

Characterization of the complete chloroplast genome of *Emmenopterys henryi* (Gentianales: Rubiaceae), an endangered relict tree species endemic to China

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Abstract The chloroplast genome sequence of an endangered relict tree species endemic to China, *Emmenopterys henryi*, was determined based on the Illumina data. The chloroplast genome of *E. henryi* was sequenced (155,379 in length, GenBank accession number KY273445). It contains 62.36% A+T base pairs and consists of a pair of inverted repeats (IRs, 25,790 bp) separated by the large single-copy (LSC, 85,554 bp) and small single-copy (SSC, 18,245 bp) regions. The chloroplast genome contains 132 unique genes, including 87 protein-coding genes, 37 transfer RNA genes and 8 ribosomal RNA genes, respectively. The phylogenetic analysis based on chloroplast genomes indicates that *E. Henryi* is most closely related to *coffea arabica* and *coffea canephora*.

Keywords *Emmenopterys henryi* · Chloroplast genome · Phylogenetic analysis

Emmenopterys henryi (Rubiaceae), a native species to China, is a Tertiary relict deciduous tree species and it is the only extant species in its genus (*Emmenopterys*), but fossils of the genus from the Eocene are found in North America and Germany (Manchester et al. 2009). The species is known to be distributed only in disjunct montane valleys at elevations ranging between 400 and 2000 m

above sea level of temperate deciduous forests scattered across subtropical China (Zhang et al. 2016). Because of the small number of populations, a low ability of seed germination, a low rate of natural regeneration and fragment environment, *E. henryi* is listed as the near threatened (NT) species according to the China Species Red List (Wang and Xie 2004). Understanding the chloroplast genome information of this rare and endangered tree may provide valuable guidelines for the development of management strategies for both in situ and ex situ conservation activities. In this study, we have mapped and determined the complete chloroplast genome sequence of *E. henryi* based on the Illumina data.

Young and healthy leaf samples of *E. henryi* were sampled for DNA isolation with the DNeasy Plant Mini Kit (QIAGEN, German) from Yaoluoping National Nature Reserve, Anhui Province, China. The whole-genomic DNA data was sequenced using the Illumina Hiseq 2500 platform. We assembled the complete cp genome with MITObim, version 1.8 with that of the *Coffea arabica* (EF044213) (Samson et al. 2007) as the reference. The cp genome was annotated with GENEIOUS R10 (Biomatters Ltd., Auckland, New Zealand) using default parameters to predict the protein-coding genes, transferRNA (tRNA) genes, and ribosome RNA (rRNA) genes. The complete cp genome sequence was submitted to the GenBank to get the accession number KY273445 (Fig. 1).

The length of the complete cp genome is 155,379. The cp genome consists of a pair of inverted repeat (IRA and IRb) regions of 25,790 bp, small single copy (SSC) and large single copy (LSC) regions with the lengths of 18,245 and 85,554 bp, respectively (Fig. 1). The cpDNA of *E. henryi* encodes an identical set of 132 unique genes, including 87 protein coding, 8 ribosomal RNA genes, and 37 transfer RNA, respectively. The AT content of the

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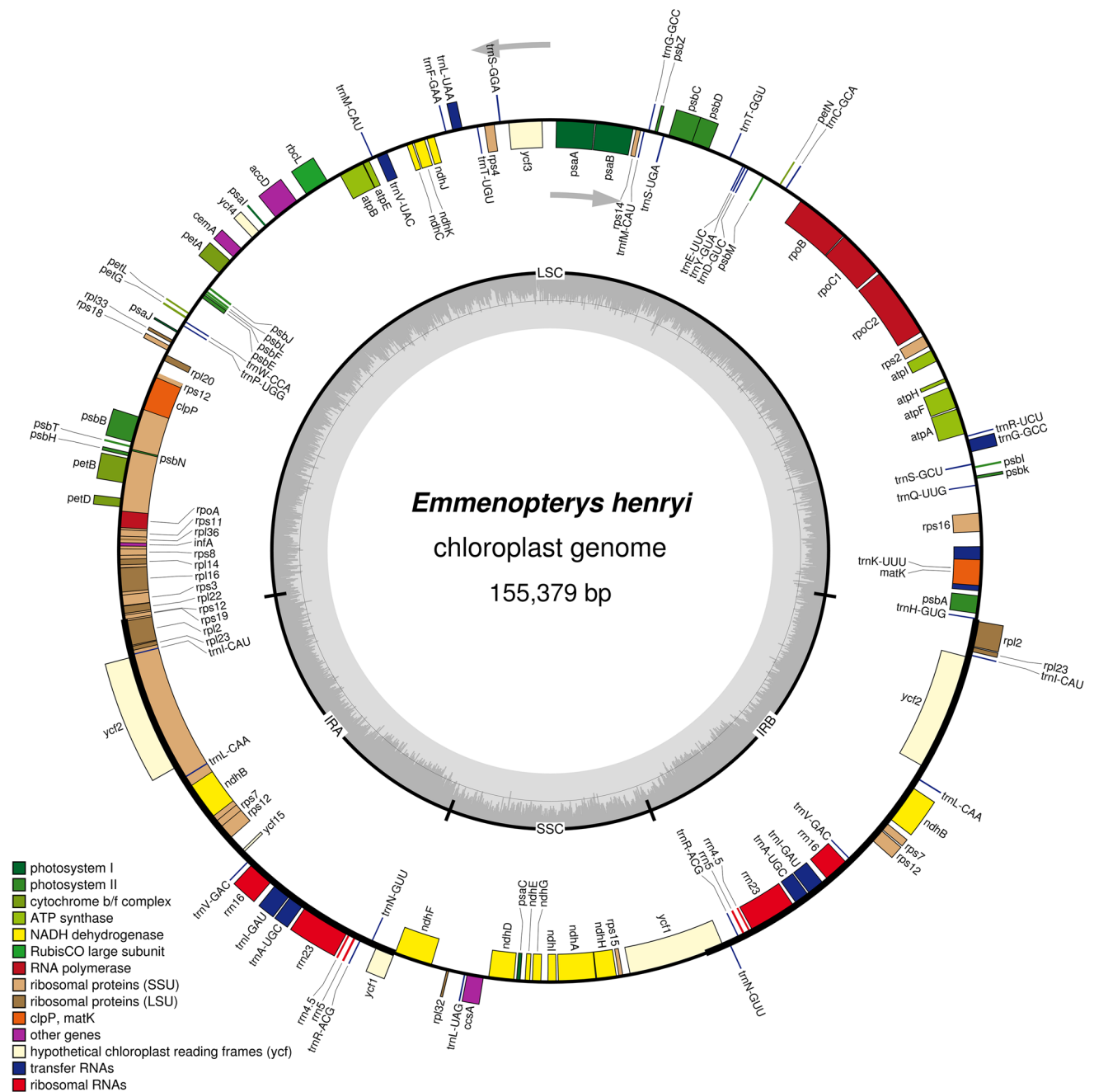


Fig. 1 Physical map of the *Emmenopterys henryi* chloroplast genome. Genes shown outside the outer circle are transcribed in the clockwise direction, whereas those inside are transcribed in the

counterclockwise direction. Areas dashed darker and light gray in the inner circle indicates the G+C and A+T contents of the genome, respectively

whole cpDNA of *E. Henryi* is 62.36%, while the corresponding values of the SSC, LSC, IR regions are 68.10, 64.51 and 56.74% respectively. To identify the phylogenetic position of *E. henryi*, a neighbor-joining (NJ) tree using MEGA 6.0 program (Tamura et al. 2013) was

performed based on the 87 protein-coding genes from 18 Gentianales plant taxa (4 Rubiaceae, 10 Apocynaceae and 5 Gentianaceae). The result has shown that the cp genome of *E. henryi* is closely related to that of *Coffea arabica* and *Coffea canephora* (Fig. 2).

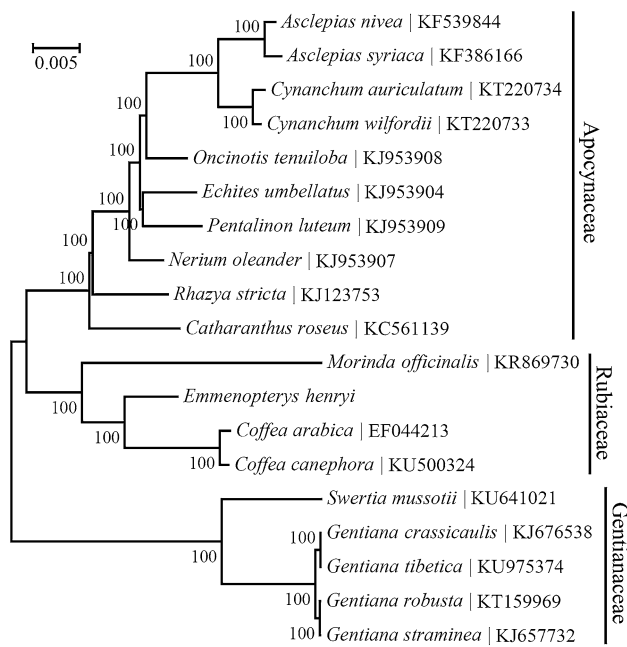


Fig. 2 Phylogenetic trees from the analyses of the nucleotide substitutions of the completely sequenced chloroplast genomes in 18 cpDNA taxa. Phylogenetic relationships of 18 species within the order Gentianales based on the NJ of the concatenated coding sequences of 87 chloroplast PCGs. The bootstrap values were based on 100 replicates, and are shown next to the branches

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