiple locations.

subunits of DNA-dependent RNA polymerase (*rpoA*, *rpoB*, *rpoC1*, *rpoC2*). Eight rRNA genes and 38 tRNA genes with different anticodons were found.

Photosynthesis-related genes formed another major category, comprising multiple functional groups. Five genes (*psaA*, *psaB*, *psaC*, *psaI*, *psaJ*) were associated with photosystem I, while 15 genes (e.g., *psbA*, *psbB*, *psbC*, *psbD*) encoded components of photosystem II. The NADPH dehydrogenase complex was represented by 12 genes (*ndhA*–*ndhK*), and six genes (*petA*, *petB*, *petD*, *petG*, *petL*, *petN*) were involved in the cytochrome b/f complex. Genes encoding subunits of ATP synthase included six loci (*atpA*, *atpB*, *atpE*, *atpF*, *atpH*, *atpI*), while the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was encoded by a single gene (*rbcL*). Two genes (*ycf3* and *ycf4*) were associated with photosystem assembly.

Other functional genes included those involved in protein processing, transcription, and membrane function. These comprised *clpP* (protease), *matK* (maturase), *cemA* (envelope membrane protein), *ccsA* (cytochrome c synthesis), and *infA* (translation initiation factor). In addition, conserved open reading frames such as *ycf15* were identified.

Several genes contained introns, with some harboring one intron (e.g., *rpl16*, *ndhA*, *atpF*) and others containing two introns (e.g., *rps12*, *ycf3*). A subset of genes was duplicated within the inverted repeat (IR) regions, including rRNA genes and certain tRNA and protein-coding genes. The *rps12* gene exhibited a trans-spliced structure, with its exons located in separate regions of the genome.

Amino Acid Composition

Relative synonymous codon usage (RSCU) analysis revealed consistent codon usage patterns across *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17) (Figure 2). For most amino acids, synonymous codons were used unequally, indicating codon bias within the plastomes.

Codons ending with A or T generally showed higher RSCU values. For example, GCT and GCA (alanine), AGA and AGG (arginine), AAT (asparagine), GAT (aspartate), CAA (glutamine), and GAA (glutamate) were more frequently used than their GC-ending counterparts. Similar patterns were observed for leucine (TTA, TTG > CTC, CTG), valine (GTT, GTA > GTC, GTG), and phenylalanine (TTT > TTC).

Among glycine codons, GGA showed relatively higher usage, while GGC was less frequent. Serine and threonine codons also exhibited bias, with TCT/TCA and ACT/ACA being more commonly used than other synonymous codons. Methionine (ATG) and tryptophan (TGG) each showed an RSCU value of 1 across all samples. Overall, codon usage patterns were highly similar among the three chloroplast genomes, with only minor variation in RSCU values.

Codon Usage Bias (RSCU Analysis)

Relative synonymous codon usage (RSCU) analysis revealed similar codon usage patterns across *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17) (Figure 3). Most amino acids showed unequal usage of synonymous codons. Codons ending with A or T generally exhibited higher RSCU values than those ending with G or C. For example, GCT and GCA

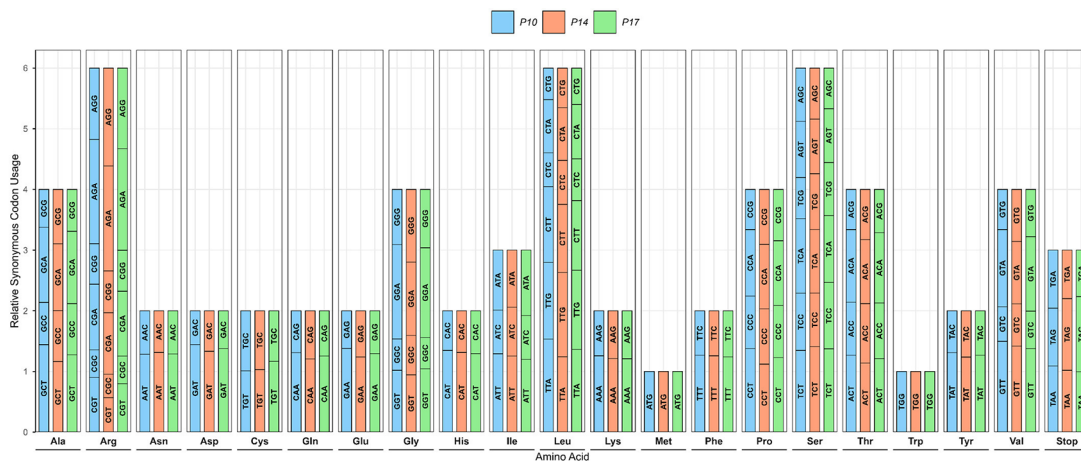


Figure 2. Amino acid composition of chloroplast-encoded proteins in *Poa angustifolia* and *Poa lipskyi*.

(alanine), AGA (arginine), AAT (asparagine), GAT (aspartate), CAA (glutamine), and GAA (glutamate) were more frequently used. Among leucine codons, TTA and TTG showed higher values compared to CTC and CTG, while for valine, GTT and GTA were more preferred than GTC and GTG. Glycine codon GGA displayed relatively high usage, whereas GGC was less frequent. Serine and threonine also showed codon bias, with TCT/TCA and ACT/ACA being more commonly used. Methionine (ATG) and tryptophan (TGG), each encoded by a single codon, had an RSCU value of 1 in all samples. Stop codons exhibited slight variation, with TAA and TAG showing moderate differences, while TGA had relatively lower values. Overall, RSCU values were highly consistent among the three plastomes, with only minor variation observed across corresponding codons.

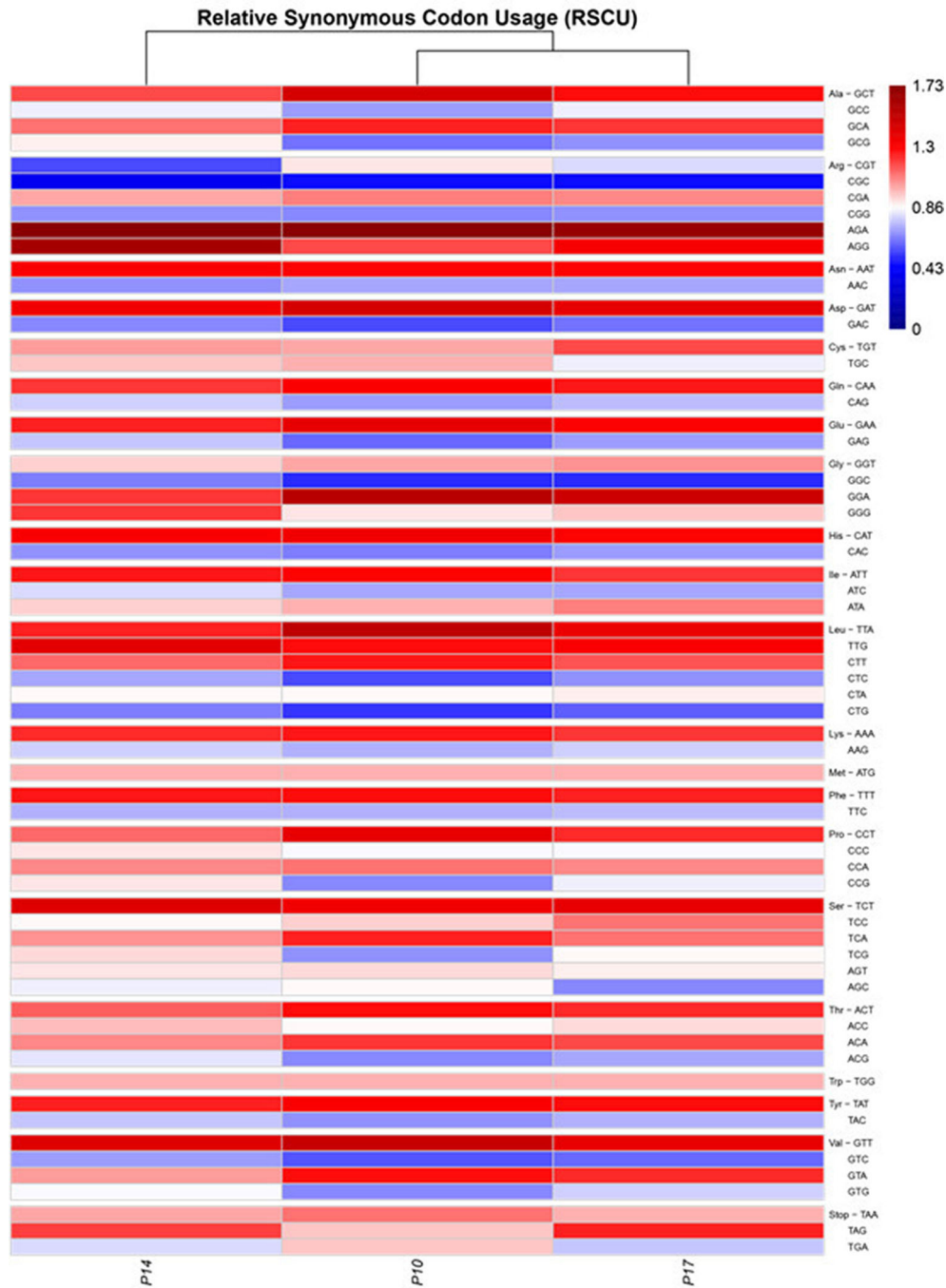


Figure 3. Relative Synonymous Codon Usage (RSCU) Patterns in Chloroplast Genomes of *Poa angustifolia* and *Poa lipskyi*.

The amino acid composition of chloroplast-encoded proteins was highly similar across *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17) (Figure 4). Leucine was the most abundant amino acid in all samples (10.7556–10.7593%), followed by isoleucine (8.3212–8.3291%), glycine (7.4476–7.4550%), and serine (7.0750–7.0808%). Moderate proportions were observed for alanine, arginine, valine, glutamate, lysine, threonine, and phenylalanine. Lower frequencies were recorded for amino acids such as cysteine (~1.105%), tryptophan (~1.768%), histidine (~2.23%), and methionine (~2.34%). The remaining amino acids, including asparagine, aspartate, glutamine, proline, and tyrosine, showed intermediate values. The relative proportions of all amino acids were nearly identical among the three plastomes, with only minimal variation observed across samples.

Sliding window analysis of nucleotide diversity (π) across the chloroplast genomes revealed several regions of elevated sequence variation (Figure 5). Using a window length of 800 bp and a step size of 200 bp, π values ranged from 0.03861 to 0.04631 (top 5). The highest nucleotide diversity was observed in the region spanning 109,046–110,053 bp ($\pi = 0.04631$, *rpl32-trnL-UAG*). Additional regions with relatively high variability were identified at positions 14,054–16,029 bp ($\pi = 0.04077$, *trnC-GCA-rpoB*).

Simple sequence repeat (SSR) analysis revealed similar repeat patterns across *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17) (Figure 6). Mononucleotide repeats were the most abundant type, with A/T repeats predominating (20–22), while C/G repeats were rare (1 in all samples).

Among dinucleotide repeats, AG/CT and AT/AT motifs were consistently detected, with AT/AT repeats occurring at higher frequencies (4 in all samples). Trinucleotide repeats such as AAG/CTT were present at low frequency (1), whereas

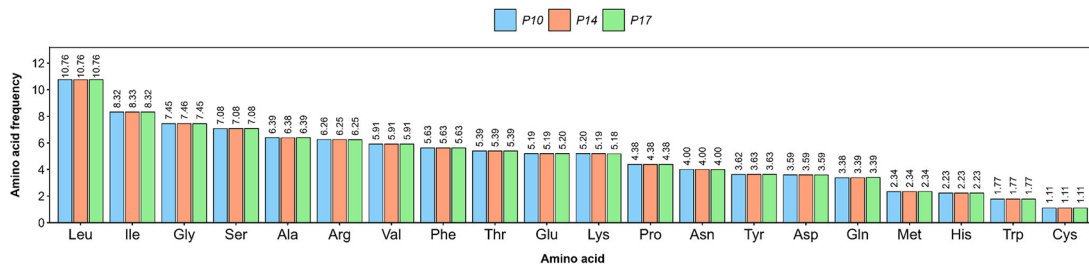


Figure 4. Amino acid composition of chloroplast-encoded proteins in *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17). Values represent the relative proportion (%) of each amino acid in the predicted protein-coding sequences of the three chloroplast genomes.

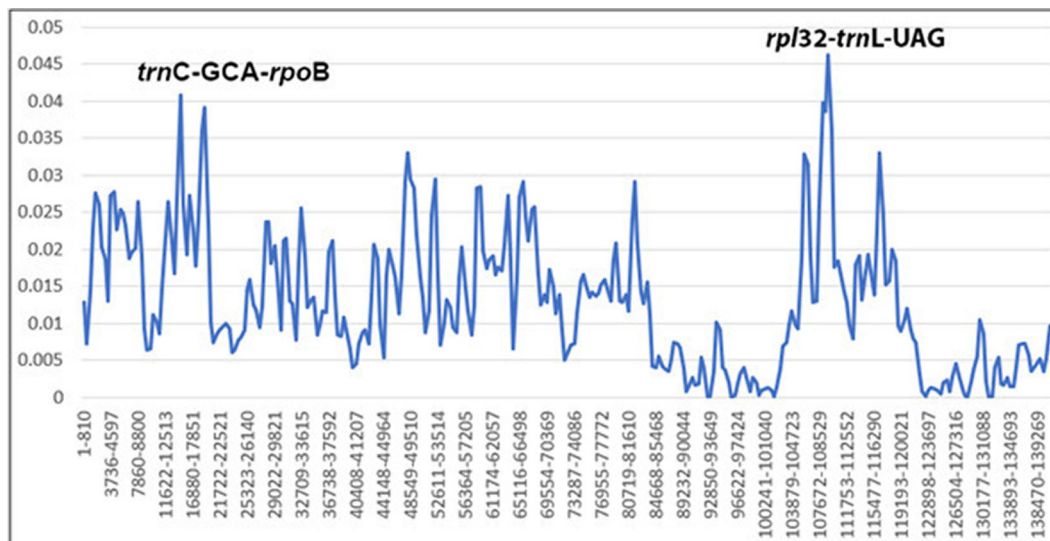


Figure 5. Sliding window analysis of nucleotide diversity (π) across chloroplast genomes of *Poa* species.

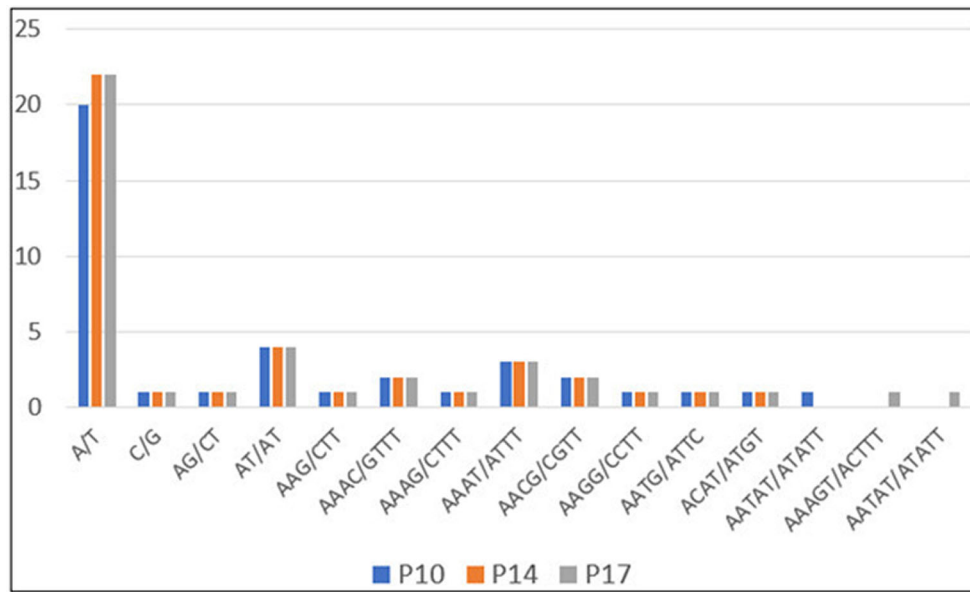


Figure 6. Distribution and frequency of simple sequence repeats (SSRs) in chloroplast genomes of *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17).

tetranucleotide repeats, including AAAC/GTTT and AACG/CGTT, were observed with moderate counts (2 each). Other motifs, such as AAAT/ATTT, occurred three times in all samples.

Pentanucleotide repeats showed slight variation among taxa. The AATAT/ATATT motif was detected only in *P. angustifolia* (P10), whereas AAAGT/ACTTT and AATAT/ATATT were identified exclusively in *P. lipskyi* (P17).

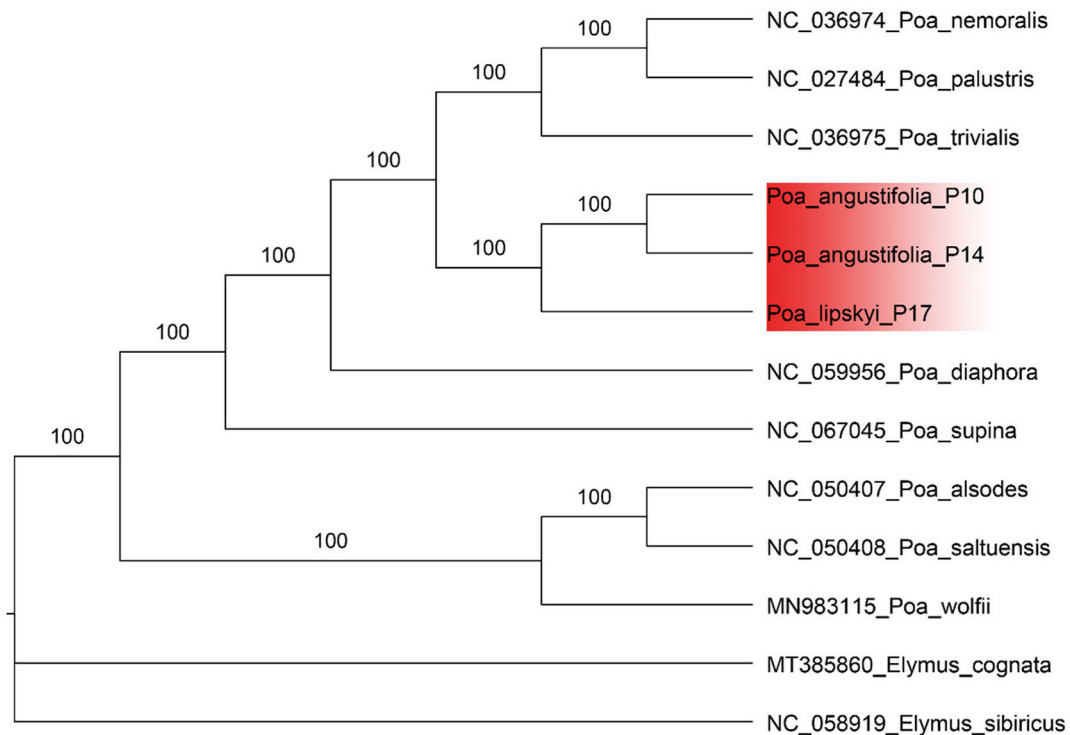


Figure 7. Maximum likelihood (ML) phylogenetic tree based on complete chloroplast genome sequences of *Poa* species. Bootstrap support values are indicated at each node. *Poa angustifolia* (P10, P14) and *Poa lipskyi* (P17) are highlighted and form a well-supported clade within the genus *Poa*. Species of *Elymus* were used as outgroups.

Phylogenetic relationships inferred from complete chloroplast genome sequences revealed well-resolved clades within the genus *Poa* (Figure 7). The analyzed taxa formed distinct groups with strong statistical support, as indicated by high bootstrap values (100) across most nodes.

The studied accessions of *Poa angustifolia* (P10, P14) clustered together, forming a strongly supported clade. This clade was closely related to *Poa lipskyi* (P17), which grouped as a sister lineage to *P. angustifolia*, also with strong bootstrap support. Together, these taxa formed a distinct subclade within the section *Poa*.

This subclade was positioned in proximity to other *Poa* species, including *P. trivialis*, *P. palustris*, and *P. nemoralis*, which formed a separate but closely related group. Additional species such as *P. diaphora* and *P. supina* were placed in more basal positions within the genus.

A separate clade comprising *P. alsodes*, *P. saltuensis*, and *P. wolfii* was also observed, indicating further phylogenetic structuring within *Poa*. The genus *Elymus* (*E. cognata* and *E. sibiricus*) formed an outgroup and clearly separated from the *Poa* clade.

Discussion

The chloroplast genomes of *Poa angustifolia* and *P. lipskyi* were highly similar in overall size, gene content, and functional organization, indicating strong structural conservation within the studied taxa. Such conservatism is typical of land-plant plastomes, which generally retain a quadripartite structure, stable gene order, and a relatively conserved set of genes associated with photosynthesis and self-replication.^{20,21} The plastome sizes recovered in this study, ranging from 135,310 bp to 135,609 bp, fall within the range commonly observed for grasses and suggest that variation among the analyzed samples is limited to small-scale differences rather than major genomic restructuring. The slight size difference between the two accessions of *P. angustifolia* and *P. lipskyi* most likely reflects variation in non-coding regions, such as intergenic spacers or small insertions and deletions, rather than differences in gene complement. This interpretation is supported by the identical total number of annotated genes across all three plastomes.

The 130 annotated genes included the major classes expected in photosynthetically active chloroplast genomes, namely genes related to the plastid genetic apparatus, photosynthetic metabolism, and several conserved open reading frames. The duplicated rRNA and tRNA genes, together with duplicated protein-coding genes such as *rpl2*, *rpl23*, *rps7*, and *ndhB*, are in agreement with their localization in the inverted repeat regions, which are known to play an important role in stabilizing plastome structure.²⁰ Likewise, the presence of intron-bearing genes such as *atpF*, *ndhA*, and *rpl16*, and the trans-spliced structure of *rps12*, corresponds closely to the standard plastome architecture described for seed plants. The absence of obvious gene loss or pseudogenization in the analyzed plastomes indicates that these chloroplast genomes retain a highly conserved functional repertoire.

The codon usage patterns detected in *P. angustifolia* and *P. lipskyi* further emphasize the conservative nature of these plastomes. Relative synonymous codon usage showed a consistent preference for codons ending in A or T, a pattern widely documented in chloroplast genomes of higher plants.^{22,23} This bias is generally attributed to the combined influence of nucleotide composition and selection on translational efficiency, although mutational bias is thought to play a major role in plastids. In the present study, the preferential use of A/T-ending codons was evident across multiple amino acids, including alanine, arginine, leucine, valine, and phenylalanine, and only minor differences were observed among the three plastomes. Such similarity suggests that the underlying forces shaping synonymous codon usage have remained stable across these taxa. Because plastid coding regions are functionally constrained, the observed codon bias likely reflects long-term compositional tendencies rather than recent lineage-specific shifts.²⁴

The amino acid composition of chloroplast-encoded proteins showed an equally high level of consistency. Leucine, isoleucine, glycine, and serine were the most abundant amino acids in all three plastomes, whereas cysteine, tryptophan, histidine, and methionine were present in lower proportions. This pattern is unsurprising given the strong conservation of plastid protein-coding genes across seed plants and the similar codon usage profiles observed in the present data.²⁵ The near-identical amino acid profiles of *P. angustifolia* and *P. lipskyi* therefore reinforce the conclusion that their plastid coding regions are highly conserved at both the nucleotide and protein levels.

The SSR analysis provided additional insight into plastome variation at a finer scale. As in many angiosperm chloroplast genomes, mononucleotide repeats were the dominant class, and A/T repeats were overwhelmingly more abundant than C/G repeats. This pattern has been widely reported in cpSSR studies and reflects the AT-rich nature of plastid non-coding regions.²⁶ Chloroplast microsatellites are of particular interest because they can evolve rapidly relative to coding sequences and often provide useful markers for species delimitation, phylogeography, and population genetics.²⁷ In

the present study, the repeat profiles of the three plastomes were broadly similar, but a few low-frequency pentanucleotide motifs differed among taxa. Although these differences are minor, they may still serve as informative markers for distinguishing plastid lineages within closely related *Poa* taxa. Their value will likely increase when tested across a broader sample of species and populations.

The sliding-window analysis of nucleotide diversity identified several regions of elevated variation, with the highest π value detected in the 109,046–110,053 (*rpl32-trnL-UAG*) bp interval. Additional peaks were observed in the regions 14,054–16,029 (*trnC-GCA-rpoB*) bp. Uneven distribution of nucleotide diversity is a common feature of plastomes, in which mutational hotspots are often concentrated in intergenic spacers and other non-coding regions rather than in highly conserved coding loci.²¹ Such variable regions are particularly valuable for developing molecular markers for low-level phylogenetics and species identification. In *Poa*, where morphological overlap, hybridization, and polyploidy can obscure species boundaries, these hotspots may represent promising targets for future barcode development or for more focused analyses of recent divergence. However, the precise utility of these regions will depend on whether they maintain consistent discriminatory power across a wider sampling of taxa within the genus.

The phylogenetic results are important for understanding sectional relationships in *Poa*. The two accessions of *P. angustifolia* formed a strongly supported clade, with *P. lipskyi* recovered as its sister lineage. Since both species belong to sect. *Poa*, this topology supports their close relationship and shows that complete chloroplast genomes can resolve relationships at the sectional level depending on plant groups.^{1,28,29}

At the same time, the broader topology suggests that sectional boundaries in *Poa* are not always clear-cut. *Poa nemoralis* and *P. palustris* (sect. *Stenopoa*) grouped together, while *P. trivialis* (sect. *Coenopoa*) was placed nearby, indicating possible deeper affinities among these lineages. Similar patterns have been reported in earlier molecular studies, which showed that plastid data do not always recover sharply delimited sections in *Poa*.²⁸

The more isolated placement of *P. supina* and *P. diaphora* is also consistent with the distinctiveness of sect. *Ochlopoa* and sect. *Eremopoa*. However, previous work has shown that some traditional sections, especially *Ochlopoa*, are not strictly natural groups, and that hybridization, reticulation, and polyploidy complicate relationships across the genus.^{1,2,30}

Data availability statement

Underlying data

Chloroplast genome assemblies (or annotations) can be found in NCBI under PZ656403 (P10 - *Poa angustifolia*), PZ656404 (P14 - *Poa angustifolia*) and PZ656405 (*Poa lipskyi*) accession numbers.

Extended data

There is no extended data associated with this study.

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