

Complete chloroplast genome sequences of the medicinal plant *Piper hancei*

Lvshui Zhang^a, Xiaokang Hu^b and Mu Liu^a

^aCollege of Landscape Architecture and Arts, Jiangxi Agricultural University, Nanchang, China; ^bGannan Arboretum, Ganzhou, China

ABSTRACT

Chloroplast genome sequences have been used in phylogenetic and population genetics studies. Here, we assembled the chloroplast genome of *Piper hancei* Maxim. that is a traditional Chinese medicine. The genome length was 161,476 bp and included a pair of inverted repeats of 27,058 bp, a large single-copy region of 89,144 bp and a small single-copy region of 18,216 bp. It contained 113 different genes, including 79 protein-coding genes, 30 transfer RNA (tRNA), and four ribosomal RNA genes. Moreover, we also identified 82 SSRs. The phylogenetic inference based on the whole chloroplast genome of 20 taxa showed *P. hancei* was sister to *P. kadsura*.

ARTICLE HISTORY

Received 29 April 2021
Accepted 30 July 2021

KEYWORDS

Chloroplast genome; *Piper hancei*; molecular markers; SSR; phylogeny

Piper hancei Maxim. is used as a traditional Chinese medicine within the family Piperaceae and is natively distributed in Fujian, Guangdong, Guangxi, Guizhou, Hunan, Yunnan, and Zhejiang provinces (Li and Hah). It is mainly used as a medicine to relieve pain, dispel wind and swelling, and treat rheumatic arthritis, weakness of the back and knees, and cough and asthma (Li and Hah). *Piper hancei* is also used as gardening plant in tropical and subtropical regions of China. At present, there was less genetic information about this species, which brings difficulties to cultivation and utilization. In this study, we sequenced and analyzed the chloroplast genome of *P. hancei* based on the next-generation sequencing method (Dong, Sun, et al. 2021; Dong, Xu, et al. 2021; Wang et al. 2021). The objective of this study was to describe the chloroplast genome structure and feature for this species.

Fresh and young leaves of *P. hancei* was collected from Gannan Arboretum, Jiangxi, China (25°51'10"~114°22'25"), which was introduced from Guangdong. A specimen and DNA were deposited at the herbarium of Jiangxi Agricultural University under the voucher number of LM750014. Total genomic DNA was isolated using a mCTAB protocol (Li et al. 2013) for constructing a 350 bp insert library and sequencing on an Illumina HiSeq X ten platform. Raw data were qualified using Trimmomatic (Bolger et al. 2014) and the chloroplast genome was assembled with GetOrganelle (Jin et al. 2020) using clean data. The complete chloroplast genome was annotated with Plann using *P. auritum* (GenBank accession number: KY085906) as reference (Huang and Cronk 2015). The annotated chloroplast genome of *P. hancei* has been deposited into GenBank with the accession number of MZ046380.

The chloroplast genome of *P. hancei* was a circular DNA molecule of 161,476 bp and had a typical quadripartite structure with a pair of inverted repeats of 27,058 bp, a large

single-copy region of 89,144 bp and a small single-copy region of 18,216 bp. The GC content was 38.3%, which was similar to other Piperaceae species (Cai et al. 2006). The *P. hancei* chloroplast genome encoded 113 different genes, including 79 protein-coding genes, 30 transfer RNA (tRNA), and 4 ribosomal RNA genes. Eighteen genes contain introns, 16 of which contain one intron and two (*clpP* and *ycf3*) with two introns.

Simple sequence repeats in the *P. hancei* chloroplast genomes were detected using GMAT (Wang and Wang 2016) with the minimum repeats of mono-, di-, tri-, tetra-, penta- and hexa-nucleotides being set to 10, 5, 4, 3, 3, and 3, respectively. A total of 82 perfect chloroplast genome SSR were identified. The number of mono-, di-, tri-, tetra-, penta- and hexa-nucleotides were 49, 13, 8, 7, 4 and one, respectively.

To estimate the phylogenetic relationships of *P. hancei* with other *Piper* species. Phylogenetic analysis was performed using the whole chloroplast genome sequence from 20 Piperales species. Whole chloroplast genome sequences were aligned with MAFFT v7 (Kato and Standley 2013) and ambiguous alignment regions were trimmed by trimAl v1.2 (Capella-Gutiérrez et al. 2009). Maximum Likelihood (ML) tree was performed using RAXML-NG (Kozlov et al. 2019). The GTR + G was chosen as the best-fit DNA substitution model according to the Akaike Information Criterion correction in ModelFinder (Kalyaanamoorthy et al. 2017). The reconstructed phylogeny revealed that *P. hancei* was sister to *P. kadsura* (Figure 1).

Disclosure statement

No potential conflict of interest was reported by the author(s).

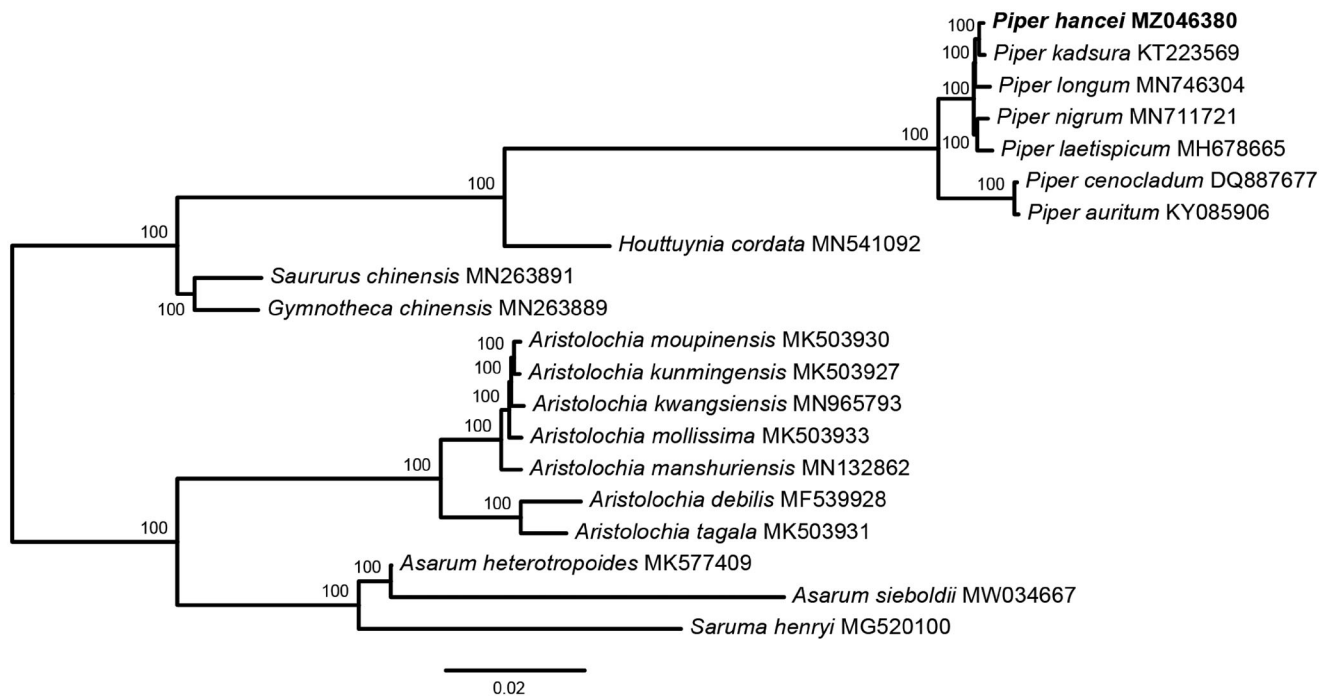


Figure 1. Maximum likelihood tree of Piperales based on the complete chloroplast genome sequences. ML bootstrap support value presented at each node.

Funding

This work was supported by Social Science Planning Project of Jiangxi Province [18YS07], Social Science Planning Project of Nanchang City [WH202003], Research on Evaluation of Ecological Sensitivity of Key Ecological Resources in Nanchang City Based on GIS [GJJ190234] and Study on Forest Landscape Regulation and Control of Poyang Lake Basin Based on Ecosystem Services.

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 no. MZ046380. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA725313, SRR14328341, and SAMN18875907, respectively.

References

- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30(15):2114–2120.
- Cai Z, Penaflor C, Kuehl JV, Leebens-Mack J, Carlson JE, dePamphilis CW, Boore JL, Jansen RK. 2006. Complete plastid genome sequences of *Drimys*, *Liriodendron*, and *Piper*: implications for the phylogenetic relationships of magnoliids. *BMC Evol Biol*. 6:77.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*. 25(15):1972–1973.
- Dong W, Sun J, Liu Y, Xu C, Wang Y, Suo Z, Zhou S, Zhang Z, Wen J. 2021. Phylogenomic relationships and species identification of the olive genus *Olea* (Oleaceae). *J System Evol*.doi: 10.1111/jse.12802
- Dong W, Xu C, Liu Y, Shi J, Li W, Suo Z. 2021. Chloroplast phylogenomics and divergence times of *Lagerstroemia* (Lythraceae). *BMC Genom*. 22(1):434.
- Huang DI, Cronk QCB. 2015. Plann: A command-line application for annotating plastome sequences. *Appl Plant Sci*. 3(8):1500026.
- Jin J-J, Yu W-B, Yang J-B, Song Y, dePamphilis CW, Yi T-S, Li D-Z. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol*. 21(1):241.doi:10.1186/s13059-020-02154-5. PMC: 32912315
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods*. 14(6):587–589.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30(4):772–780.
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*. 35(21):4453–4455.
- Li J, Wang S, Jing Y, Wang L, Zhou S. 2013. A modified CTAB protocol for plant DNA extraction. *Chinese Bullet Bot*. 48:72–78.
- Li S-M, Hah G-Q. 1987. Studies on the chemical constituents of *Piper hancei* Maxim III. *J Integr Plant Biol*. 29(3):293–296.
- Wang X, Wang L. 2016. GMATA: an integrated software package for genome-scale SSR mining, marker development and viewing. *Front Plant Sci*. 7:1350.
- Wang M, Wang X, Sun J, Wang Y, Ge Y, Dong W, Yuan Q, Huang L. 2021. Phylogenomic and evolutionary dynamics of inverted repeats across *Angelica* plastomes. *BMC Plant Biol*. 21(1):26.