

The complete chloroplast genome sequence of *Zanthoxylum ailanthoides* Sieb. et. Zucc (Rutaceae): an important medicinal plant

Yan-Ni Liang^a, Nan Cui^b, Xiao-Bin Liang^c, Xi-Yang Huang^b, Wei Zhang^a and Hong Li^b

^aModern Industry College of Liupao Tea, Wuzhou University, Wuzhou, China; ^bGuangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin, China; ^cGuangxi Zhongheng Chinese Medicinal Crops Industry Development Co., Ltd., Wuzhou, China

ABSTRACT

Zanthoxylum ailanthoides is a deciduous tree, with important medicinal and economic values. The complete chloroplast genome sequence of *Z. ailanthoides* was assembled and the phylogenetic relationship to other species was inferred in this study. The chloroplast genome is 157,209 bp in length, including two inverted repeats of 26,408 bp, a large single-copy of 86,099 bp and a small single copy of 18,294 bp. Moreover, the chloroplast genome contains 129 genes, including 84 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. The overall GC content of the chloroplast genome is 38.4%. The phylogenetic analysis indicated that *Z. ailanthoides* was grouped with a clade containing the species of *Z. multijugum*, *Z. calcicola*, *Z. oxyphyllum*, *Z. stenophyllum*, and the genus was closely related to *Phellodendron*. This study contributes to a better understanding of the phylogenetic relationships among *Zanthoxylum* species.

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Introduction

Zanthoxylum ailanthoides Sieb. et. Zucc 1846 in Rutaceae is distributed mainly in China, Korea Japan, and the Philippines (Wu et al. 2011). The root and stem barks of *Z. ailanthoides* are used as important medicinal materials. Various chemicals such as benzo, phenanthridines, coumarins, lignans, flavonoids, quinolines, benzenoids, and triterpenoids are contained in these plants (Cheng et al. 2003; Chen et al. 2009; Ching et al. 2013). *Z. ailanthoides* has been widely used for the treatment of rheumatic bone pain, viral infection, cold, etc (State Administration of Medicine and Drug of PRC (Compilation Committee of Zhonghua Bencao) 1999). Previous studies have focused on its planting and chemical composition extraction, but the complete chloroplast genome of *Z. ailanthoides* has not been reported yet. In this study, we assembled the complete chloroplast genome of *Z. ailanthoides* and inferred phylogenetic relationships of this species to other species in the genus *Zanthoxylum* and the genus among other close relatives. The study may have important implications for understanding the phylogeny, population, and biodiversity of this species.



Materials and methods


The samples of *Z. ailanthoides* (Figure 1) were collected from a transplanted individual in Guilin Botanical Garden

(25°07'04" N, 110°29'91" E). The voucher specimen was deposited at the Herbarium of Guangxi Institute of Botany (<http://www.gxib.cn/splBK/>, contact person name: Chun-Rui Lin, Email: chunruilin@tom.com; identifier: HongLi, Email: hongligxib@126.com), with the voucher number IBK00439685. Total DNA from the silica-dried leaves was extracted using the CTAB method (Doyle 1987). Approximately, 15 µg of gDNA was isolated and delivered to Beijing Genomics



Figure 1. A branch of *Zanthoxylum ailanthoides* shows the morphology of leaves and unopened flowers. Photographed by the first author.

CONTACT Hong Li  hongligxib@126.com  Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China

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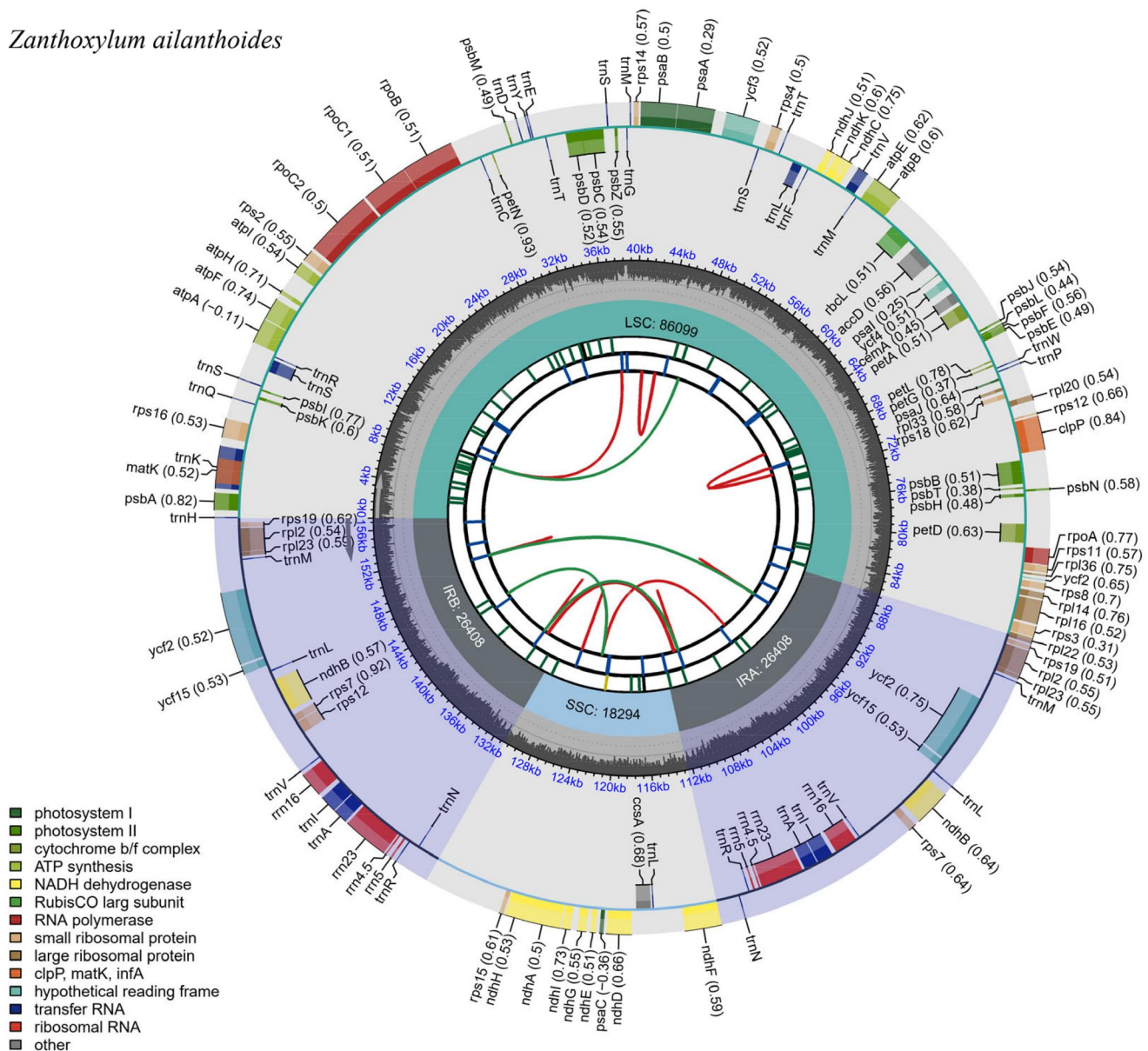
Zanthoxylum ailanthoides

Figure 2. Chloroplast reference genome of *Zanthoxylum ailanthoides*. The map contains six tracks by 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 small single-copy (SSC), inverted repeat (IRa and IRb), and large single-copy (LSC) regions are shown on the fourth track. The GC content along the genome is plotted on the fifth track. The base frequency at each site along the genome will be shown between the fourth and fifth tracks. 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.

Institute (BGI) for genomic sequencing by using the BGI-500 platform (Shenzhen, China). After quality filtering using FastQC (Wingett and Andrews 2018), we obtained 16,231,070 clean reads. The chloroplast genome of *Z. ailanthoides* was assembled with SPAdes v3.13.0 (Bankevich et al. 2012) and annotated using GeSeq online program (Tillich et al. 2017). A circular map of the chloroplast genome and a schematic map of the cis- and trans-splicing genes were generated by CPGView (<http://www.1kmpg.cn/cpgview/>). The annotated chloroplast genome sequence was registered in GenBank with an accession number (NC061688).

To select highly variable regions, six chloroplast genome sequences of *Zanthoxylum* species were compared using DnaSP 5.10 (Rozas et al. 2017) and mVISTA (Frazer et al.

2004). In the DnaSP software, nucleotide diversity was calculated using sliding windows with a window length of 600 bp and a step size of 200 bp to evaluate the divergence of the six *Zanthoxylum* chloroplast genome sequences. The mVISTA in the Shuffle-LAGAN mode was used to compare the chloroplast genomes of *Z. ailanthoides* with the other five chloroplast genomes using the annotation of *Z. acanthopodium* as the reference.

To investigate the phylogenetic position of *Z. ailanthoides*, 26 complete chloroplast genome sequences from Rutaceae and one outgroup species (*Casimiroa edulis*) were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov>). The sequences were extracted and aligned by MAFFT (Katoh and Standley 2013). The resulting alignment was subjected to ML

(maximum likelihood) analyses performed using IQ-TREE (<http://iqtree.cibiv.univie.ac.at/>) with the best-predicted model TVM + F + I + G4 and 1000 bootstrap replicates.

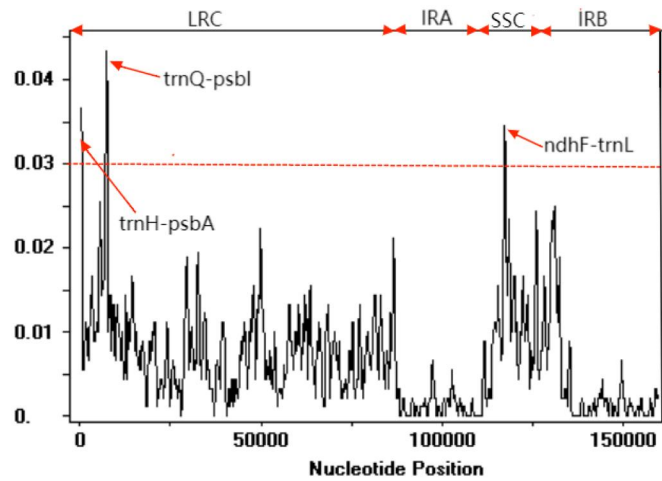


Figure 3. Results of comparative chloroplast genomic analyses for six cp genome sequences of the *Zanthoxylum* species.

Results

The average read mapping depth of the assembled genome is $199\times$ (Supplementary Figure S1). The complete chloroplast genome of *Z. ailanthoides* is 157,209 bp in length (Figure 2). It has a typical quadripartite structure with a large single-copy region (LSC; 86,099 bp), a small single-copy region (SSC; 18,294 bp), and two inverted repeats (IRA and IRB; 26,408 bp each). The GC content of the entire chloroplast genome is 38.4%. The circular chloroplast genome contains 129 genes, including 84 protein-coding, 37 tRNA and 8 rRNA genes. Among these genes, six tRNA genes (*trnK-UUU*, *trnG-UCC*, *trnL-UAA*, *trnV-UAC*, *trnI-GAU*, and *trnA-UGC*) and nine protein-coding genes (*rps16(2)*, *atpF*, *rpoC1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB(2)*, and *ndhA*) contain one intron, and two genes (*clpP*, *ycf3*) each have two introns. Totally 12 cis-splicing genes, including *rps16*, *atpF*, *rpoC1*, *ycf3*, *clpP*, *petD*, *rpl16*, *rpl2*, *ycf15*, *ndhB*, *ndhA*, and *ycf15* (Supplementary Figure S2), and one trans-splicing genes *rps12* (Supplementary Figure S3) were detected by CPGView (<http://www.1kmpg.cn/cpgview/>).

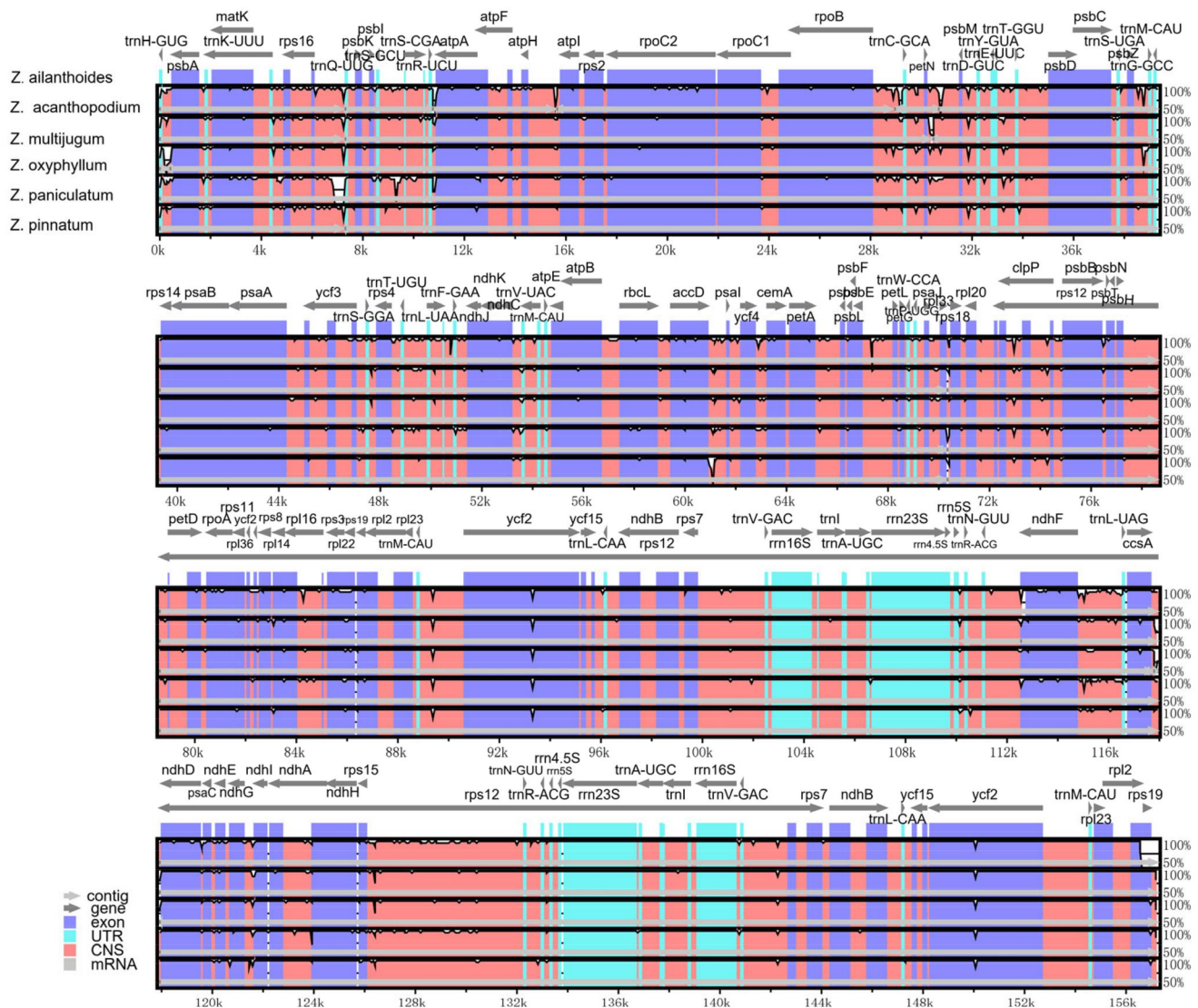


Figure 4. Sliding window analyses of the entire cp genome. Gray arrows indicate genes and their orientation. The similarity among cp genomes was shown on a vertical scale ranging from 50%–100%.

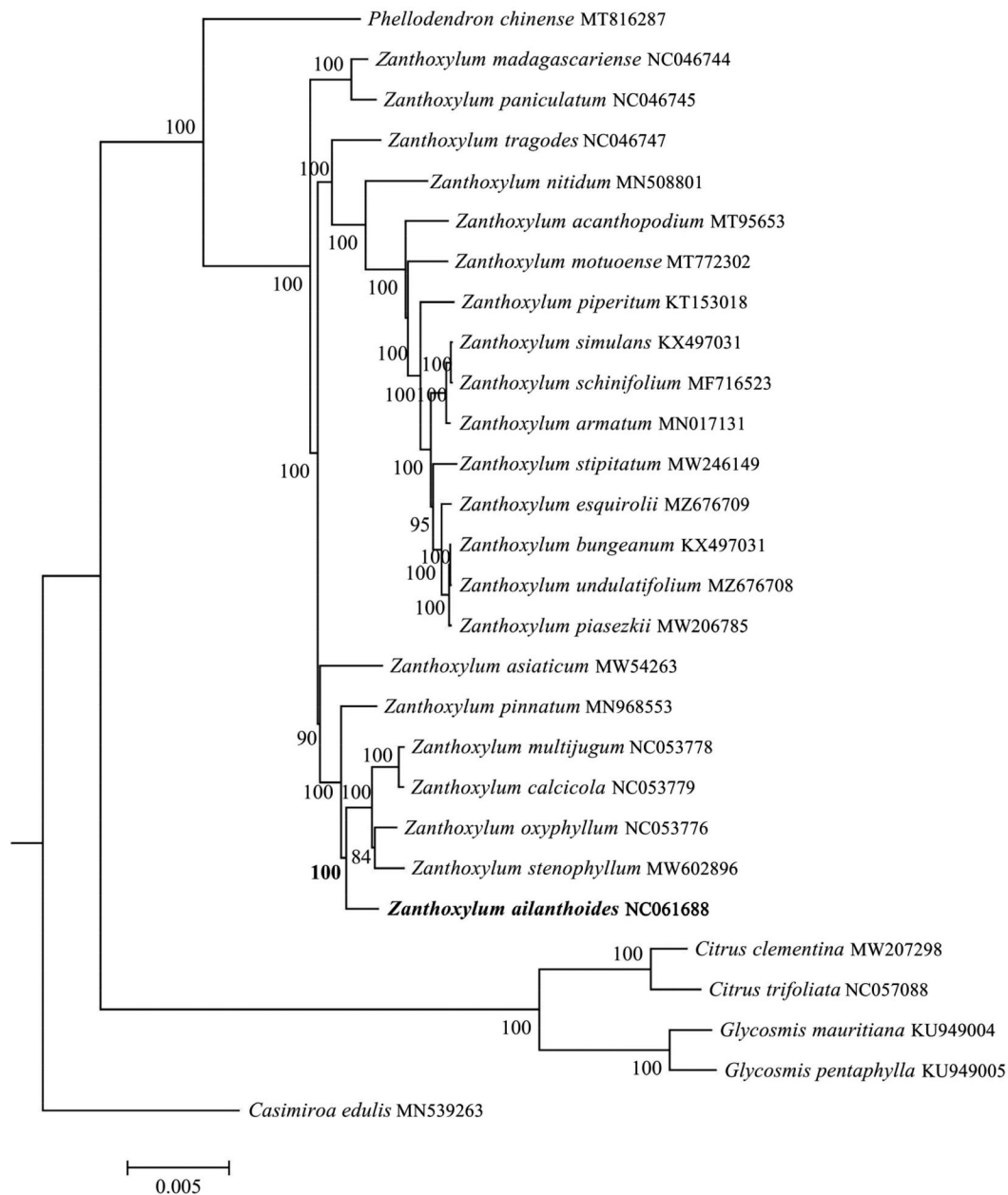


Figure 5. A maximum-likelihood phylogenetic tree based on the complete chloroplast genome sequences (with 1000 bootstrap replications). *Casimiroa edulis* was set as the outgroup. Numbers on the nodes represent bootstrap values. The following sequences were used: *Zanthoxylum ailanthoides* NC061688 (in this study), *Phellodendron chinense* MT816287 (Yang et al. 2020), *Zanthoxylum madagascariense* NC046744 (unpublished), *Zanthoxylum paniculatum* NC046745 (unpublished), *Zanthoxylum tragodes* NC046747 (unpublished), *Zanthoxylum nitidum* MN508801 (Shi et al. 2019), *Zanthoxylum acanthopodium* MT95653 (Li et al. 2020), *Zanthoxylum motuoense* MT772302 (Li et al. 2022), *Zanthoxylum piperitum* KT153018 (unpublished), *Zanthoxylum simulans* KX497031 (Hou et al. 2018), *Zanthoxylum schinifolium* MF716523 (unpublished), *Zanthoxylum armatum* MN017131 (Wang et al. 2019), *Zanthoxylum stipitatum* MW246149 (unpublished), *Zanthoxylum esquirolii* MZ676709 (Liu et al. 2022), *Zanthoxylum bungeanum* KX497031 (unpublished), *Zanthoxylum undulatifolium* MZ676708 (Sun et al. 2022), *Zanthoxylum piasezkii* MW206785 (Sun et al. 2021), *Zanthoxylum asiaticum* MW54263 (unpublished), *Zanthoxylum pinnatum* MN968553 (unpublished), *Zanthoxylum multijugum* NC053778 (unpublished), *Zanthoxylum calcicola* NC053779 (unpublished), *Zanthoxylum oxyphyllum* NC053776 (unpublished), *Zanthoxylum stenophyllum* MW602896 (Huang et al. 2022), *Citrus clementina* MW207298 (Sun and Lin 2021), *Citrus trifoliata* NC057088 (unpublished), *Glycosmis mauritiana* KU949004 (unpublished), *Glycosmis pentaphylla* KU949005 (unpublished), *Casimiroa edulis* MN539263 (Yang et al. 2019).

To compare the differences in *Zanthoxylum* chloroplast genomes, we used DnaSP and mVISTA to detect sequence variations. The PI (Nucleotide diversity) value of nucleotide diversity ranged from 0 to 0.0433 (Figure 3). In the present study, the chloroplast genomes of six *Zanthoxylum* species were aligned and compared to screen single-nucleotide polymorphisms with higher nucleotide diversity. As a result, we found that *trnH-psbA* (PI = 0.0367), *trnQ-psbI* (PI = 0.0433), and *ndhF-trnL* (PI = 0.0311) were highly divergent regions.

After visual analyses of these six chloroplast genome sequences by mVISTA using *Z. acanthopodium* as a reference sequence, the results were fairly consistent with those found obtained from DnaSP (Figure 4).

The ML phylogenetic tree showed that *Z. ailanthoides* was grouped with a clade containing *Z. multijugum*, *Z. calcicola*, *Z. oxyphyllum*, and *Z. stenophyllum* in the genus, and the genus was most closely related to *Phellodendron* with 100% bootstrap support (Figure 5). The complete chloroplast

genome of *Z. ailanthoides* contributes to a better understanding of the phylogenetic relationships among *Zanthoxylum* species. And it provides basic data for the utilization of this important medicinal plant.

Discussion

The study reports the chloroplast genome of *Z. ailanthoides* for the first time, it's quite similar to other *Zanthoxylum* species in terms of the overall organization, GC content, and gene/intron arrangement (Hou et al. 2018; Shi et al. 2019). Chloroplast genomes have been demonstrated the potential to be one important source of genetic markers for evolutionary and species identification (Lee et al. 2019). Our research results could be used for authenticating *Z. ailanthoides* and analyzing the genetic diversity and phylogenetic relationships in *Zanthoxylum*.

Author contributions

Hong Li and Xi-Yang Huang designed the study. Yan-Ni Liang and Nan Cui carried out the data analyses and interpretation of the data. Yan-Ni Liang wrote the manuscript and photo-taking. Xiao-Bin Liang and Wei Zhang revised the manuscript and contributed reagents/materials/analysis tools. All authors agree to publish this work.

Ethics statement

This article does not contain any studies with animals performed by any of the authors. Permission was granted by the Guangxi Zhuang Autonomous Region and the Chinese Academy of Sciences research the species and there was no endangered or protected species were involved in this study.

Disclosure statement

No potential conflict of interest was declared by the authors.

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Data availability statement

The genome sequence data that supports the findings of this study are openly available in GenBank at (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession No. NC061688. The associated BioProject, SRA, and BioSample numbers are PRJNA780794, SRR17001278, and SAMN23175903, respectively.

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