MITOCHONDRIAL DNA PART B: RESOURCES
2025, VOL. 10, NO. 4, 325–329
https://doi.org/10.1080/23802359.2025.2482254

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PLASTOME REPORT

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The complete chloroplast genomes of *Petrocodon mirus* and *Petrocodon hancei* (Gesneriaceae)

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ABSTRACT

The genus *Petrocodon* is uniquely distributed in karst areas and exhibits high floral morphological diversity. We assembled and characterized the complete chloroplast genomes of *Petrocodon mirus* X.Z.Shi, J.X.Fu & L.H.Yang 2024 and *Petrocodon hancei* (Hemsl. 1890) A.Weber & Mich.Möller 2011. The genome sizes are 153,547 bp and 153,294 bp, respectively. Phylogenetic analysis revealed that *P. hancei* is closely related to *P. multiflorus*, while the position of *P. mirus* remains unclear. These findings provide genomic resources for studying genetic diversity in *Petrocodon* and Gesneriaceae.

ARTICLE HISTORY
Received 17 December 2024
Accepted 14 March 2025

KEYWORDS
Chloroplast genome sequencing; Gesneriaceae; *Petrocodon hancei*; *Petrocodon mirus*; phylogenetics analysis

Introduction

The genus *Petrocodon*, belonging to the family Gesneriaceae, is primarily distributed in the karst regions of southern and southwestern China (Li et al. 2024). After years of revisions and the continuous discovery of new species, the genus has expanded to 55 species and four varieties (GRC 2024). Plants of this genus exhibit diverse flower morphologies and vibrant colors, with the flower corolla often featuring color halos, stripes, spots, or net patterns in various colors, making it an ideal group for horticultural and ornamental purposes (Lu et al. 2017; Huang et al. 2023).

Chloroplast (cp) genomes aid in studying phylogenetic relationships among plant groups and provide important insights into their ecological adaptability (Hu et al. 2015; Li et al. 2019). However, sequencing studies on the cp genomes of *Petrocodon* species are still scarce (Xin et al. 2019; Hsieh et al. 2022). To enrich the study of cp genomes in the *Petrocodon* genus, we have assembled and annotated the cp genomes of *P. mirus* and *P. hancei*. Notably, *P. mirus* (Shi et al. 2024) and *P. gracilis* (Ding et al. 2024) are the same species, with *P. mirus* taking precedence as the earliest validly published name under the International Code of Botanical Nomenclature (ICBN). This study provides a foundational genomic resource for future research in the *Petrocodon* genus and the Gesneriaceae family.

Materials and methods

Plants of *P. mirus* were collected from Shangying Town, Tiandeng County, Guangxi Zhuang Autonomous Region (23°4′5″N, 106°59′6″E) (Figure 1(A,B)); and specimens of *P. hancei* were obtained from Danxia Mountain, Shaoguan, Guangdong (25°1′21″N, 113°44′1.44″E) (Figure 1(C,D)). All voucher specimens (IBK00470320, IBK00470321) were deposited at the Herbarium of Guangxi Institute of Botany (IBK, http://www.gxib.cn/spIBK/, Chunrui Lin, E-mail: chunruilin@tom.com; Identifier: Pengwei Li, E-mail: li_pengweii@126.com).

Total DNAs were extracted from the silica-dried leaves of *P. mirus* and *P. hancei* using a modified CTAB method (Doyle and Doyle 1987). After constructing the sequencing library, paired-end sequencing was completed on the NovaSeq 6000 system (in Novogene Corp., Tianjin, China). Generally, 1.9 GB of high-quality clean data were obtained after filtering with Trimmomatic v0.39 (Bolger et al. 2014). *De novo* assembly was performed with GetOrganelle v1.7.7.1 (Jin et al. 2020) and the resulting scaffolds were visualized using Bandage v0.7.1 (Wick et al. 2015). Clean reads were mapped to the two assembled genomes using BWA v0.7.18 (Jung and Han 2022) to determine the coverage depth of each base. The newly assembled cp genomes of *P. mirus* and *P. hancei* were annotated using CPGAVAS2 (Shi et al. 2019) using

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2025.2482254.

P. coriaceifolius (NC_065790.1) as the reference. Finally, the circular map of the cp genome was generated using the CPGView program (Liu et al. 2023).

Based on different construction methods, the phylogenetic tree of the genus *Petrocodon* was built using sequenced cp genome data, with representative species from closely related genera *Primulina* and *Oreocharis* (*P. pengii*, *P. cordata*, *O. cotinifolia*, *O. esquirolii*) used as outgroups to ensure the correctness of the root and the reliability of the results. Sequences were aligned with MAFFT v 7.471 (Katoh and Standley 2013) and manually adjusted in BioEdit 5.0.9 (Hall 1999). Subsequently, nucleotide substitution models were selected using ModelFinder (Kalyaanamoorthy et al. 2017) based on the Akaike information criterion (AIC). Maximum-likelihood (ML) and Bayesian inference (BI) analyses were performed to construct phylogenetic trees based on the complete cp genome data. Using the same optimal substitution model (GTR + F + I + G4), ML analyses were performed with RAxML-NG (Kozlov et al. 2019), while BI analyses were performed with MrBayes 3.2.7 (Ronquist et al. 2012).

Results

The cp genomes of *P. mirus* (PQ636880) and *P. hancei* (PQ636881) are 153,547 bp and 153,294 bp in length, with an average GC content of 37.42% and 37.48%, and mean depths of 402× and 1812×, respectively (Figure 2 and Figure S1). Each genome contains a large single-copy (LSC) region of 84,358 bp and 84,142 bp in the two species, and a small single-copy (SSC) region of 18,281 bp and 18,290 bp, respectively. These regions are separated by two inverted repeat (IR) regions, which are 25,454 bp and 25,431 bp long in these two species (Figure 2). The structure of the cis-splicing and trans-splicing genes is provided in Figures S2 and S3. Both genomes encode 130 genes, including 85 protein-encoding genes, eight ribosomal RNA genes, and 37 transporter RNA genes, with the order of the genes identical in both species. Among them, one intron is in nine genes (i.e. *atp*F, *ndh*A, *ndh*B, *pet*B, *pet*D, *rpl*16, *rpl*2, *rpo*C1, and *rps*16), while three genes (*clp*P, *rps*12, and *ycf*3) possess two introns, and only *rps12* exists trans-splicing (Figure S3). Additionally, 17 genes are completely duplicated in the IRs, including six protein-

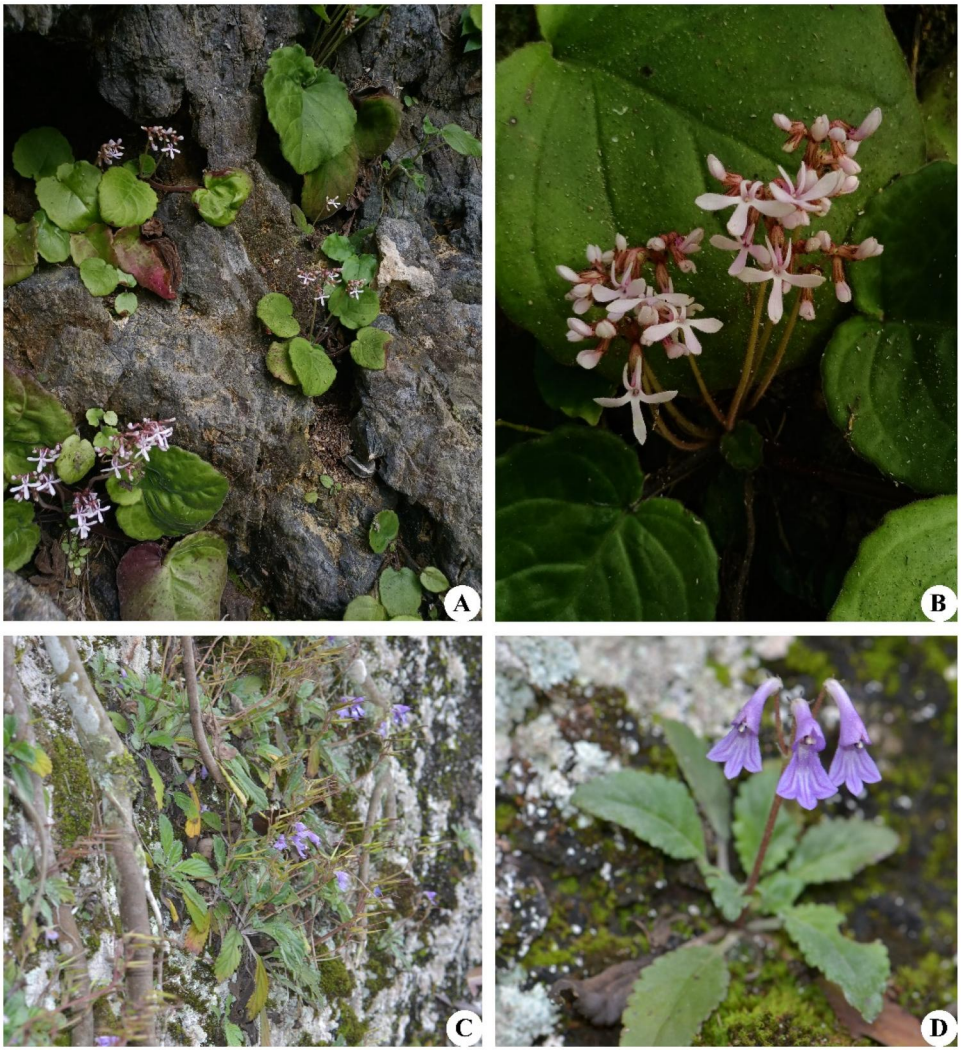

Figure 1. Photographs of *P. mirus* and *P. hancei*. (A) Karst habitat of *P. mirus* (Shangying Town, Tiandeng County, Guangxi Zhuang Autonomous Region). (B) Distinctive floral morphology of *P. mirus*. (C) Karst habitat of *P. hancei* (Danxia Mountain, Shaoguan, Guangdong province). (D) Unique floral structure of *P. hancei*. All photographs were taken by Yu-Chuan Qi.

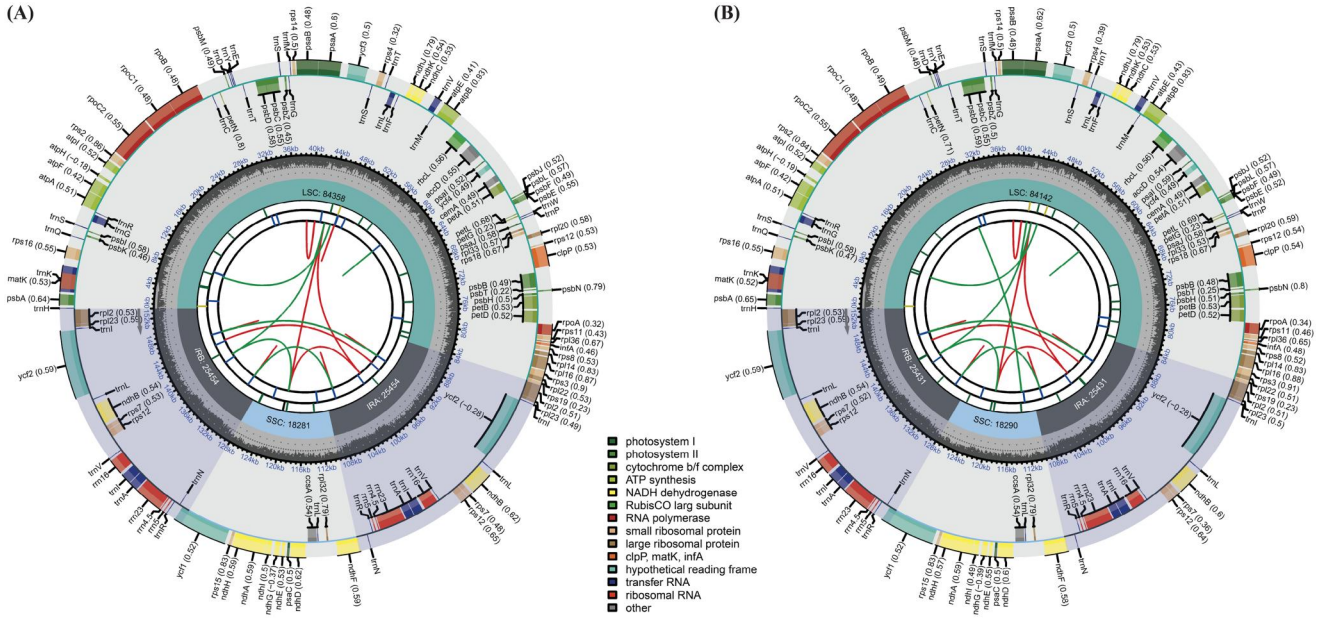

Figure 2. The schematic maps of the chloroplast genomes of *P. mirus* (A) and *P. hancei* (B). From the center outward, the first track shows the dispersed repeats. The second track displays the long tandem repeats. The third track represents the short tandem repeats or microsatellites. The fourth track illustrates the small single-copy (SSC) region, inverted repeat regions (IRA and IRB), and large single-copy (LSC) region. The GC content along the genome is plotted on the fifth track. Genes are presented on the sixth track, with optional codon usage bias indicated in parentheses following the gene name. Genes are color-coded based on their functional classification. The transcription directions of the inner and outer genes are clockwise and counterclockwise, respectively. The functional classification of the genes is shown in the middle.

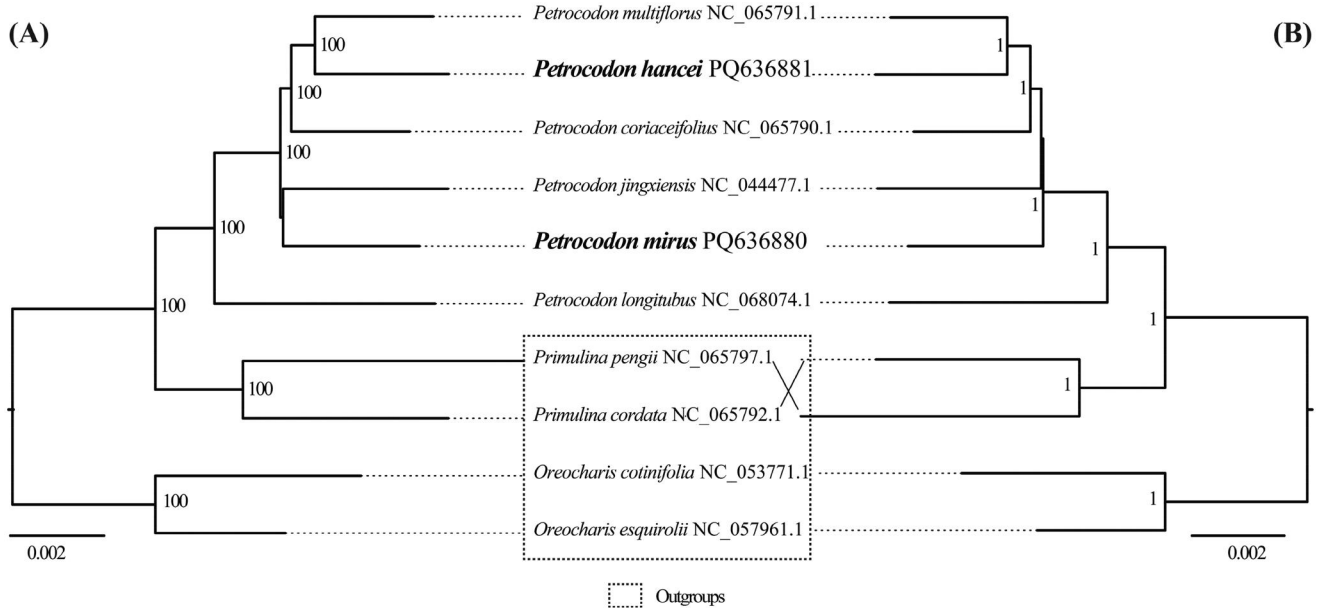

Figure 3. Maximum-likelihood (A) and Bayesian inference (B) trees based on 10 complete chloroplast genome sequences in Gesneriaceae. The bootstrap values (>50%) and the posterior probability values (>0.80) are shown around the corresponding nodes. The newly assembled species are highlighted in bold. The GenBank accession numbers and references of all species in the tree are *Petrocodon multiflorus* NC_065791.1 (Hsieh et al. 2022), *Petrocodon hancei* PQ636881 (this study), *Petrocodon coriaceifolius* NC_065790.1 (Hsieh et al. 2022), *Petrocodon jingxiensis* NC_044477.1 (Xin et al. 2019), *Petrocodon mirus* PQ636880 (this study), *Petrocodon longitubus* NC_068074.1 (Luo et al. 2024); outgroups: *Primulina pengii* NC_065797.1 (Hsieh et al. 2022), *Primulina cordata* NC_065792.1 (Hsieh et al. 2022), *Oreocharis cotinifolia* NC_053771.1 (Tang et al. 2021), and *Oreocharis esquirolii* NC_057961.1 (Gu et al. 2020).

coding genes (*rpl*2, *rpl*23, *ycf*2, *ndh*B, *rps*7, and *rps*12), four rRNA genes (*rrn*16, *rrn*23, *rrn*4.5, and *rrn*5), and seven tRNA genes (*trnI*-CAU, *trnL*-CAA, *trnV*-GAC, *trnI*-GAU, *trnA*-UGC, *trnR*-ACG, and *trnN*-GUU) (Figure 2).

To elucidate the phylogenetic positions of the newly assembled *P. mirus* and *P. hancei* genomes within the *Petrocodon* genus, ML and BI phylogenetic trees were constructed based on eight other complete cp genomes from the Gesneriaceae family. Both trees exhibit consistent topological structures. The six sequenced *Petrocodon* species form a monophyletic clade, with *P. hancei* closely related to *P. multiflorus* (Figure 3). In contrast, the systematic position of *P. mirus* remains unclear. In the ML tree, *P. mirus* and *P. jingxiensis* form a sister relationship with low support. If

this node collapses, it will align with the BI tree, indicating that the relationship among *P. mirus*, *P. jingxiensis*, and (*P. coriaceifolius* + (*P. hancei* + *P. multiflorus*)) remains unresolved (Figure 3).

Discussion and conclusions

In this study, we successfully assembled the cp genomes of *P. mirus* and *P. hancei*. These two genomes share similar lengths and GC content, as well as consistent gene composition and arrangement (130 genes, including 85 protein-coding genes, 37 tRNA genes, and eight rRNA genes), which are similar to the characteristics observed in those of other *Petrocodon* species (Xin et al. 2019; Hsieh et al. 2022).

The phylogenetic analysis based on the complete cp genome in this study suggests that the relationship between *P. mirus* and *P. jingxiensis* remains uncertain. Fortunately, the latest comprehensive morphological comparison study revealed that *P. mirus* can be distinguished from *P. jingxiensis* by its shorter pedicels, slender tubular corolla, shorter corolla tube, unequal corolla lobes (upper ones linear and reflexed, lower ones oblanceolate), and included chiritoid-like stigma (Ding et al. 2024). In future research, additional data from nuclear genomes will be needed to confirm the relationship between these two species.

Studies have shown that the complete cp genome provides important insights into the evolutionary processes of species (Zhai et al. 2019; Wu et al. 2024). The complete genome sequences of the two species of *Petrocodon* provided in this study will serve as an important foundation for elucidating the evolutionary relationships of *Petrocodon* and the entire Gesneriaceae family. In addition, plants of the genus *Petrocodon* are exclusively distributed in the karst regions of southern and southwestern China. The complete cp genome data provided in this study also provide a foundation for further exploration of the mechanisms of their adaptation to the karst environment.

Acknowledgments

Yu-Chuan Qi and Li-Hui Liu conceived and supervised the study. Yu-Chuan Qi and Ying Wei collected samples, coordinated sequencing, and performed chloroplast assembly and analysis. Li-Hui Liu conducted the phylogenetic analysis. Yu-Chuan Qi and Ying Wei wrote the manuscript, which was revised and approved by all authors.

Author contributions

CRediT: **Yu-Chuan Qi**: Investigation, Resources, Writing – original draft, Writing – review & editing; **Ying Wei**: Resources, Software, Writing – review & editing; **Li-Hui Liu**: Methodology, Software, Writing – review & editing.

Ethical approval

The materials used in this study have no ethical concerns. Neither *P. mirus* nor *P. hancei* are classified as protected or endangered species, and therefore no special permits or licenses were required for their collection. Field research was carried out in full compliance with all applicable legal and regulatory requirements.

Disclosure statement

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

Funding

This study was supported by the Natural Science Foundation of Guangxi (No. 2019GXNSFBA245004).

Data availability statement

Genome sequence data supporting this study are available in GenBank (NCBI) under accession numbers PQ636880 (*P. mirus*) and PQ636881 (*P. hancei*) at https://www.ncbi.nlm.nih.gov/. The BioProject, SRA, and BioSample numbers are as follows: PRJNA1188752, SRR31535544, and SAMN44848296 for *P. mirus*, and PRJNA1188754, SRR31419614, and SAMN44848297 for *P. hancei*, respectively. The specific data links are as follows: https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1188752+or+PRJNA1188754; https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA1188752%2CPRJNA1188754%20

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