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PLASTOME REPORT

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Analysis of the complete plastomes of *Albizia kalkora* (Roxb.) Prain 1897 (Fabaceae)

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ABSTRACT:
Albizia kalkora (Roxb.) Prain 1897, belonging to the family Fabaceae, is not only a landscape tree but also a medicinal plant. At present, few plastomes have been reported from *Albizia*, which delays the in-depth phylogenomic studies and the development of high-resolution discriminating markers for this genus. Herein, we sequenced the first plastome of *A. kalkora* by NGS technology. The genome is a circular structure (176,158 bp), containing a large single-copy (LSC) region (91,521 bp), a small copy (SSC) region (5237 bp), and two inverted repeat (IR) regions (39,700 bp each). It has 35.45% GC content and encodes 109 unique genes, which are 76 protein-coding, 4 rRNA, and 29 tRNA genes. The genetic distance analysis of the intergenic spacer regions for *A. kalkora*, *A. odoratissima* and *A. bracteate* shows four intergenic regions with very high K2p values, namely, *ccs*A-*ndh*D (15.04), *mat*K-*rps*16 (10.77), *rps*11-*rpl*36 (17.63) and *rps*3-*rps*19 (20.08), which can discriminate the three *Albizia* species. In addition, we identified ten pairs of regions that could be utilized to design primers to discriminate the three *Albizia* species. The phylogenetic analysis showed *Albizia* was closely related to *Samanea*. The results in this study will provide valuable information to elucidate the classification, identification and evolutionary history of *Albizia*.

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Albizia kalkora; DNA barcode; fabaceae; plastome; phylogeny

Introduction

Albizia kalkora (Roxb.) Prain 1897 is a deciduous tree or shrub with 3–8 m tall, belonging to the family Fabaceae (Legume). Its native range is in northeastern India, China North-Central, China South-Central, China Southeast, East Himalaya, Bangladesh, Japan, Korea, and Vietnam. *A. kalkora* trees are not only an ornamental plant, but also has medicinal value. Some of its chemical components can be used as raw materials and have potential applications in the pharmaceutical industry. Several researches on the chemical composition of *A. kalkora* have been reported. Leaves of *A. kalkora* contain a large number of chemical components, such as squalene, epinephrine, trans-squalene, stachydrine and other chemical components with high medicinal value (Hou et al. 2020).

To develop the valuable plant resource, we should delineate the phylogenetic relationship of the species in its family, and then develop high-resolution markers that can be used to distinguish orthodox species from other substitute and adulterant species. In morphology, the *A. kalkora* is similar to the *A. julibrissin*. Although the two species can be distinguished by some morphological characteristics, more objective data of molecular biology is helpful to distinguish these two species, especially in the case of intermediate hybridization of the two species. In previous studies, the plastome has been used for species identification, taxonomic classification, evolution investigation, and genetic engineering because of their highly conserved nature and role in monolepsis (Mariga 2004). However, there is no reported plastome of *A. kalkora* up to now. The lack of complete plastomes is severely limiting our ability to perform the phylogenomic and DNA barcoding studies of this species. In this study, we sequenced, assembled, and characterized the first complete plastome of *A. kalkora*, developed DNA barcodes for distinguishing *Albizia*

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species and compared the plastome with others from the tribe Ingeae of Fabaceae. The study results will lay a solid foundation for future phylogenomic study and bioprospecting of *Albizia* species.

Material

Fresh leaves of *A. kalkora* (Figure 1) were collected from Central China Medicinal Botanical Garden, EnShi, China (Geospatial coordinates: N 30.180978°, E 109.756823°). The specimen was deposited at the Institute of Medicinal Plant Development (contact person: Haimei Chen, hmchen@implad.ac.cn) under the specimen number of implad201808110. Total genomic DNA was extracted by using the plant genomic DNA kit (Tiangen Biotech, Beijing, China). The purity and the concentration of the extracted DNA was determined by using a Nanodrop spectrophotometer 2000. The extracted DNA with the ratio of OD_{260}/OD_{280} between 1.8-2.0 was seen as high-quality samples and was further used to construct the DNA sequencing library.

Methods

The sequencing library with an insert size of 350 bp fragments was constructed and sequenced by using the Illumina HiSeq 2500 platform. The obtained 2×350 reads were filtered by the software to obtain high-quality reads (Bolger et al. 2014). The high-quality reads were used to assemble the plastome sequence by using NOVOPlasty (v4.2) (Nicolas et al. 2017) with *mat*K (GenBank: APA32793.1) as a seed sequence. The CpGAVAS2 web service (Shi et al. 2019) was utilized to annotate the plastome with the dataset of "43-plastomes Reference Dataset". And the plastome was visualized as a circular map by using CPGview-RSG (http://www.herbalgenomics.org/cpgview/). Finally, the plastome annotation results were deposited in the GenBank under accession number MW420923.

Figure 1. Panorama (A) and detail (B) photos of *Albizia kalkora*. The photos were shot by Liqiang Wang and the coordinates of the plant was N 30.180978°, E 109.756823°. Main identifying traits: Trees, deciduous, 3–8 m tall. Branchlets dark brown, pubescent, with conspicuous lenticels. Stipules in-conspicuous. Pinnae 2–4 pairs. Leaflets 5–14 pairs, oblong or oblong-ovate. Heads 2–7, axillary or terminal, arranged in panicles. Flowers dimorphic, primarily white, turning yellow, with conspicuous pedicels. Calyx tubular, 2–3 mm, 5-toothed, calyx and corolla villous. Corolla 6–8 mm; lobes lanceolate. Stamens 2.5–3.5 cm, basally connate into a tube; tube shorter than corolla tube. Seeds 4–12, obovoid or suborbicular; pleurogram oblong.

The plastomes of *A. kalkora* and other 13 reported plastomes of Fabaceae (tribe Ingeae) were downloaded from the GenBank database for the phylogenetic analysis. The plastome of *Pterolobium punctatum* was selected as an outgroup. Then the shared CDS sequences of the 15 plastomes were extracted and concatenated by the PhyloSuite (v 1.1.16) (Zhang et al. 2020) and were aligned by the MAFFT (v 7.313) (Katoh and Standley 2013) with default parameter of "–auto". Phylogenetic analysis was carried out based on maximum likelihood (ML) model implemented in the IQ-TREE (v 1.6.8) (Nguyen et al. 2015) under the TVM + F + I + G4 nucleotide substitution model. The significance level for the phylogenetic tree was evaluated by bootstrap testing with 1000 replications. To discriminate the *Albizia* species, potential DNA barcoding markers were identified by using the ecoPrimers software (Tiayyba et al. 2011).

Results

The plastome of *A. kalkora* is 176,158 bp in size with a large single copy (LSC) region of 91,521 bp, a small copy (SSC) region of 5237 bp and two inverted repeats (IRa and IRb) regions of 39,700 bp by each (Figure 2). The reliability of genome assembly was strongly supported by the results of mapping experiment (Figure S1). The length of the protein-coding sequence in the plastome of *A. kalkora* is 88,494 bp, representing 50.24% of the total genome length (Table S1). In contrast, the length of the ribosomal RNA (rRNA) and transfer RNA (tRNA) genes is 9242 bp and 2725 bp, representing 5.25% and 1.55% of the total genome length (Table S1). The GC content analysis showed that the overall GC content of the whole plastome is 35.45% (Table S1). The GC content of the IR region (38.66%) is greater than that of the SSC region (28.55%) and that of the LSC region (33.06%) (Table S1).

We detected 109 unique genes in the *A. kalkora* plastome (Table S2), including 76 protein-coding genes (PCGs), 4 rRNA genes, and 29 tRNA genes. Fifteen PCGs (*rps*15, *rps*7, *rpl*2, *rpl*23, *ndh*A, *ndh*B, *ndh*D, *ndh*E, *ndh*G, *ndh*H, *ndh*I, *psa*C, *ycf*1, *ycf*15 and *ycf*2), 7 tRNA genes (*trn*A-UGC, *trn*E-UUC, *trn*L-CAA, *trn*M-CAU, *trn*N-GUU, *trn*R-ACG and *trn*V-GAU) and 4 rRNA genes (*rrn*16S, *rrn*23S, *rrn*4.5S, *rrn*5S) are located at the IR regions. Among these genes, 9 PCGs (*rps*16, *atp*F, *rpo*C1, *pet*D, *pet*B, *rpl*16, *rpl*2, *ndh*B, and *ndh*A) contain one intron, 2 PCGs (*ycf*3 and *clp*P) contain 2 introns, and 6 tRNA genes (*trn*K-UUU, *trn*T-CGU, *trn*L-UAA, *trn*C-ACA, *trn*E-UUC and *trn*A-UGC) contain 1 intron. The structures of the cis-splicing PCG genes were shown in Figure S2.

To investigate the plastomic divergence of *Albizia* species, we conducted a genetic distance analysis of intergenic spacer regions for the plastomes of *A. kalkora*, *A. odoratissima* and *A. bracteate*. The result showed that four intergenic spacer regions had very high K2p values, namely, *ccs*A-*ndh*D

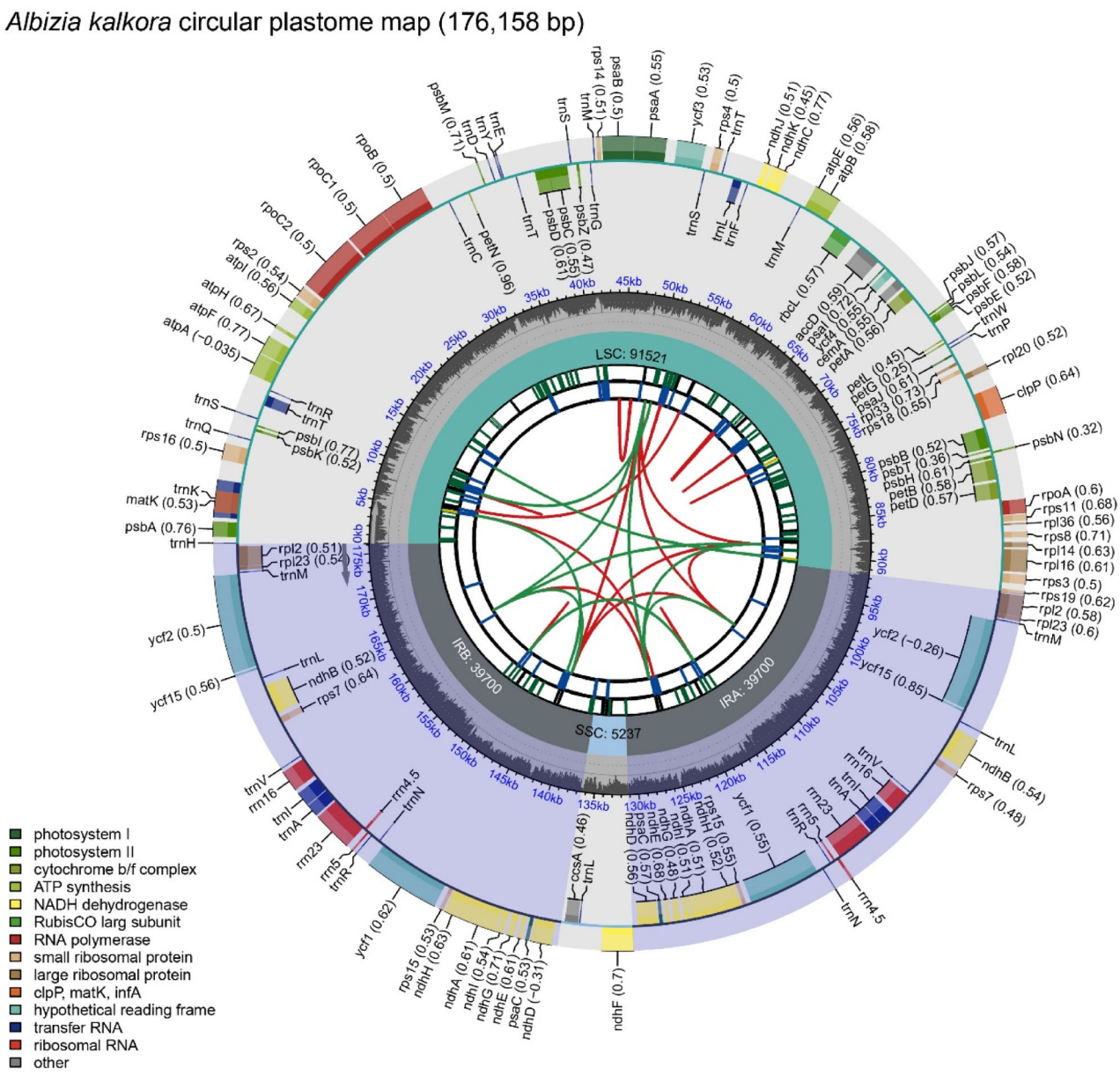

Figure 2. Circular map of overall features of the *Albizia kalkora* plastome. The map contains six tracks in default. From the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D) and Palindromic (P) repeats, connected with red and green arcs. The second track shows the long tandem repeats as short blue bars. The third track shows the short tandem repeats or microsatellite sequences as short bars with different colors. The colors, the type of repeat they represent, and the description of the repeat types are as follows. Black: c (complex repeat); Green: p1 (repeat unit size = 1); Yellow: p2 (repeat unit size = 2); Purple: p3 (repeat unit size = 3); Blue: p4 (repeat unit size = 4); Orange: p5 (repeat unit size = 5); Red: p6 (repeat unit size = 6). The small single-copy (SSC), inverted repeat (IRa and IRb), and large single-copy (LSC) regions are shown with their length on the fourth track. The GC content along the genome is plotted on the fifth track. The genes are shown on the sixth track. The optional codon usage bias is displayed in the parenthesis after the gene name. Genes are color-coded by their functional classification. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively. The functional classification of the genes is shown in the bottom left corner.

(15.04), *mat*K-*rps*16 (10.77), *rps*11-*rpl*36 (17.63) and *rps*3-*rps*19 (20.08) (Figure S3), indicating a high variation among the three *Albizia* species. In addition, we identified ten pairs of regions that could be utilized to design primers to discriminate the three *Albizia* species (Table 1), which were mainly from intergenic regions, indicating the high practicability of intergenic regions in distinguishing relatively close species as DNA barcodes.

The plastome provides valuable resources for the phylogeny research of angiosperms. Here, we carried out an ML phylogenetic analysis for the 14 species belonging to the tribe Ingeae (Fabaceae). The phylogenetic tree showed the three *Albizia* species were clustered together (Figure 3), consistent with the expected relationships. The relationship between *A. odoratissima* and *A. bracteate* was the closest. Furthermore, the *Albizia* species were most closely related to *Samanea* species with a bootstrap value of 100. The topological structure of the ML tree was tested using Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa 1999) and proximately unbiased (AU) (Shimodaira 2002) methods embedded in IQ-TREE. The topological structure of the ML tree was reliable with p-SH and p-AU values of 0.504 and 0.511 respectively.

Table 1. Potential regions to design PCR primers for the discrimination of *Albizia odoratissima* (KX852437), *A. bracteata* (MN709837) and *A. kalkora* (MW420923).

No.	Species	Forward region start-end	Inverse region start-end	Location of DNA barcodes	Forward sequences	Inverse sequences
1	*A. odoratissima*	11122–11177	11227–11276	*atp*A	AAAACATTAAATGAGATTTTTAATCAATTTTTTTCTACTTGTTCCTGAAGTAAAAA	AAAACATTAAATGAGATTTTTAATCAATTTTTTTCTACTT GTTCCTGAAGTAAAAA
	A. bracteata	12134–12189	12239–12288			
	A. kalkora	12056–12111	12161–12210			
2	*A. odoratissima*	12004–12038	12082–12134	*atp*A	ACTACAATAGTGTATTCCATTGCTCCCCTTTCTTG	ACTACAATAGTGTATTCCATTGCTCCCCTTTCTTG
	A. bracteata	13016–13050	13094–13146			
	A. kalkora	12938–12972	13016–13068			
3	*A. odoratissima*	14953–15088	15141–15197	*atp*H-*atp*I	CCGACGGCCAACCCAGCAGCAATAACCGAAGCGGCAGAAATCAGTGGATTCATG ATAAGTTCCTCGCACCAAAAATAAAGAAATAGTTAATGATAC AATCAACCAACAAATTATGACTTAATTATTCCATCATTAA	CCGACGGCCAACCCAGCAGCAATAACCGAAGCGGCAGAAATCAGT GGATTCATGATAAGTTCCTCGCACCAAAAATAAAGAAATAGTTAA TGATACAATCAACCAACAAATTATGACTTAATTATTCCATCATTAA
	A. bracteata	16000–16135	16186–16242			
	A. kalkora	15867–16002	16052–16108			
4	*A. odoratissima*	16903–17138	17203–17260	*atp*I-*rps*2	GAACAGCTATAATGGCTGAAACTAATAAAATAGCAATTACAACCCAAGAAGTAA TAAGTACTTGGGCATGGACTTGGAAACCCCCTATTTGCCAATAGAAATGTTGG CCTACTTCCACAGCCGATATATCGTATAATCTTTTTAATGTGTTGATGGAACATAAT AGAACATTCATATTGCCCTCTGAAAGAAATACAACTTTAAAAGGAATTATTTTGA TTCAACCATCTCTTTTT	GAACAGCTATAATGGCTGAAACTAATAAAATAGCAATTACAACCC AAGAAGTAATAAGTACTTGGGCATGGACTTGGAAACCCCCTATTT GCCAATAGAAATGTTGGCCTACTTCCACAGCCGATATATCGTATAATC TTTTTAATGTGTTGATGGAACATAATAGAACATTCATATTGCCCTCT GAAAGAAATACAACTTTAAAAGGAATTATTTTGATTCAACCATCTCTTTTT
	A. bracteata	18162–18397	18470–18527			
	A. kalkora	17979–18214	18287–18344			
5	*A. odoratissima*	17203–17260	17312–17609	*atp*I-*rps*2 and *rps*2	TTTTTTGCGCGATTCAGTAATAGTATAGTAACCGATTCCATAAATCTATGGAGTTCCC	TTTTTTGCGCGATTCAGTAATAGTATAGTAACCGATTCCATAAATC TATGGAGTTCCC
	A. bracteata	18470–18527	18585–18882			
	A. kalkora	18287–18344	18396–18693			
6	*A. odoratissima*	18053–18078	18123–18225	*rps*2-*rpo*C2	GATCAGGTATCCTGAAATAGAAATAA	GATCAGGTATCCTGAAATAGAAATAA
	A. bracteata	19323–19348	19393–19490			
	A. kalkora	19135–19160	19205–19302			
7	*A. odoratissima*	29593–29618	29664–29713	*rpo*B-*trn*C-GCA	AATACTGATTAGTTATGTCTCAATTT	AATACTGATTAGTTATGTCTCAATTT
	A. bracteata	30855–30880	30926–30975			
	A. kalkora	30674–30699	30749–30798			
8	*A. odoratissima*	29664–29713	29782–29830	*rpo*B-*trn*C-GCA	ATTGAAGAATCATTCATGAATAGTTAATGGTTCCGATTTGTCCTGAATTT	ATTGAAGAATCATTCATGAATAGTTAATGGTTCCGATTTGTCCTGAATTT
	A. bracteata	30926–30975	31042–31090			
	A. kalkora	30749–30798	30866–30914			
9	*A. odoratissima*	52029–52062	52137–52211	intron of *trn*L-UAA	ATCGAAACTCCAGAAAAGAAAGGATCAAGGATGA	ATCGAAACTCCAGAAAAGAAAGGATCAAGGATGA
	A. bracteata	52899–52932	53003–53077			
	A. kalkora	52790–52823	52898–52972			
10	*A. odoratissima*	5103–5133	5205–5326	*mat*K-*rps*16	AAAAGAAATAAAGAAAGAAAAGAAGTTCTTT	AAAAGAAATAAAGAAAGAAAAGAAGTTCTTT
	A. bracteata	5135–5165	5242–5363			
	A. kalkora	5137–5167	5234–5355			

Note: The regions were sorted by the K-2p distance of the DNA barcodes between each pair of regions.

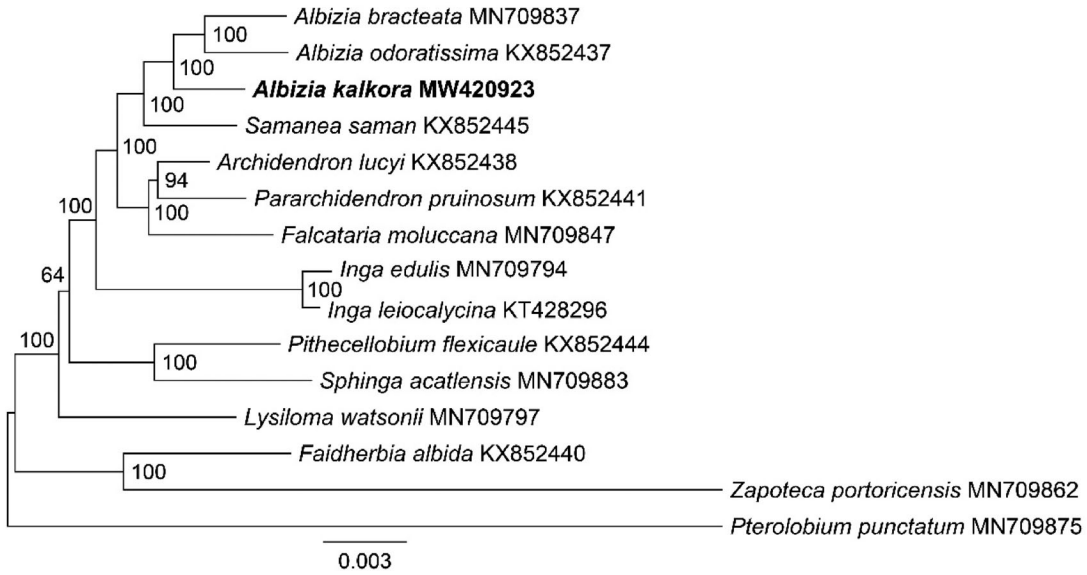

Figure 3. The phylogenetic tree of 14 Ingeae species and the outgroup of *Pterolobium punctatum*. The tree was constructed with sequences of 77 shared CDS sequences present in all 15 species using the maximum likelihood method. The 15 species were *Albizia bracteate* (MN709837) (Zhang et al. 2020), *A. odoratissima* (KX852437) (Huan et al. 2017), *A. kalkora* (MW420923, new plastome in this study), *Samanea saman* (KX852445) (Huan et al. 2017), *Archidendron lucyi* (KX852438) (Huan et al. 2017), *Pararchidendron pruinosum* (KX852441) (Huan et al. 2017), *Falcataria moluccana* (MN709847) (Zhang et al. 2020), *Inga edulis* (MN709794) (Zhang et al. 2020), *Inga leiocalycina* (KT428296) (Dugas et al. 2015), *Pithecellobium flexicaule* (KX852444) (Huan et al. 2017), *Sphinga acatlensis* (MN709883) (Zhang et al. 2020), *Lysiloma watsonii* (MN709797) (Zhang et al. 2020), *Faidherbia albida* (KX852440) (Huan et al. 2017), *Zapoteca portoricensis* (MN709862) (Zhang et al. 2020) and *Pterolobium punctatum* (MN709875, outgroup) (Zhang et al. 2020). The scale of the scale bar represents a substitution frequency of 0.003 for each site base of the shared CDS sequences. Bootstrap supports were calculated from 1000 replicates. The *A. kalkora* was labeled by bold font in the phylogenetic tree.

Discussion and conclusion

The present study provided useful information for species identification, genetic diverse analysis, and phylogenetic analysis of *A. kalkora*. However, several limitations still exist in the study. For instance, the distinguishing ability of the DNA barcodes has not been validated extensively because only three complete *Albizia* plastomes are available at this time. As mentioned earlier, *A. kalkora* is similar to *A. julibrissin*, the two species could be distinguished when the plastome of *A. julibrissin* is reported. In the future, we will plan to collect more *Albizia* samples for validating the reliability of the developed DNA barcodes and deeply study the phylogenetics of *Albizia* genus.

In this study, the complete plastome of *A. kalkora* was sequenced and assembled for the first time. The structure of the plastome was typical tetrad structure. Its length was 176,158 bp and contained 109 unique genes. Ten regions from highly varied intergenic regions could be used to design primers to provide potential DNA barcodes for discriminating *Albizia* species. Finally, the phylogenetic analysis indicated that *Albizia* species were clustered together, which were most closely related to *Samanea* species. The results of this study will provide valuable information to elucidate the classification, identification, and evolutionary history of *Albizia*.

Ethical statement

All samples in this study were collected with the permission from the Central China Medicinal Botanical Garden, EnShi, China. This study complies with relevant institutional, national, and international guidelines and legislation.

Authors' contributions

The manuscript includes contributions of all authors. CL conceived the study; MJ and LQW collected the sample of *Albizia kalkora*; HMC and LQW extracted DNA for next-generation sequencing; SHS and SYL assembled the genome, drafted the manuscript, and performed literature research; BW and QD performed the statistics of the gene content and analyzed the genome structure; HYY and YL annotated the plastome, constructed the phylogenetic tree and revised the manuscript. All authors have read and agreed on the contents of the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors. Dr. CL is an author of this manuscript and also serves as the Editor-in-Chief of Mitochondrial DNA Part B journal. Dr. CL declares no conflicts of interest regarding the research findings in this study.

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Data availability Statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession number MW420923. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA704524, SRR13775224, and SAMN18043475, respectively.

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