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PLASTOME REPORT

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The complete chloroplast genome of *Hemiboea pterocaulis* (Gesneriaceae) exclusively distributed in Guilin, Guangxi, China

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ABSTRACT

Hemiboea pterocaulis is a unique species only found in Guilin, Guangxi, China. In this study, we sequenced and assembled the complete chloroplast genome of *H. pterocaulis* and revealed its phylogenetic relationship with other *Hemiboea* species. The chloroplast genome sequence of *H. pterocaulis* is 153,159 bp in length and comprises a large single-copy (LSC) region of 84,178 bp, a small single-copy (SSC) region of 18,087 bp, and a pair of inverted repeat (IR) regions, each with a length of 25,447 bp. It has a total GC content of 37.6% and encodes 132 genes, including 87 protein-coding genes, 37 tRNA genes, and eight rRNA genes. The phylogenetic relationships based on the complete chloroplast genome sequences of *Hemiboea* taxa indicate that *H. pterocaulis* is most closely related to *H. suiyangensis*, indicating that *H. pterocaulis* is an independent species and is separated from the *H. subcapitata* complex. These results provide valuable insights into the phylogeny, species divergence, and delimitation of the *Hemiboea* genus.

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Introduction

Hemiboea (Gesneriaceae) is predominantly found in southern China and northern Vietnam. Species belonging to *Hemiboea* mainly inhabit evergreen broad-leaved forests or mixed evergreen and deciduous broad-leaved forests, growing in limestone-rich habitats, such as rock crevices or humus-covered rock surfaces, as well as habitats containing conglomerates, granites, and/or sandstones (Wei et al. 2010). Currently, databases document 44 species and seven varieties in *Hemiboea* (Checklist of *Hemiboea*, https://padme.rbge.org.uk/grc/data/checklists). Among the species in this genus, *H. pterocaulis* (Z. Y. Li) J. Huang, X. G. Xiang & Q. Zhang 2017 is unique, and is characterized by multiple longitudinal ridges, with narrow wings along these ridges (Figure 1). It is exclusively found in the limestone habitats of Guilin in Guangxi, China, and was initially published as *H. subcapitata* var. *pterocaulis* (Li 2004). Subsequently, based on comprehensive analyses of its morphological, phonological, and molecular characteristics, *H. subcapitata* var. *pterocaulis* was characterized as a separate species, *H. pterocaulis* (Z.Y. Li) J. Huang, X.G. Xiang & Q. Zhang (Huang et al. 2017); however, was still assumed to be closely related to *H. subcapitata*. A recent study revealed that

Figure 1. Image of *Hemiboea pterocaulis* with elliptical leaves and obtusely denticulate margins growing on karst limestone. This photograph was taken by Zhang-Ping Huang in Jinzhong Mountain, Luojin Town, Yongfu, Guilin, Guangxi, China (110° 17′ E, 25° 4′ N. Alt.: 154 m), and was used with his permission.

H. pterocaulis forms a species complex with *H. yongfuensis* and some other unidentified populations, sharing highly

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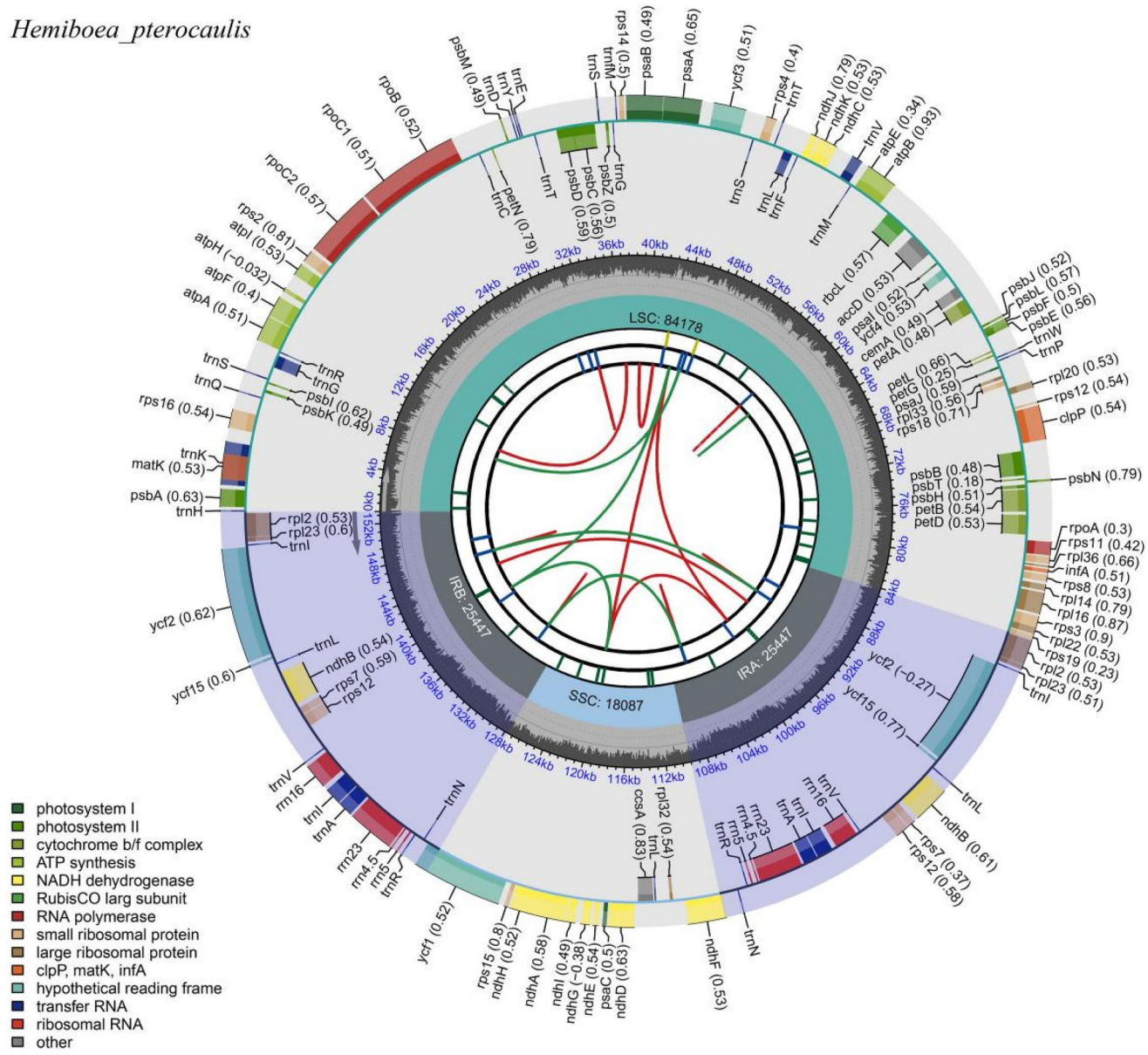

Figure 2. The schematic map illustrates the overall features of the chloroplast genome of *H. pterocaulis*. The map contains six tracks. In the first circle from the center outward, the dispersed repeats are represented. The second circle depicts the long tandem repeats. The short tandem repeats or microsatellite sequences are displayed in the third circle. The quaternary structures, namely the large single copy (LSC), small single copy (SSC), and inverted repeats (IR)a, and IRb regions are indicated in the fourth circle. The fifth circle represents the GC content. Finally, the sixth circle showcases the genes.

similar floral characteristics and overlapping distribution patterns in Guilin (Li et al. 2020).

Nevertheless, the phylogenetic position and genetic diversity of *H. pterocaulis* remain unknown, as previous studies were based only on chloroplast fragment *trn*L-F and nuclear ribosomal internal transcribed spacer (ITS) region sequences (Huang et al. 2017). With the rapid development of high-throughput sequencing techniques and assembling methods, it has become easier to assemble chloroplast genomes and is widely used in phylogenomic studies (Li et al. 2019). In this study, we successfully assembled and annotated the complete chloroplast genome of *H. pterocaulis* and reevaluated its phylogenetic relationship to other *Hemiboea* taxa whose complete chloroplast genomes have been published and are openly available. We believe that this study enhances our understanding of the phylogeny, species divergence, and genetic diversity of *H. pterocaulis*, and provides data that will be useful for future research on the taxonomy, genomics, and related studies of *Hemiboea*.

Materials and methods

The voucher specimen and molecular materials of *H. pterocaulis* were collected from Jinzhong Mountain, Luojin Town, Yongfu, Guangxi, China (110° 17′ E, 25° 4′ N. Alt.: 154 m). The voucher specimen was deposited at the Guangxi Institute of Botany with the number IBK00407875 (http://flora.gxib.cn; contact person: Chunrui Lin, email: chunruilin@tom.com). The silica-dried molecular materials of *H. pterocaulis* were used for the isolation of total genomic DNA using the improved cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). High-throughput sequencing was conducted at

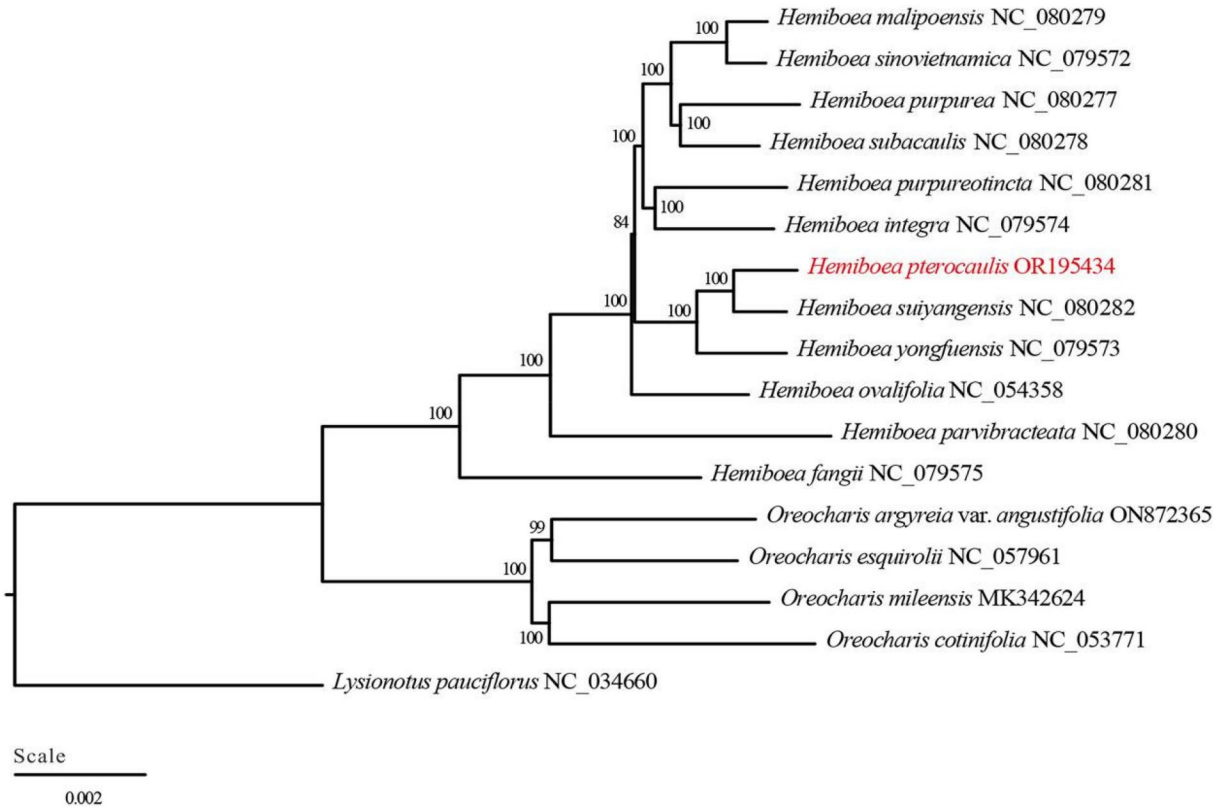

Figure 3. Maximum likelihood (ML) phylogenetic tree based on 17 complete chloroplast genome sequences. The bootstrap support values surrounding the nodes provide confidence estimates for the branching patterns in the phylogenetic tree. The species in this study is highlighted in red. The following sequences were used: *H. pterocaulis* OR195434 (in this study); *H. malipoensis* NC_080279, *H. sinovietnamica* NC_079572, *H. purpurea* NC_080277, *H. subacaulis* NC_080278, *H. purpureotincta* NC_080281, *H. integra* NC_079574, *H. suiyangensis* NC_080282, *H. yongfuensis* NC_079573, *H. ovalifolia* NC_054358, *H. parvibracteata* NC_080280, *H. fangii* NC_079575 (Cui et al. 2023). Outgroups: *O. argyreia* var. *angustifolia* (https://www.ncbi.nlm.nih.gov/nuccore/ON872365.2); *O. esquirolii* NC_057961 (Gu et al. 2020); *O. mileensis* MK342624 (Meng et al. 2019); *O. cotinifolia* NC_053771 (Tang et al. 2021); *L. pauciflorus* NC_034660 (Ren et al. 2017). the scale indicates the base substitution ratio.

Novogene (Tianjin, China) using the Illumina NovaSeq 6000 system (Illumina, San Diego, CA, USA), yielding a total of 2.79 Gb paired-end clean data. The *de novo* assembly of the clean data was performed using GetOrganelle with default parameters (Jin et al. 2020). The annotation of the assembled chloroplast genome was performed using the CPGAVAS2 online web server (http://www.herbalgenomics.org/cpgavas2) with *H. yongfuensis* (GenBank: NC_079573) as a reference. To visualize the circular map of the chloroplast genome, the Chloroplast Genome Viewer (CPGView, http://www.1kmpg.cn/cpgview/) was employed. The genome sequence of *H. pterocaulis* has been deposited in GenBank (accession number: OR195434). The coverage depth of each base was calculated by mapping all clean reads to the assembled chloroplast genome using BWA-MEM (Li 2013) and SAMtools (Danecek et al. 2021), and plot results were obtained using OriginPro 2020 (OriginLab Corporation, Northampton, MA, USA).

To conduct phylogenetic analysis, the complete chloroplast genome sequences of 16 other species of Gesneriaceae (including 11 *Hemiboea* species, four *Oreocharis* species, and one *Lysionotus*) were obtained from the National Biotechnology Information Center (NCBI) to elucidate the evolutionary relationships. The complete chloroplast genome sequences were aligned using MAFFT 7.409 with default settings (Katoh and Standley 2013). The hypervariable regions were removed by using BMGE v1.12 (Criscuolo and Gribaldo 2010). A molecular phylogenetic tree was constructed using RAxML with the GTRGAMMA model and 1000 bootstrap replicates (Stamatakis 2014).

Results

The complete chloroplast genome of *H. pterocaulis* is 153,159 bp in length, with a large single-copy (LSC) region spanning 84,178 bp, a small single-copy (SSC) region covering 18,087 bp, and two inverted repeat (IR) regions of 25,447 bp each (Figure 2). The average coverage depth is 5782.2 (Figure S1). The total GC content of the chloroplast genome is 37.6%. It encodes a total of 132 genes, including 87 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Among these genes, 19 genes possess one intron each, including *rps*16, *atp*F, *rpo*C1, *pet*B, *pet*D, *rpl*16, *rpl*2 (2), *ndh*B (2), *ndh*A, *trn*K-UUU, *trn*G-UCC, *trn*L-UAA, *trn*V-UAC, *trn*A-UGC (2), and *trn*I-GAU (2). Additionally, four genes contain two introns each, namely *rps*12 (2), *ycf*3, and *clp*P. A total of 11 cis-splicing genes, namely *rps*16, *atp*F, *rpo*C1, *ycf*3, *clp*P, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, *ndh*A, were discovered and a trans-splicing gene *rps*12 was identified (Figure S2).

The phylogenetic analysis, which is displayed in Figure 3, demonstrated that a well-supported lineage (BS = 100%) exclusively comprising all *Hemiboea* species and the topology within *Hemiboea* is consistent with previous findings (Cui

et al. 2023). Within *Hemiboea*, *H. pterocaulis* was identified as the strong supported sister to *H. suiyangensis* (BS = 100%), and this clade, in turn, formed the sister group to *H. yongfuensis*, with strong support (BS = 100%).

Discussion and conclusion

This study marks the first-ever assembly of the chloroplast genome sequence of *H. pterocaulis* and provides an annotation of its structural characteristics. The phylogenetic results indicated that *H. pterocaulis* is most closely related to *H. suiyangensis*. This study sheds light on the taxonomy, phylogeny, and bioconservation of *Hemiboea*.

Ethical approval

H. pterocaulis is a highly endangered species though it is not yet listed as a protected species. Therefore, this study adds a valuable biodiversity resource to the science. At the same time, the authors collected the material from the public area of Jinzhong Mountain, Luojin Town, Yongfu, Guangxi, China. Following the current restrictions, there was no requirement for specific approval for domestic collection though it might need to change to protect this valuable species in the future.

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Author contributions

Yu-Mei Zheng conducted the experiment and wrote the manuscript. Xin-Mei Qin collected the materials, acquired the data, and guided data analysis. Xi-Yang Huang and Yuan analyzed the data. Yong-Bin Lu and Hui Tang designed the research and modified the manuscript. All authors agreed to be accountable for and publish the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 accession number OR195434. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA990553, SRR25132865, and SAMN36270339, respectively.

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