

The complete chloroplast genome sequences of *Hippuris vulgaris* (Plantaginaceae)

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ABSTRACT

Two complete chloroplast genomes of *Hippuris vulgaris* (*H. vulgaris_A* and *H. vulgaris_B*), representing two distinct clades in China, were sequenced and assembled in this study. The circular genomes were 152,763 and 152,713 bp in length and exhibit a typical quadripartite structure of the large single-copy (LSC, 82,983/82,949 bp) and small single-copy (SSC, 18,294/18,278 bp) regions, separated by a pair of inverted repeats (IRs, both 25,743 bp). Both two cp genomes identically contain 133 genes, including 88 protein-coding genes, 37 *tRNA*, and eight *rRNA* genes. The phylogenetic analysis within *Plantaginaceae* demonstrated *Hippuris* an independent clade included in the expanded Plantaginaceae.

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Hippuris vulgaris L., previously belonging to the monogeneric family Hippuridaceae, now is included in an expanded *Plantaginaceae* (Olmstead et al. 2001; Albach et al. 2005). *H. vulgaris* is an aquatic perennial herb with a circumboreal distribution confined in temperate, boreal, and sub-arctic regions (Wan 2000; Elven et al. 2012). In Inner Mongolia and Tibet of China, *H. vulgaris* is used as an endemic Chinese medicine to cure tuberculosis and cough (Shang et al. 2012). In this study, we firstly assembled and characterized the complete chloroplast genomes of *H. vulgaris*, which will provide organelle molecular basis for further research of this medicinal aquatic plant.

Fresh leaves of two individuals (*H. vulgaris_A* and *H. vulgaris_B*), representing two distinct clades revealed in previous research (Lu et al. 2016) were collected separately from Jiagedaqi District (124°32.556'E, 51°50.416'N) and Fuyu County (124°32.954'E, 47°44.450'N) in Heilongjiang Province of China, and dried with silica gel. The vouch specimens (No. Xu7157 and Xu 639) were deposited in Herbarium of the Wuhan University. Total genomic DNA was extracted from ~3 mg materials using DNA Plantzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Purified DNA was sheared into ~500 bp fragments, and the paired-end sequencing libraries were constructed according to the Illumina standard protocol (Illumina, San Diego, CA). Genomic DNA of two samples was sequenced using an Illumina BGISEQ-500 (Illumina, San Diego, CA) at Beijing Genomics Institute (BGI; Shenzhen, China). Illumina paired-end sequencing generated a total of 20,176,296 and 20,662,298 bp raw

reads after removing adapters. The raw reads were then used to assemble the cp genomes using NOVOPlasty version 2.7.2 (Dierckxsens et al. 2017), with ATP synthase alpha subunit (*atpA*) gene from *Veronica nakaiana* (GenBank accession no. NC_031153) as the seed. Chloroplast genome annotation was performed using transferring annotations in Geneious Prime (Kearse et al. 2012), with the cp genome of *V. nakaiana* (GenBank accession no. NC_031153) as the reference. Where necessary, the positions of start and stop codons and boundaries between introns and exons were manually corrected. The two annotated complete cp genomes of *H. vulgaris* were deposited in GenBank under the accession no. MW044609 and MW044610. Chloroplast genomes of *H. vulgaris_A* and *H. vulgaris_B* were circular DNA molecules of 152,763 and 152,713 bp in length. Both cp genomes had a typical quadripartite structure, consisting of a pair of inverted repeats (IRa and IRb: both 25,743 bp) separated by a large single-copy region (LSC: 82,983 and 82,949 bp) and a small single-copy region (SSC: 18,294 and 18,278 bp). The overall GC content was both 37.6%. The IR regions had higher GC content (43.1 and 43.2%) than the LSC (35.6 and 35.7%) and SSC (30.6 and 30.8%) regions. Both two cp genomes encoded a set of 133 genes, containing 88 protein-coding genes, 37 *tRNA* genes, and eight *rRNA* genes.

Eleven species in *Plantaginaceae* and three species of *Scrophularia* with available chloroplast genomes were selected to study the phylogenetic placement of *H. vulgaris* (Figure 1). The sequence alignment was conducted using

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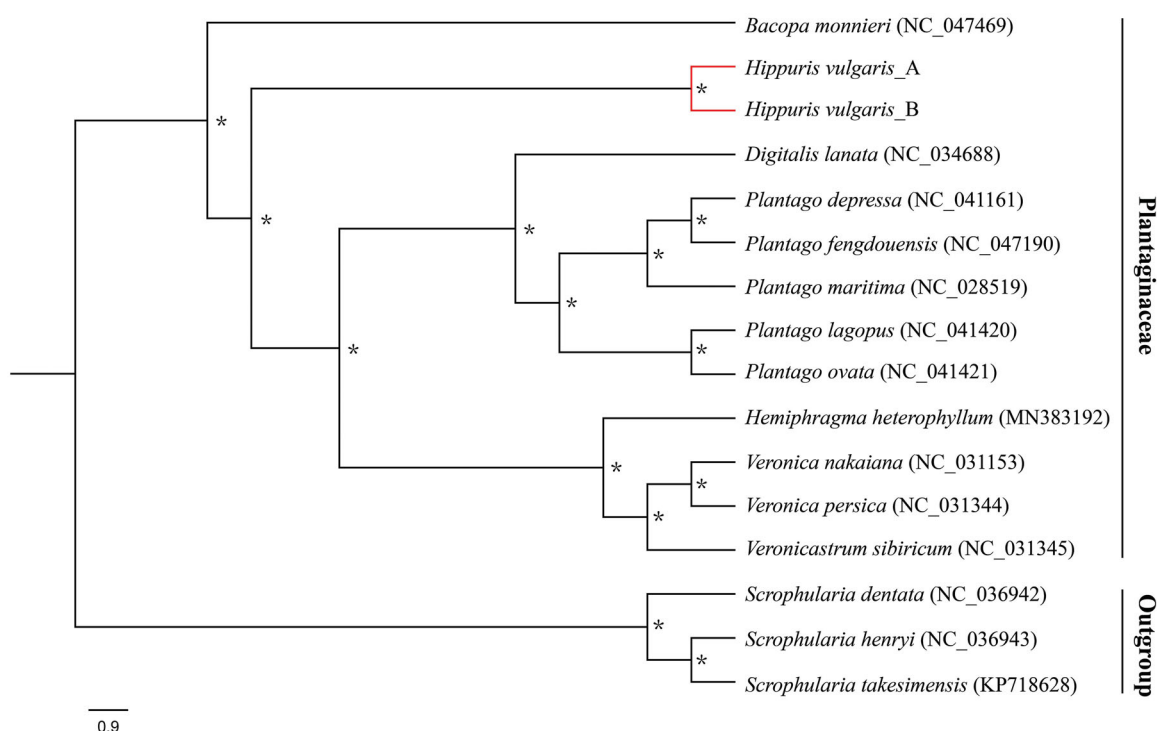


Figure 1. The best maximum likelihood (ML) phylogram inferred from 16 complete chloroplast genome sequences in *Plantaginaceae* and *Scrophularia* (accession numbers were listed and *** indicates the bootstrap support values = 100%).

MAFFT version 7.3 (Kato and Standley 2013). The phylogenetic tree was constructed using IQTREE version 1.6.7 (Nguyen et al. 2015), with the best selected GTR + F + R3 model and 5000 bootstrap replicates. Three *Scrophularia* species were used as outgroups. The result confirmed that *H. vulgaris* was included in *Plantaginaceae* and forms an independent clade in present data extent.

Disclosure statement

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

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Data availability statement

The DNA matrix and phylogenetic tree that support the findings of this study are openly available via the DOI <http://dx.doi.org/10.25833/qdhr-0c03>.

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