The complete chloroplast genome of Pterobryopsis orientalis (Pterobryaceae, Hypnales) and its phylogenetic implications

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ABSTRACT

Pterobryopsis orientalis (Müll. Hal.) M. Fleisch. 1917, a subtropical moss from the family Pterobryaceae, is native to the western Himalaya. In this study, the complete chloroplast genome of \textit{P. orientalis} was sequenced using the Illumina NovaSeq 6000 platform, as a resource for future research on the classification and evolution of the Pterobryaceae. The genome was 124,719 bp in length, consisting of a large single-copy (LSC: 87,401 bp), a small single-copy (SSC: 18,530 bp), and two inverted repeat regions (IRs: 18,788 bp). The genome consisted of 126 unique genes, including 82 protein-coding genes, 36 tRNA genes, and eight rRNA genes. The overall GC content of the whole chloroplast genome was 52.09%. Phylogenetic analysis showed that \textit{P. orientalis} is closely related to the genus Calypothecium.

The genus Pterobryopsis (Hypnales: Pterobryaceae), a poorly known pantropical moss genus with approximately 30 species (Frey and Stech 2009), is one of the largest genera in Pterobryaceae (Kindberg 1897). It has traditionally been identified by frondose stems, single costa, and flagelliform branches. However, due to a lack of molecular data, the boundaries between Pterobryopsis and Calypothecium have become blurred, as several species could not even be assigned at the genus level according to the generic definition. Han et al. (2022) reported the first plastome sequence of Calypothecium while no chloroplast genome of Pterobryopsis has been published. Pterobryopsis orientalis (Müll. Hal.) M. Fleisch. (first published as Neckera orientalis Müll. Hal. in 1856), is a western Himalayan moss and native to South Asia (Fleischer 1917). As \textit{P. orientalis} has specific geographic distribution, it is important to understand the background of the evolutionary biology of the species. In this study, we assembled and characterized the first complete chloroplast genome sequences of \textit{P. orientalis}, as a resource for future taxonomic and systematic studies of the Pterobryaceae.

Plants of \textit{P. orientalis} were collected from Yaoshang village in Maguan County, Yunnan Province, China (23°06′41″N, 104°18′36″E) and dried with silica gel. A specimen was deposited in National Herbarium, Institute of Botany, the Chinese Academy of Sciences (PE, contact: Ningning Yu, email: yuning@ibcas.ac.cn) under the voucher number PE02131066. Genomic DNA was extracted from tender leaves of the specimen. The libraries were constructed with an average length of 350 bp by using the NexteraXT DNA Library Preparation Kit (Illumina, San Diego, CA). Then, the libraries were sequenced on Illumina NovaSeq 6000 platform (Beijing, PR China), and 7.04 GB of 350 bp paired-end sequence reads were collected. After filtration of the raw data, 90.14% of Q30 bases were obtained and 23,390,196 bases of clean reads were used for further assembly. With Calliergonella cuspidata (NC049070) as a reference, the qualified clean reads were de novo assembled using NOVOWrap 1.20 (Wu et al. 2021) with default parameters. Finally, the complete chloroplast genome of \textit{P. orientalis} was annotated using the GeSeq online tool (Tillich et al. 2017), and then submitted to GenBank with the accession number of OL372263.

The complete chloroplast genome of \textit{P. orientalis} was 124,719 bp in length, consisting of a large single-copy (LSC: 87,401 bp) and a small single-copy (SSC: 18,530 bp) separated by two inverted repeats (IRa and IRb: 9394 bp, respectively). The overall GC content is 52.09%. A total of 126 genes were predicted, consisting of 82 protein-coding genes, 36 tRNA genes, and eight rRNA genes.

Phylogenetic analysis was performed based on complete plastid genome of \textit{P. orientalis}, and 12 other species in the Hypnales. All the chloroplast genomes were aligned...
using MAFFT v7.307 (Katoh and Standley 2013), and the maximum-likelihood (ML) phylogenetic tree was analyzed with RAxML v8 under the GTR + I + G nucleotide substitution model and 1000 rapid bootstraps (Stamatakis 2014). The phylogeny analysis showed that *P. orientalis* is closely related to species of *Calyptothecium* (Figure 1). This study will provide valuable molecular information for future studies on taxonomic and evolution of the Pterobryaceae.

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**Author contributions**

Ningning Yu, Yu Jia, and Li Zhang were mainly responsible for the study design; Ningning Yu and Wei Han participated in the collection of experimental materials; Wei Han and Yin Li analyzed and interpreted the data; Wei Han and Ningning Yu wrote and revised the manuscript; Yu Jia and Li Zhang approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work.

**Disclosure statement**

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

**Data availability statement**

The genome sequence data that obtained at this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) under accession no. OL372263. The associated BioProject, SRA, and BioSample numbers are PRJNA775849, SRR16937479, and SAMN22933330, respectively.

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Figure 1. The phylogenetic tree was constructed using maximum-likelihood (ML) method based on complete chloroplast genome of *P. orientalis* and 12 representative species. ML bootstrap support values were shown at each node. *Haplocladium microphyllum* was selected as outgroup.