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Characterization and Phylogenetic Analysis of the Complete Chloroplast Genome of Koeleria Macrantha

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ABSTRACT

Koeleria is a genus of great ecological and economic importance in the tribe Poeae. The taxonomy of *Koeleria* has long been recognized as problematic due to its remarkable morphological plasticity, simplicity of the morphological structures, and overall taxonomic similarity. To address this issue, the complete chloroplast (cp) genome of *Koeleria macrantha* was sequenced, assembled, and compared to previously sequenced *Koeleria* species (*Koeleria nitidula* and *Koeleria glauca*) and closely related other Poeae species. The *K. macrantha* whole cp genome was 136,065 bp in length and had a typical quadripartite structure comprising a large single copy (LSC; 80,203 bp), a small single copy (SSC; 12,592 bp), and a pair of inverted repeats (IRs; 21,635 bp). The genome consisted of 129 genes, including 83 protein-coding genes, 38 transfer RNA genes, and 8 ribosomal RNA genes. A total of 44 simple sequence repeats (SSRs) were detected. The highest nucleotide variability among *Koeleria* was observed in the *trnP-psaJ* region with the Pi value > 0.025. Moreover, the coding regions were found to be more conserved than non-coding regions. Phylogenetic analysis revealed that *Koeleria* is monophyletic and that *K. macrantha* is sister *Koeleria nitidula* and *Koeleria glauca*. Overall, the complete cp genome sequence of *K. macrantha* will provide a solid foundation for future phylogenetic studies, as well as for the analysis of the evolution of *Koeleria* species.

Keywords: *Koeleria*; chloroplast genome; SSR; nucleotide variability; phylogenetic relationship

INTRODUCTION

Koeleria is a genus belonging to the tribe Poeae in the grass family (Family: Poaceae). Species included in this genus are generally called June grasses and they are distributed in temperate regions all over the world, as well as on tropical mountains and rocky soils (Clayton and Renvoize, 1986). *Koeleria* species provide good forage in mountain steppe.

The whole *Koeleria macrantha* (common name: Prairie June grass) has astringent properties. To heal wounds in folk medicine, it is applied externally to the skin in the form of a poultice (Moerman, 1998). Due to its high fiber content, the plant is used as a bakery raw material, while in livestock it is used to feed the animals. It is also commonly used in seed mixes for the restoration of native prairie, savanna, coastal scrub, chaparral, and open forest habitats across the majority of North America. Good drought tolerance and fibrous roots make it useful for revegetation and erosion control on mined lands, over septic systems, in construction areas, on burned sites, and in other disturbed areas (Wang et al. 2011).

However, because of its remarkable morphological plasticity, as well as the simplicity of its morphological structures and overall similarity among taxa, *Koeleria* taxonomy has long been recognized as problematic (Pecinka, 2006; Brullo, 2009). Persoon (1805) established the genus *Koeleria*, which includes five perennial and

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annual species, at the beginning of the 19th century. More taxa were described during the 19th century, and Clayton and Renvoize considered *Koeleria* to include approximately 35 species in 1986. Domin, the first 20th-century botanist to study *Koeleria* species from all over the world, classified them into two groups based on distribution (Domin 1907), Bulbosae Domin and Caespitosae Domin (Quintanar, 2013). Ujhelyi (1961) divided the genus into 15 species complexes distributed across a large geographical area and published various names to accommodate all ploidy levels in each series. Among them, the existence of a large group of taxa closely related to *K. macrantha* and *K. pyramidata* (Lam.) P. Beauv. has received a lot of attention in the literature (Arnow, 1994; Quintanar, 2013) . Therefore, proper DNA-based classification of *Koeleria* species is required to clarify their taxonomic positions.

In this study, we present the complete chloroplast genome of *Koeleria macrantha*, obtained using the Illumina platform. The genome was compared to relatives within the genus *Koeleria* and the tribe Poeae to identify valuable variable regions that can serve as molecular markers. Additionally, a phylogenetic analysis of selected complete chloroplast genomes from the tribe Poeae was conducted, revealing close relationships among *Koeleria* species. Our findings provide a valuable resource for future research on *Koeleria*, facilitating accurate species identification and improving the understanding of phylogenetic relationships among related species.

RESULTS

Cp Genome Features of K. macrantha

The complete cp genome of *Koeleria macrantha* was 136,065 bp long, and displayed a typical quadripartite structure containing a large single-copy (LSC) region (80,203 bp), a short single-copy (SSC) region (12,592 bp) and a pair of inverted repeat (IRA and IRB) regions (21,635 bp). It consisted of 83 protein-coding genes, 38 tRNA genes, and eight rRNA genes, for a total of 129.

Six protein-coding genes (*rps15*, *rps7*, *ndhB*, *rpl23*, *rpl2*, and *rps19*), eight tRNA genes (*trnN-GUU*, *trnR-ACG*, *trnA-UGC*, *trnI-GAU*, *trnV-GAC*, *trnL-CAA*, *trnI-CAU*, and *trnH-GUG*), and four rRNA genes were duplicated in the IR regions. Ten protein-coding genes, including two duplicated genes (*rpl2* and *ndhB*) and eight tRNA genes, including two duplicated genes (*trnI-GAU* and *trnA-UGC*) contained one intron, whereas the protein-coding gene *ycf3* had two introns. Similar to most of the other angiosperms, the 5' end of *rps12* was located in the LSC region and 3' end was duplicated in the IR regions (Figure 1 and Table 1).

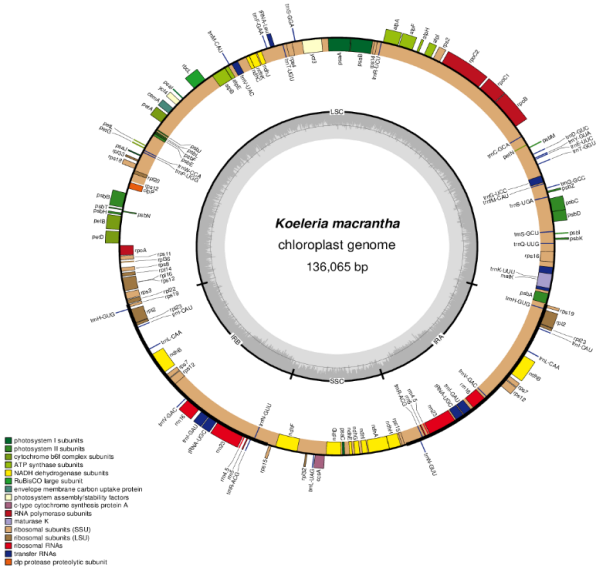

Figure 1. Gene map of *K. macrantha* chloroplast genomes. Genes on the inside of the map are transcribed in the clockwise direction and genes on the outside of the map are transcribed in the counterclockwise direction. The darker gray in the inner circle represents to GC content whereas the light gray corresponds to AT content. Different functional groups of genes are shown in different colors.

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Table 1. Genes with introns in the *K. macrantha* cp genome

Gene	Region	No of introns	Exon I (bp)	Exon II (bP)	Exon III (bp)
atpF	LSC	1	145	407	
ycf3	LSC	2	126	226	161
petB	LSC	1	6	642	
petD	LSC	1	8	475	
rpl16	LSC	1	9	402	
rpl2	IRB	1	391	431	
rpl2	IRA	1	391	431	
ndhB	IRB	1	777	756	
ndhB	IRA	1	777	756	
ndhA	SSC	1	550	539	
rps12	IRB	2	114	232	29
rps12	IRA	2	114	232	29
trnK-UUU	LSC	1	37	35	
trnG-UCC	LSC	1	23	48	
tRNA-Leu	LSC	1	35	50	
trnV-UAC	LSC	1	39	37	
trnI-GAU	IRB	1	37	35	
trnI-GAU	IRA	1	37	35	
trnA-UGC	IRB	1	38	35	
trnA-UGC	IRA	1	38	35	

Comparative Analysis of cp Genome Structures

The complete cp genome structure of *K. macrantha* was compared to the cp genomes of two other *Koeleria* species (*Koeleria nitidula* and *Koeleria glauca*). The findings revealed that similar characteristics were shared by all *Koeleria* species. Despite differences in total cp length, all three shared the same IR region length (Table 2). To resolve the structural evolutionary history of the cp genomes of the subtribe Aveninae (tribe: Poeae) with the *K. macrantha*, we compared IR/SSC and IR/LSC junctions across five selected complete plastomes from the subtribe Aveninae including *Koeleria nitidula*, *Koeleria glauca*, *Avena chinensis*, *Lagurus ovatus*, *Trisetum spicatum*. The IRscope analysis revealed that there was no significant variation in junction sites among three *Koeleria* cp genomes, as well as *Koeleria* cp genomes with *Lagurus ovatus* compared to *A. chinensis* and *T. spcatum*. All species used in this study had an IRa/b region of ~21,600 bp and an SSC region of ~12,600 bp

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(Figure 2).

Table 2: Comparison of general features of *Koeleria* genomes

Genome features	***Koeleria macrantha***	***Koeleria nitidula***	***Koeleria glauca***
Accession Number	OP894923	NC_042404	NC_059965
Total length (bp)	136,065	136,085	136,051
LSC length (bp)	80,203	80,251	80,179
IR length (bp)	21,635	21,635	21,635
SSC length (bp)	12,592	12,564	12,592
GC content (%)	38.5	38.5	38.5
Total genes	129	129	129
Genes duplicated in IR	18	18	18
Protein coding genes	77	77	77
tRNA	30	30	30
rRNA	4	4	4

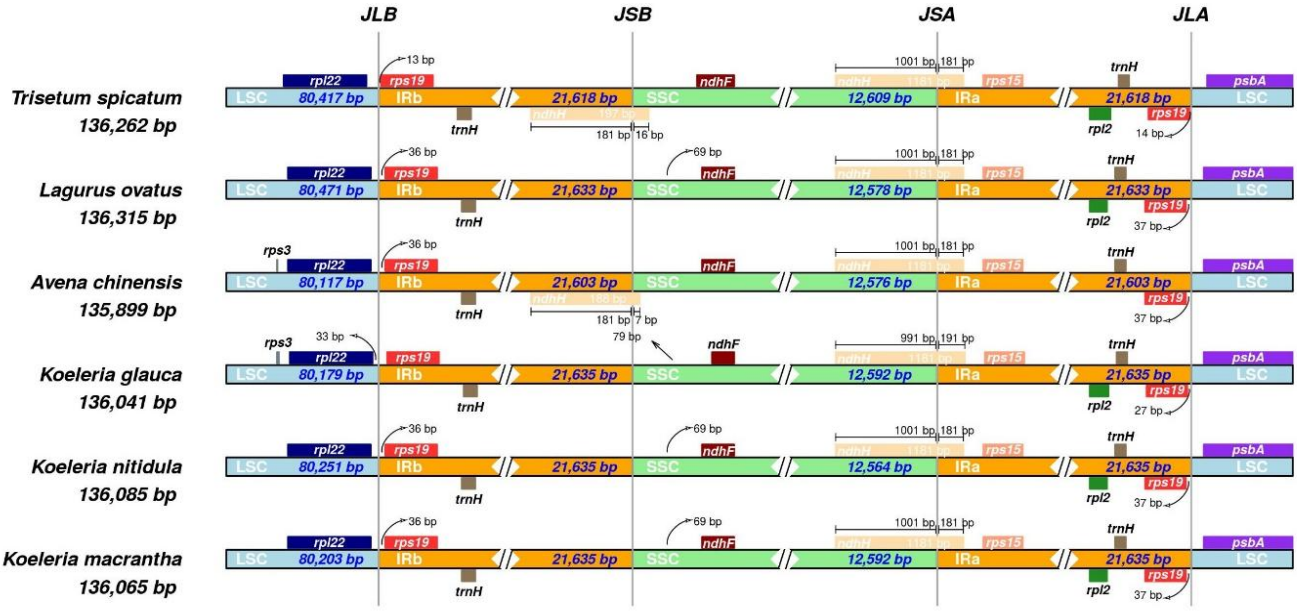

Figure 2. Comparison of the LSC, SSC, and IR regions among six selected cp genomes in the Aveninae. Genes are denoted by colored boxes. The gaps between the genes and boundaries are proportional to the distances in bps.

Analysis of simple sequence repeats

Simple sequence repeats in three *Koeleria* species were identified using MISA tool. A total of 44, 40, and 39

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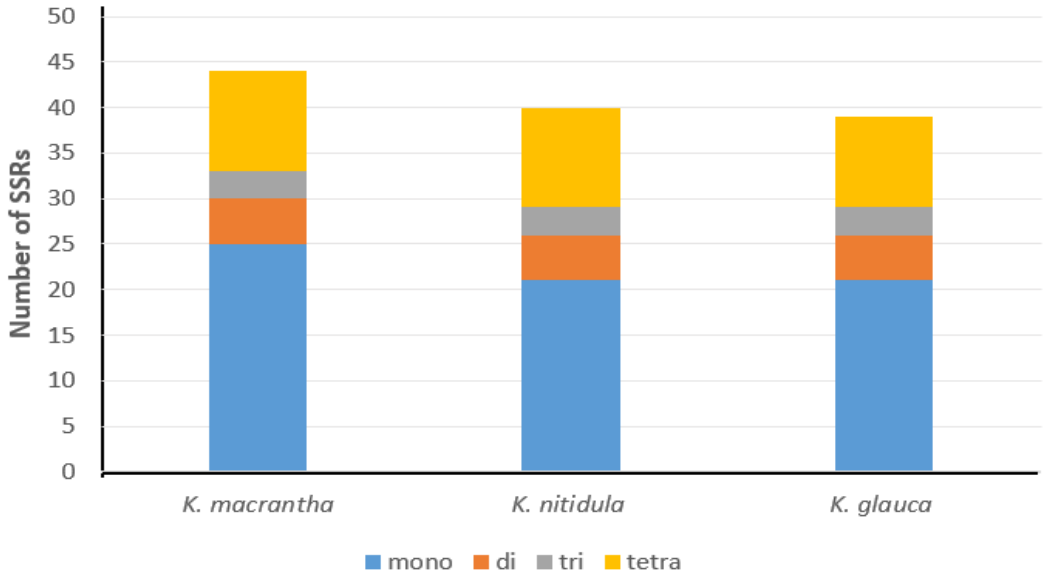
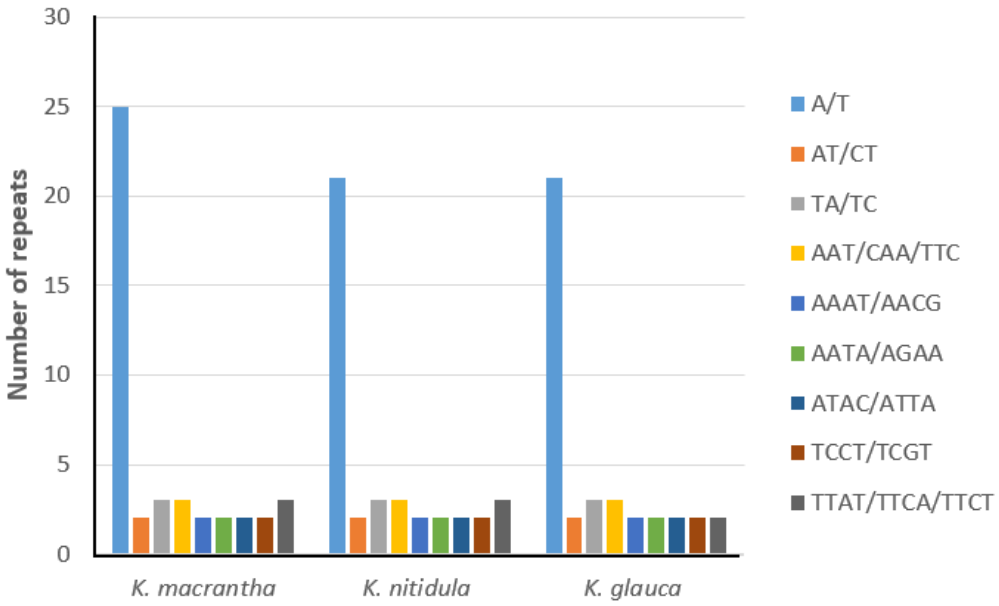
SSRs were identified in *K. macrantha*, *K. nitidula* and *K. glauca* cp genomes, respectively. Mononucleotide repeat A/T was the most abundant in all three cp genomes, countered 25, 21, and 21 times in *K. macrantha, K. nitidula* and *K. glauca,* respectively. The numbers of dinucleotide SSRs were similar across all species and were greater than the number of trinucleotide SSRs, which were three in each. No penta and hexanucleotide SSRs existed in these three species (Figure 3).



(a)



(b)

Figure 3. Simple sequence repeats (SSRs) in *Koeleria* cp genomes. (a) Number of different SSR types. (b) Distribution of SSRs in the chloroplast genomes of *Koeleria.*

Analysis of Sequence Divergence

The sequence divergence among three *Koeleria* species was assessed by calculating nucleotide variability (Pi) using DnaSP software. The average Pi value was 0.00134. The highest variable region *trnP-psaJ* was observed with the Pi value > 0.025 at the LSC region. Remarkably, *rbcL-psal*, *rpl16,* and *ccsA-ndhD* regions also showed a high Pi value of ~0.01 among the three species. Less variability was detected in the IR regions (Figure 4).

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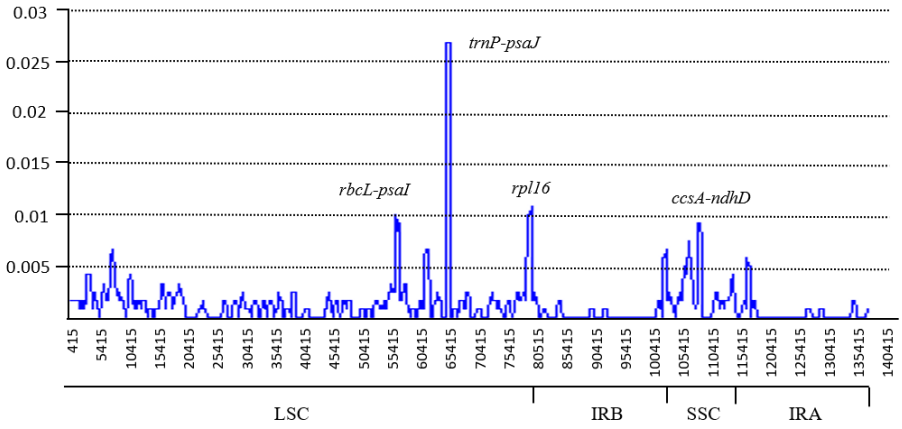

Figure 4. Sliding window analysis among *Koeleria* species. Pi values are indicated in the y-axis.

Phylogenetic analysis

To reveal the phylogenetic position of *K. macrantha* with other Poeae species, the ML tree was constructed using 22 Poeae species, including two Poinae types as out group. Results showed that *K. nitidula* and *K. glauca* were in the same clade and both were sister to *K. macrantha* with the bootstrap value of 100%. Furthermore, species from the *Avena* clustered together and were closely related to *Arrhenatherum elatius* (Figure 5).

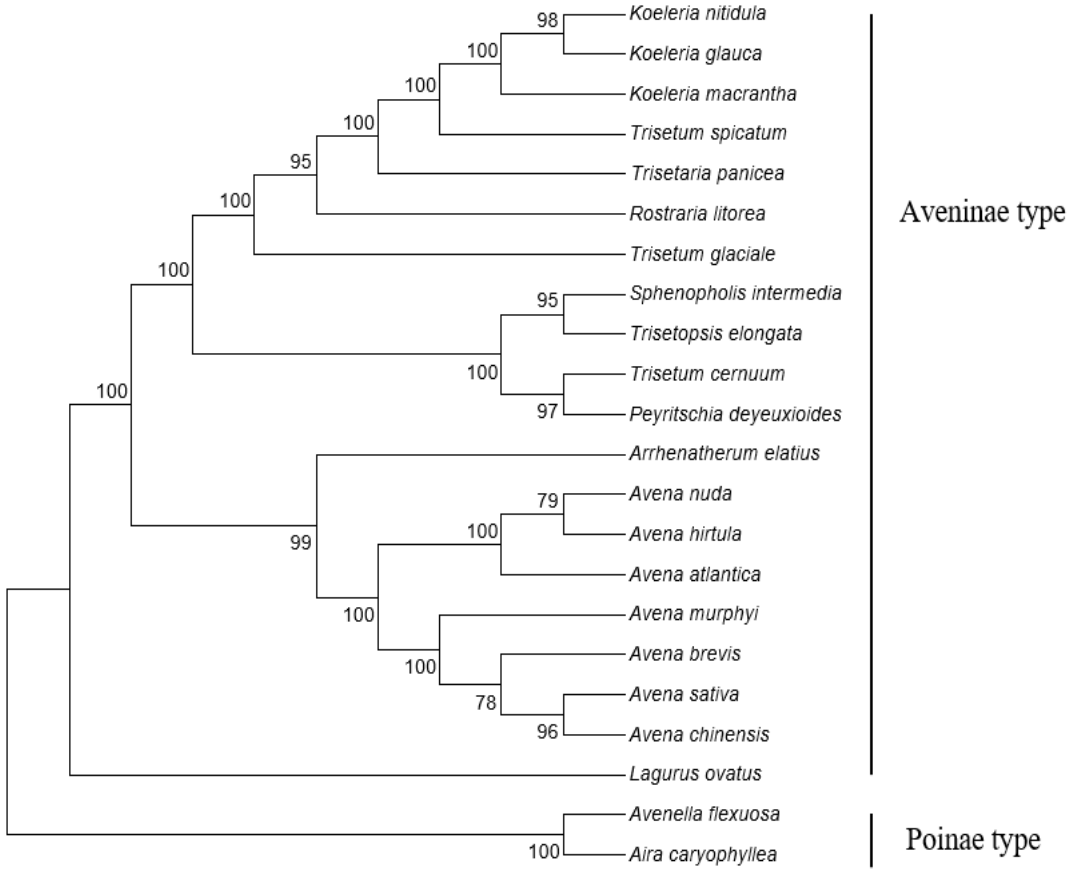

Figure 5. The maximum likelihood (ML) phylogenetic tree based on complete chloroplast genomes. Number beside nodes indicate bootstrap values.

DISCUSSION

The grass family Poaceae, formerly known as Gramineae, is the most economically important plant family, providing staple foods such as maize, wheat, rice, barley, and millet as well as feed for meat-producing animals.

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Some Poaceae members are used as building materials (bamboo, thatch, and straw); others can be used to produce biofuel, primarily through the conversion of maize to ethanol. They have adapted to the range of environmental extremes that plants occupy, from the coldest regions and highest elevations where plants grow to equatorial heat, and from fully aquatic habitats to deserts.

Despite ongoing efforts to reconstruct the phylogeny of the Poaceae, phylogenetic relationships have yet to be fully resolved (Feng et al. 2021). Recently, there has been rapid progress in comparative chloroplast genomics, which has been widely applied to plant phylogenomics research (Huang et al. 2014; Gao et al. 2019). Thus, a large number of grass complete chloroplast genome resources are urgently needed to enable future phylogenomic analyses of the grass family (Gao et al. 2016; Hutang and Gao, 2017; Saarela et al. 2018).

In order to achieve this purpose, we resolved the chloroplast genome of *Koeleria macrantha*, which had a typical quadripartite structure of 136,065 bp in length and contained a pair of IRs, LSC, and SSC regions, similar to most other species (Ren J et al. 2022; Somaratne et al. 2019; Daniell et al. 2016). Previous research found that the GC content in the cp genomes of Pooideae species was unevenly distributed, with the IR regions having a higher GC content than the two single copy regions (Ren J et al. 2022). This could be attributed to the fact that four rRNA genes with high GC content were found in the IR regions, supporting previous research. The *accD* gene was lost in cp genomes of Pooideae species, while *ycf1*, *ycf2*, *ycf15*, and *ycf68* were pseudogenes, which is a fairly common occurrence in Poaceae (Huang et al. 2017). There is a correlation between gene loss and evolution, and some research suggests that it could be an adaptive strategy with benefits to survival and reproduction. The *trnK-UUU* has the longest intron that completely wraps the *matK* gene, which is consistent with previous findings (Ren J et al. 2022; Li X. et al. 2019).

In most land plants, the *rpoC1* gene has been found to contain introns. However, deletion of the *rpoC1* intron has been observed in some angiosperm lineages, including most Poaceae and some Fabaceae, Cactaceae, and Aizoaceae species (Huang et al. 2017). Our findings in the subfamily Pooideae confirm that the *rpoC1* intron is absent in all Poaceae. Similarly, the *clpP* gene typically had two introns. Nonetheless, Guisinger et al. (2010) demonstrated that *clpP* intron loss was present in all Poaceae species, which was supported by our findings.

Length variation in the IR region of the chloroplast genome was a common phenomenon during land plant evolution, resulting in the formation of a variation of boundary features (Yang et al. 2010). The study found that boundary genes in the subfamily Pooideae were mostly *rpl22*, *rps19*, *rps15*, *ndhF*, *ndhH*, and *psbA*, which supported our findings.

The sequence divergence level analysis revealed that coding regions were more conserved than non-coding regions in the cp genomes of the subfamily Pooideae, and the two single copy regions had greater variation potential than the IR regions. These two findings were consistent with previous research in other plant taxa (Gu et al. 2016; Alzahrani et al. 2020). The highly variable regions discovered in this study showed promise as specific DNA barcodes for the subfamily Pooideae, which has positive implications for species identification.

Simple sequence repeats (SSRs) were often used as a molecular marker to explore population relationships and evolutionary history due to its polymorphism, co-dominance and reliability. A total of four types of SSRs were detected in the cp genomes of *K. macrantha* and its related species, of which mono-nucleotide repeats were the most common. Furthermore, this study found that most SSR types were mono-nucleotide repeats with an A/T preference. This phenomenon has been observed in a several of other taxa (Wheeler et al. 2014).

In the current study, complete chloroplast genomes were used for the first time to explore the phylogenetic position of *K. macrantha*. The phylogenetic analysis strongly demonstrated that *K. macrantha* formed a sister branch with the genus *Trisetum* (BS = 100), which further justified the results of previous morphological treatments and phylogenetic studies based on chloroplast fragments (Saarela et al. 2017). Although numerous authors have proposed taxonomic classifications of *Trisetum*, classification for *Trisetum* and *Trisetaria* has been challenging. Previously reported that *Koeleria* is closely related to *Trisetum*. However, according to our findings only *Trisetum spicatum* is closely related to *K. macrantha* and other *Trisetum* species were found in different positions in the Aveninae. A similar topology was found in the plastid tree in Wölk and Röser (2017). These different topologies in plastid trees may be due to ancient hybridization.

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Previous studies have identified *Lagurus* as the sister group of the clade Avena which was supported by our findings (Saarela et al. 2017). Therefore, phylogenetic evidence is consistent with the inclusion of *Lagurus* in Aveninae. However, more sampling of *Koeleria* is needed to clarify its evolutionary history, especially since *Koeleria* is the most poorly sampled genus in the Poaceae.

MATERIALS AND METHODS

Plant Materials and DNA Sequencing

Fresh *K. macrantha* leaves were collected at Yan'an, Shaanxi, China (179°36°39′20″N 109°24′26″E). Total genomic DNA was extracted from silica-dried leaves using the modified cetyltrimethylammonium bromide (CTAB) method (Doyle, 1987). Purified DNA was used to prepare paired-end libraries using the Illumina Paired-End DNA Library Kit according to the manufacturer's instructions (Illumina, CA, USA). Genomic DNA was sequenced on a HiSeq X Ten platform.

Genome Assembly and Annotation

We obtained high-quality reads from raw reads using the Trimmomatic tool (Bolger, 2014). The high-quality reads were then assembled using the GetOrganelle software (https://github.com/Kinggerm/GetOrganelle/blob/master/get organelle from reads.py). Assembly was polished by mapping raw reads back to the retrieved sequence using the map reference tool in Geneious 10.0.5 (http://www.geneious.com). The generated consensus sequence was further adjusted by alignments with related species (Lauren et al. 2021). Dual Organellar GenoMe Annotator online tool (https://dogma.ccbb.utexas.edu/) was used to annotate all protein-coding sequences and tRNA and mRNA genes (Wyman, 2004). Annotations of tRNA genes were confirmed with tRNAscan-SE (Lowe, 2014). Finally, the circular cp genome of *K. macrantha* was drawn using the online tool Organellar Genome DRAW (ORDRAW, https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Lohse, 2007). The complete chloroplast genome of *K. macrantha* was deposited in GenBank with the accession OP894923.

SSR Analysis

The MISA tool (Beier et al. 2017) was used to detect SSRs, with the parameters set to ten repeated units for mononucleotide SSRs, six repeated units for dinucleotide SSRs, and five repeated units for tri-, tetra-, penta-, and hexanucleotide SSRs. IR border regions were visualized using the IRscope online program (https://irscope.shinyapps.io/irapp/) (Amiryousefi et al. 2018).

Sequence divergence analysis

Sequence divergence values were determined for the aligned *Koleraria* species using the sliding window method in DnaSP v5.10. (Librado et al. 2009) with a 200bp step size and an 800 bp window length.

Phylogenetic Analysis

Phylogenetic analysis was carried out based on an alignment of completed cp genomes from 22 angiosperms including twenty Aveninae type Poeae species and two Poinae type Poeae species as out group. MAFFT (Katoh et al. 2002) was employed for multiple sequence alignment under the default paremeters. The ML tree was constructed in MEGA 6.0 (Tamura et al. 2013) based on the Tamura-Nei model using a heuristic search for initial trees. Bootstrap analysis was performed with 1,000 replicates. A discrete gamma distribution was used with four categories. Initial ML tree(s) were obtained using the neighbor joining and BIONJ algorithms.

CONCLUSIONS

In this study, we sequenced the complete cp genome sequence of *K. macrantha*, a grass belonging to the tribe Poeae. SSRs and specific genome features were identified and compared to those of other *Koleraria* species. This is the first study to compare complete cp genomes of *Koleraria* species, using all complete *Koleraria* cp genomes available in the NCBI database to date. Phylogenetic analysis shows that cp genome data sets can be

used to resolve *Koleraria* phylogeny using a large sample size. This study adds to the literature by providing new information on the genetic relationships among *Koleraria* species, supporting future efforts in species identification, phylogenetic studies, and conservation.

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Conflicts of Interest

The authors declare no conflict of interest.

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