Complete plastid genome characterization and phylogenetic analysis of *Pentasachme caudatum* Wallich ex Wight (Gentianales: Apocynaceae) from Guangdong, China

Shenyu Miao\(^a\), Yuqian Luo\(^a,b\), Mary Ann C. Bautista\(^b,c,d\) and Tao Chen\(^b,d\)

\(^a\)School of Life Sciences, Guangzhou University, Guangzhou, China; \(^b\)Fairy Lake Botanical Garden, Chinese Academy of Sciences, Shenzhen, China; \(^c\)South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China; \(^d\)Graduate School, University of Chinese Academy of Sciences, Beijing, China

**ABSTRACT**

*Pentasachme caudatum* Wallich ex Wight is considered as one of the Asian enigmatic genera classified in the Asclepiadoideae (Apocynaceae). To determine its evolutionary relationship in the family, we sequenced and characterized the complete chloroplast genome of *P. caudatum*. The plastid genome of *P. caudatum* is 158,487 bp in length, containing a large single-copy (90,380 bp), a small single-copy (18,585 bp), and a pair of inverted repeats (24,761 bp). It has 127 annotated genes, consisting of 83 protein-coding, eight rRNA and 36 tRNA genes. Phylogenetic analysis using 76 protein-coding regions of the plastid genomes of related taxa showed that *P. caudatum* was resolved in a fully supported clade with *Orthotheca albida*. The newly sequenced *P. caudatum* provides essential genetic information that is useful for future phylogenetic studies in the family Apocynaceae.

**ARTICLE HISTORY**

Received 20 December 2020
Accepted 31 January 2021

**KEYWORDS**

Apocynaceae; Asclepiadoideae; Pentasachme caudatum; phylogeny; plastid genome

In the Apocynaceae, the Asclepiadoideae has been established as a monophyletic group, however, systematic positions of the Asian genera like *Pentasachme* is still uncertain (Surveswaran et al. 2014). *Pentasachme* are characterized as rheophytic herbs, with slender, elliptic leaves and sessile inflorescences that bear few flowers with long, slender corolla (Surveswaran et al. 2014). In China, only one species of *Pentasachme* is recognized, *Pentasachme caudatum* Wallich ex Wight (Li et al. 1995). Morphologically, *P. caudatum* is easily confused with *Cynanchum stauntonii*, which complicates species identification (Li et al. 1995). Both species are considered medicinal, but they are used to treat different diseases. Hence, misidentifications for use as an herbal medicine may lead to potential safety risks. Authentication of the plant material is the initial and most important step in conducting either taxonomic or pharmaceutical studies. Thus, this study aimed to provide the complete plastid sequence of *P. caudatum* to be used as a genetic resource for future plant authentication.

Total DNA was extracted from fresh leaves of *Pentasachme caudatum* (2020061510, SZG, Tao Chen, taochen@szbg.ac.cn) collected from Sanzhuo and Shenzhen District, Shenzhen, Guangdong, China (E114°16′09″, N22°37′39″) using a modified CTAB method (Murray and Thompson 1980). Sequencing was performed on the Illumina Novaseq 600 platform producing paired-end sequences with an average read length of 150 bp and average sequencing depth of 2,369. Using the NGS-QC toolkit (Patel and Jain 2012), the raw Illumina reads were filtered and the resulting high quality reads were assembled via the de novo assembler SPAdes 3.11.0 (Vasiljevic et al. 2015). The assembled cp genome was annotated using Plann software (Huang and Cronk 2015), RNAmmer 1.2 Server (Lagesen et al. 2007) and tRNAscan-SE (Chan and Lowe 2019) for protein-coding genes, rRNA and tRNA genes. *Oncinotis tenuiloba* (KJ953908.1) was used as a reference in the plastid genome annotation.

The complete plastid sequence of *P. caudatum* has a total length of 158,487 bp and exhibit a typical quadripartite structure containing a large single copy region (90,380 bp) and a short single copy region (18,585 bp) separated by two inverted repeat regions (24,761 bp). The genome has 127 annotated genes, including 83 protein-coding, eight rRNA and 36 tRNA genes. Similar to other Asclepiadoideae plastid genomes, there are 17 genes with introns, 15 genes (trnK-UUU, rps16, trnG-UGC, atpF, rpoC1, trnL-UAA, trnV-UAC, petB, petD, rpl16, rpl2, ndhB, trnL-GAU, trnA-UGC, ndhA) have a single intron, whilst clpP and ycf3 have two introns. The rps12 gene was also transspliced, with the 5′ exon located in the LSC region and 3′ exon duplicated in the IR regions. The overall GC content of the genome was 37.8%.

Phylogenetic position of *P. caudatum* was determined by constructing a Maximum Likelihood tree using RAxML 8.2.11 (Stamatakis 2014). The *P. caudatum* cp genome sequence was aligned with 19 other sequences from the Apocynaceae and ML tree was inferred using the GTR+I+G nucleotide substitution model with 1,000 replicates. *Gentiana* was designated as the outgroup. The resulting ML tree (Figure 1) showed that *P. caudatum*
occupies a sister position to *Orthanthera albida*. The tree also confirms the sister tribe relationship between Ceropegieae (P. caudatum, O. albida) and Marsdenieae (*Marsdenia astephanoides, Hoya liangii, H. potsii*) (Endress et al. 2007; Surveswaran et al. 2014). Both tribes are characterized by having erect pollinaria (Endress et al. 2007). Moreover, this phylogenetic tree based on plastid genomes further supports the monophyly of the subfamily Asclepiadoideae and its close relationship to Secamonoideae.

**Disclosure statement**

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

**Funding**

This work was supported by the Science and Technology Planning Project of Guangdong Province (2018B030320007) and the Fourth National Survey of Chinese Traditional Medicine Resources Project from State Administration of Traditional Chinese Medicine (GZY-KJS-2018-004).

**ORCID**

Mary Ann C. Bautista [http://orcid.org/0000-0002-5614-7170](http://orcid.org/0000-0002-5614-7170)

Tao Chen [http://orcid.org/0000-0001-7214-5020](http://orcid.org/0000-0001-7214-5020)

**Data availability statement**

The complete chloroplast genome sequence of *Pentasachme caudatum* was deposited in NCBI GenBank with the accession number MW136321. Raw sequencing data were also available in SRA database with the BioSample number SAMN17080906, SRA number SRX9685746 and BioProject accession number PRJNA672143. (https://www.ncbi.nlm.nih.gov/nuccore/MW136321; https://www.ncbi.nlm.nih.gov/sra/PRJNA672143)

**References**

Chan PP, Lowe TM. 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol. 1962:1–14.

Endress ME, Liede-Schumann S, Meve U. 2007. Advances in Apocynaceae: the enlightenment, an introduction. Ann Missouri Bot. 94(2):259–267.

Huang DI, Cronk Q. 2015. Plann: a command-line application for annotating plastome sequences. Appl Plant Sci. 3(8):1500026.

Lagesen K, Hallin PF, Rodland E, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35 (9):3100–3108.

Li PT, Gilbert MG, Stevens WD. 1995. Pentasachme. In Wu ZY, Raven P, Hong DY, editors. Flora of China. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press; Vol. 16; p. 262–263.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8(19):4321–4326.

Patel RK, Jain M. 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLOS One. 7(2):e30619.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.

Surveswaran S, Sun M, Grimm GW, Liede-Schumann S. 2014. On the systematic position of some Asian enigmatic genera of Asclepiadoideae (Apocynaceae). Bot J Linn Soc. 174(4):601–619.

Vasilinetc I, Prijibelski AD, Gurevich A, Korobeynikov A, Pevzner PA. 2015. Assembling short reads from jumping libraries with large insert sizes. Bioinformatics. 31(20):3262–3266.