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Complete chloroplast genome sequence of *Pithecellobium clypearia* (Jack) Benth.

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ABSTRACT

Pithecellobium clypearia (Jack) Benth. 1844 belongs to the genus *Pithecellobium* in the family Fabaceae. The complete chloroplast genome of *P. clypearia* was sequenced and analyzed by Illumina sequencing in this study. The full length of the complete chloroplast genome is 176,770 bp, containing a pair of inverted repeat regions of 39,693 bp (IRa and IRb) separated by a large single-copy (LSC) region of 92,500 bp and a small single-copy (SSC) region of 4,884 bp. The *P. clypearia* chloroplast genome encodes 137 genes, comprising 92 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Phylogenetic analysis based on complete chloroplast genomes revealed that *P. clypearia* is closely related to *Archidendron lucyi* and *Pithecellobium flexicaule*. This study provides useful resources for further study and development of this species.

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Pithecellobium clypearia (Jack) Benth. 1844 (homotypic synonym: *Archidendron clypearia* (Jack) I.C.Nielsen), is a species of the genus *Pithecellobium* in the family Fabaceae. It is widely distributed in southern China, such as Fujian, Guangdong, Guangxi, and Hainan provinces (Wu and Raven 2010). *P. clypearia* contains various compounds, including flavonoids, lignans, polyphenols, terpenoids, and steroids (Kang et al. 2014; Wang et al. 2020). It has been used to treat upper respiratory tract infections and gastroenteritis in traditional Chinese medicine (Thao et al. 2016).

The taxonomic status and phylogenetics position of *P. clypearia* remain controversial, some botanists thought it belongs to the genus *Pithecellobium* (Hooker 1844), while others thought it belongs to the genus *Archidendron* (Wu and Raven 2010). In recent years, comparative analysis of the complete chloroplast genome sequences has been used as an effective tool for studying plant phylogeny and origin (Liu et al. 2019; Chen et al. 2021). Here, in order to decipher the taxonomic status and phylogeny of *P. clypearia*, we analyzed its complete chloroplast genome and presented a phylogenetic analysis.

P. clypearia used for chloroplast genome sequencing was cultivated in the Biological Garden of Zhaoqing University (N23°6′, E112°30′, Zhaoqing, China). The voucher specimen (no. BGCLSZU003) was deposited in the Herbarium of Zhaoqing University (https://www.zqu.edu.cn/, Yinghua Wang, wangyinghua@zqu.edu.cn). The total genomic DNA was extracted from fresh leaves of *P. clypearia* using the Plant Genomic DNA Kit (Tiangen, Beijing, China). Then, it was sequenced using the Illumina NovaSeq platform (Illumina, San Diego, CA). The raw data were mapped to plastome of *Inga leiocalycina* (GenBank accession number: KT428296.1), using Bowtie2 v2.2.4 (Langmead and Salzberg 2012) to exclude reads of nuclear and mitochondrial origins.

The chloroplast genome of *P. clypearia* was reconstructed with SPAdes 3.10.1 through the *de novo* assembly method (Bankevich et al. 2012), and *de novo* assembled chloroplast contigs were concatenated into larger contigs using Sequencher 5.3.2 (Gene Codes Inc., Ann Arbor, MI). Annotation of the chloroplast genome was generated by CpGAVAS (Liu et al. 2012) and a circular representation was drawn using the online tool OGDRAW (Lohse et al. 2007). The complete plastome sequence has been submitted to GenBank with the accession number of MW309810. The raw data have been uploaded to the NCBI Sequence Read Archive (SRA) database under the accession number SRR15400572.

The length of chloroplast genome sequence of *P. clypearia* is 176,770 bp, including two inverted repeat regions (IRa and IRb, each 39,693 bp) separated by a large single copy LSC (92,500 bp) region and a small single copy SSC (4,884 bp) region. The GC content of the overall chloroplast genome, IR regions, LSC, and SSC are 35.20%, 38.55%, 32.63%, and 29.30%, respectively. The chloroplast genome has 137 genes in total, including 92 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Among these identified genes, 44 genes are involved in photosynthesis, and 58 genes are involved in self replication.

To analyze the phylogenetic position of *P. clypearia*, its plastome and the complete chloroplast genomes of 15 species from Fabaceae and its sister families published on NCBI GenBank were aligned using MAFFT v7.427 (Katoh et al. 2005). The gaps in the alignment were removed using the program trimAl with '-nogaps' v 1.4 (Capella-Gutierrez et al.

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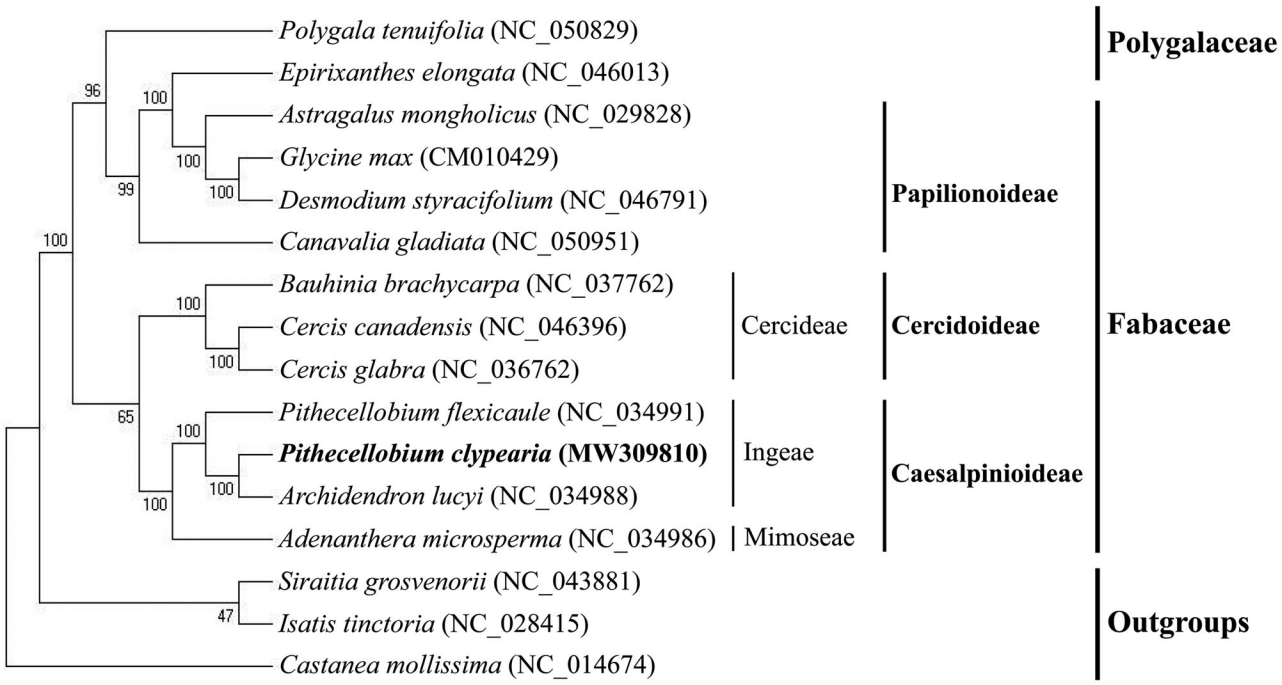

Figure 1. The maximum-likelihood (ML) tree reconstruction from whole chloroplast genomes of 16 plant species. Bootstrap support values are indicated at each node. *Siraitia grosvenorii*, *Isatis tinctoria*, and *Castanea mollissima* were used as outgroups.

2009). A maximum-likelihood (ML; 1000 bootstrap replicates) phylogenetic tree was constructed using MEGA v7.0 (Kumar et al. 2016), following the Kimura 2-parameter model of nucleotide substitution model. *Siraitia grosvenorii*, *Isatis tinctoria*, and *Castanea mollissima* were set as outgroups. The results showed that *P. clypearia* is closely related to *Pithecellobium flexicaule* and *Archidendron lucyi* in the tribe Ingeae (Figure 1). The Leguminosae is the third largest angiosperm family in terms of species numbers, with close to 770 genera and over 19,500 species (LPWG 2013). In 2017, the Legume Phylogeny Working Group (LPWG) constructed a new phylogenetic tree of the family Leguminosae using an extensive sampling of plastomes. Based on the phylogenetic tree and taxonomic characters, LPWG proposed a new six-subfamily classification system (LPWG 2017). Wang et al. reconstructed a phylogenetic framework with strong statistical support, it was useful for future studies on legume classification, evolution, and diversification (Wang et al. 2017; Zhang et al. 2020). However, relationship between genera in the family Fabaceae has been difficult to resolve. This study showed *P. clypearia* is more closely related to *A. lucyi* than to *P. flexicaule*. Therefore, we suggest that *P. clypearia* belongs to the genus *Archidendron*.

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Ethics statement: Ethical approval for the study was obtained from the Ethical Committee of Zhaoqing University.

Authors contributions

YW performed the experiments investigation, project administration, writing the original draft and data curation. GC prepared the resources, supervised the project, and made revisions to the manuscript.

Disclosure statement

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

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Data availability statement

The data that newly obtained at this study are available in the NCBI under accession number of MW309810 (https://www.ncbi.nlm.nih.gov/nuccore/MW309810). The associated BioProject, SRA, and Bio-Sample numbers are PRJNA753227, SRR15400572, and SAMN20691823, respectively.

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