The complete chloroplast genome sequence of *Bolbitis multipinna* (Dryopteridaceae)

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ABSTRACT

*Bolbitis multipinna* is an endemic fern found in Yunnan, China. In the case of the chloroplast genome, Illumina Hiseq X-Ten was used, and it revealed a conserved quadripartite structure of 152,437 bp, including a large single-copy (LSC) region of 82,618 bp, a small single-copy (SSC) region of 21,403 bp, and a pair of inverted repeat regions of 24,208 bp (IRa and IRb). The chloroplast genome of *B. multipinna* contains 132 genes, including 89 protein-coding genes, 35 tRNA genes, and eight rRNA genes. By using the maximum-likelihood method and the Bayesian analysis on the chloroplast genomes of 15 species ferns, the phylogenetic analysis revealed that *B. multipinna* is closely related to *B. heteroclita*.

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With about 80 species, *Bolbitis* is a pan-tropical genus mainly found in Asia and Pacific islands (Moran et al. 2010; Zhang et al. 2013; PPG I 2016). *Bolbitis multipinna* is endemic to Yunnan, China, and was first described by Wang et al. (2008). It presents significant morphological variability, with one to more reticulate veins appearing on simple pinnate leaves (Zhang et al. 2013). Despite recent study of *Bolbitis*, there is little information on the chloroplast genomes (Chao et al. 2019). An in-depth analysis of chloroplast genomics of this genus will contribute to the development of a phylogenetic framework for morphological and taxonomical studies of the species.

The fresh leaves of *B. multipinna* were collected in Xishuangbanna County, Yunnan, China (101°32’32.32”E, 21°8’36.54”N, altitude: 750 m) (Figure 1(A,B)), and the specimen (no. WFG5594-13) has been deposited at the South China Botanical Garden Herbarium (http://herbarium.scbg.ac.cn/, contact person: Feiyan Zeng, and email: zengfeiy@scbg.ac.cn). The genomic DNA in leaf tissue samples was extracted using CTAB following the manufacturers’ protocols (Doyle 1987), and sequenced by Illumina Hiseq X-Ten platform (Illumina Inc., San Diego, CA). As a result, the sequences were assembled using the GetOrganelle 1.7.5 toolkit, which depends on SPAdes 3.11 and bowtie2 (Bankevich et al. 2012; Langmead and Salzberg 2012; Jin et al. 2020). Annotation of complete chloroplast genome was done by using the online tool CpgAvas2 (Shi et al. 2019) and submitted to GenBank under accession number OM891302.

The raw reads of *B. multipinna* are approximately 6 G, and there are 152,437 bp in the *B. multipinna* chloroplast genome, including a large single-copy (LSC) region of 82,618 bp, a small single-copy (SSC) region of 21,403 bp, and a pair of...
inverted repeat regions of 24,208 bp (IRa and IRb). In total, 132 genes are encoded by the *B. multipinna* chloroplast genome, including 89 protein-coding genes, 35 tRNA genes, and eight rRNA genes (Figure 2). In the LSC and SSC regions as well as the IRs, the GC content was 41.3%, 38.8%, and 45.3%, respectively.

In order to infer the phylogenetic position of the newly sequenced *B. multipinna* in *Bolbitis*, we constructed a phylogeny using this newly sequenced genome in conjunction with 14 published chloroplast genomes in NCBI (Figure 3). The 71 common chloroplast protein-coding genes were extracted from the 15 complete genomes, which were then aligned using MAFFT v7 (Katoh and Standley 2013). By concatenating these alignments, phylogenetic trees were generated using maximum-likelihood (ML) and the Bayesian inference (BI) methods in IQ-TREE v1.4.2 (Nguyen et al. 2015) and MrBayes v3.2.1 (Ronquist et al. 2012). Based on the results, *B. multipinna* was found to be more closely related to *B. heteroclita* with strong support (MLBS: 100%, BIPP: 1). *Bolbitis* was well supported as the sister species of *Elaphoglossum* (MLBS: 100%, BIPP: 1), which is in agreement with previous studies (Moran 2010; Liu et al. 2016).

**Author contributions**

The paper was drafted by Xinxin Cheng who assembled and annotated the samples; data analysis and interpretation were performed by Liyun Nie and Yujie Liao; Faguo Wang was responsible for the experimental design and the revision of the paper; all authors are accountable for all aspects of the work.

**Ethics statement**

Neither the samples used in this study nor the sampling site is listed on either the IUCN Red List of Threatened Species or the List of State-protected Plant Species. Both field and laboratory studies were conducted in accordance with the guidelines formulated by the South China Botanical Garden, Chinese Academy of Sciences.

**Disclosure statement**

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

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**Figure 2.** The chloroplast genome map of *B. multipinna.*
Data availability statement

The data of this study are available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov under accession no. OM891302. The associated BioProject, SRA, and BioSample numbers are PRJNA894320, SRR22044602, and SAMN31452502, respectively.

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Figure 3. Maximum-likelihood and the Bayesian phylogeny of B. multipinna and related taxa based on 15 complete chloroplast genomes. The maximum-likelihood bootstrap support (MLBS) and the Bayesian inference posterior probability (BIPP) are on the left and right, respectively, along the branches.