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PLASTOME REPORT

Characteristics of the complete chloroplast genome of Saxifragaceae species *Bergenia purpurascens* (Hook. f. et Thoms.) Engl

Li Ao[a]*, Na Li[a]*, Azam Fardous Mohammad Safiul[a], Sha Li[a], Tinjuan Zhao[a], Jianlan Wang[a], Yuanchao Zou[a], Rui Li[a] and Shixi Chen[a,b]

[a]College of Life Science, Neijiang Normal University, Neijiang, China; [b]Conservation and Utilization of Fishes Resources in the Upper Reaches of the Yangtze River, Key Laboratory of Sichuan Province, Neijiang, China

ABSTRACT

Bergenia purpurascens (Hook. f. et Thoms.) Engl. is one species of traditional Chinese medicinal plant. This is the first publication of its complete chloroplast (cp) genome. The whole cp genome has 157,246 base pairs in length with 132 annotated genes, of which were 87 protein-coding genes, 37 *tRNA*s, and 8 *rRNA*s. According to the phylogenetic study, *B. purpurascens* and *Bergenia scopulosa* T. P. Wang. 1974 had a sister relationship. This genomic data and conclusions from *B. purpurascens* phylogenetic research will provide useful information and throw light on more in-depth investigations of the systematics and evolutionary patterns of *Saxifragaceae*.

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Introduction

Bergenia purpurascens (Hook. f. et Thoms.) Engl., commonly called Purple *Bergenia* (1868), is a popular ornamental plant belonging to the family Saxifragaceae. It is a hardy perennial native to western China and Central Asia, particularly the Himalayas, Tibet, and Mongolia (Zhao et al. 2006; Koul et al. 2020). *B. purpurascens* is an evergreen groundcover plant that is widely used in landscaping because of its attractive foliage, bright pink flowers, and ability to thrive under a variety of conditions with formaldehyde purification (Zhang et al. 2011). The plant contains galloylarbutin and other polyphenols, and several researchers have reported that it has anti-inflammatory, antibacterial, and antifungal properties, making it beneficial for treating skin conditions, arthritis, respiratory problems, as well as digestive disorders and as a tonic to improve overall health and well-being (Xin-Min et al. 1987; Zhang et al. 2011; Bajracharya et al. 2012; Patel et al. 2012; Bajracharya and Maharjan 2013; Li et al. 2013; Shi et al. 2014; Bajracharya 2015; Pandey et al. 2017; Żbikowska et al. 2017; Zhang et al. 2017; Liu et al. 2018; Jing et al. 2019; Zhao et al. 2019; Kostić et al. 2020; Koul et al. 2020; Qu et al. 2020). Moreover, previous researchers conducted extensive phytochemical and pharmacological investigations of this species. However, few molecular studies have been conducted, such as the genetic diversity and relationship of China's *Bergenia* germplasms (Lv et al. 2021), molecular markers (Zhang et al. 2016), and the chloroplast genome sequence of *Bergenia scopulosa* T. P. Wang. 1974 (Bai et al. 2017). Understanding the genetic diversity is important for the conservation of *Bergenia* and clarifying the evolution of this flowering species. Nevertheless, the complete plastome with more genetic information and its phylogenetic position remains unknown. Because of its valuable information and highly conserved nature, the complete chloroplast (cp) genome has been widely used in molecular markers, barcode identification, phylogenetic analysis, and other fields (Yang et al. 2020; Gu et al. 2022). Here, we sequenced and characterized the cp genome of *B. purpurascens* to provide more genetic information and assessed its phylogenetic position within the genus *Bergenia* for further evolutionary research.

Materials and methods

The *B. purpurascens* sample used for cp genome sequencing was identified and artificially reproduced in Aba Tibetan and Qiang Autonomous Prefecture in Sichuan Province, China (latitude 103° 29′ 57.084″, longitude 31° 10′ 4.98″). The voucher specimen (accession No. CP00001) was identified and deposited at the Herbarium of Neijiang Normal University (Neijiang City, China; Shixi Chen, saihei@foxmail.com). Young leaf specimens were collected, stored at room temperature, and packaged with 0.2 g of silicon dioxide after collection. Then, total genomic DNA was extracted from stored fresh leaves using suspension, lysis, isolation, cleaning, elution, and

CONTACT Shixi Chen saihei@foxmail.com College of Life Science, Neijiang Normal University, Neijiang 641100, China.
*These authors contributed equally to this work.

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cleanup from the modified cetyltrimethylammonium bromide (CTAB) method (Porebski et al. 1997) and stored in the Molecular Biology Lab of Neijiang Normal University. Later, the short reads of the cp genome of *B. purpurascens* were sequenced using a genomic library with insert sizes of 260 bp and sequenced on an Illumina Hi-Seq 2500 platform. Approximately 2.07 Gb of raw reads were obtained and filtered using Trimmomatic (Version 0.39) (Bolger et al. 2014). Filtered reads were used to assemble the cp genome using NOVOPlasty (version 4.3.1) (Dierckxsens et al. 2017). Furthermore, the sample generated a volume of 7.95 Gb of clean data with a guaranteed Q30 of 92.62%, which guaranteed the correct assembly of the genome (Supplementary Figures S1 and S2). Next, annotation was conducted with the GeSeq tool using the default parameters and used 3rd Party Stand-Alone Annotators of *B. purpurascens* from Chloë (version 0.1.0) and t-RNA annotation from tRNAscan-SE (version 2.0.7) and checked with the CPGAVAS2 web server (Tillich et al. 2017; Shi et al. 2019). The annotation was followed by a manual check compared with the NCBI database and deposited in the NCBI database with GenBank accession number OR004350. The cp genome was immediately visualized using Chloroplot (Zheng et al. 2020).

Afterward, to ascertain the phylogenetic position of *B. purpurascens* (OR004350) within *the family Saxifragaceae*, fourteen species were analyzed, and *Rodgersia aesculifolia* Batalin. 1893 (MW327540) and *Rodgersia sambucifolia* Hemsl. 1906 (MN496077) were chosen as the outgroups.

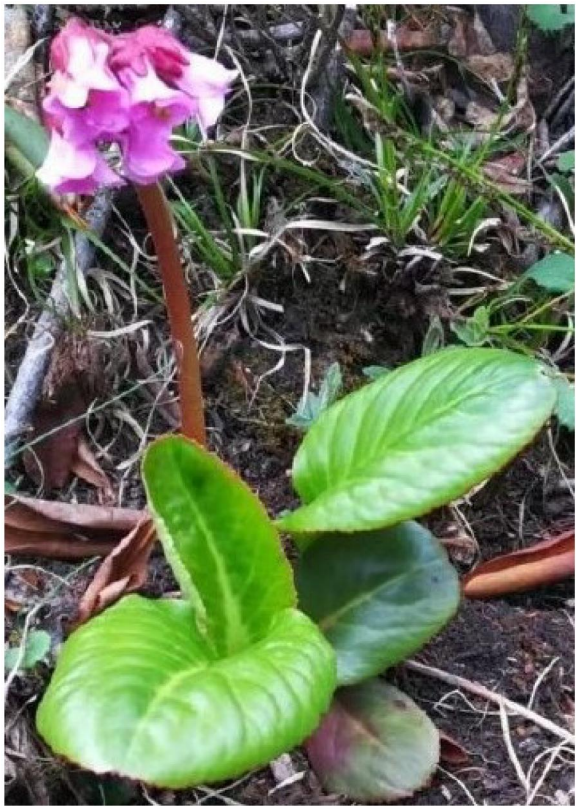

Figure 1. The photo of an individual of *B. purpurascens* was taken by Li Ao from Aba Tibetan and Qiang Autonomous Prefecture in Sichuan Province, China, including young leaves and flowers, the core feature of the species including its thick, large, and leathery of obovate leaves, cymose paniculate inflorescences, and broadly ovate purplish-red petals.

The sequences used for constructing the tree are 154,407 bp ~ 157,289 bp in length, and all sequences have the same GC content of 37%. Alignment and phylogenetic reconstructions were performed using the function 'build' of ETE3 3.1.2 (Huerta-Cepas et al. 2016) as implemented on GenomeNet (https://www.genome.jp/tools/ete/). Alignment was performed with MAFFT v6.861b with the default options (Katoh et al. 2005). The ML tree was inferred using IQ-TREE 1.5.5 run with ModelFinder and tree reconstruction (Nguyen et al. 2015). The best-fit model, according to BIC, is K3Pu + I. Tree branches were tested by SH-like aLRT with 1000 replicates.

Results

B. purpurascens features a rosette of leathery, elliptic to ovate-elliptic leaves with a glossy deep green above and purple-red underneath. Leaves form a spreading clump of foliage. Leaves are evergreen and have nodding, pink to purplish-red flowers that in cymose inflorescences atop strong purplish-red stems (Figure 1).

The circular cp genome of *B. purpurascens* is 157,246 bp in length and divided into typical quadripartite regions, including two 25,489 bp inverted repeat (IR) regions, one 88,080 bp large single-copy (LSC) region, and one 181,88 bp small single-copy (SSC) region (Figure 1). The overall GC content of the chloroplast genome of *B. purpurascens* was 38%. This genome was annotated with 132 genes, including 87 protein-coding genes, 37 *tRNA* genes, and eight ribosomal *RNA* genes, and the IR regions led to two copies of *rps*12, *rps*7, *ndh*B, *ycf*15, *ycf*2, *rpl*23, *rpl*2, *trn*M, *trn*L, *trn*V, *trn*E, *trn*A, *trn*R, *trn*N, *rrn*16, *rrn*23, *rrn*4.5, and *rrn*5 (Figure 2).

In addition, the ML tree revealed a sister relationship between *B. purpurascens* and *B. scopulosa* and formed a clade for species within the order Saxifragales (Figure 3).

Discussion and conclusion

Overall, in this study, the chloroplast genome sequence of *B. purpurascens* was assembled for the first time, and the structure of this species was annotated, which structure was the same with the other species of Saxifragaceae (Bai et al. 2017; Li et al. 2019; Wu et al. 2020; Chen et al. 2022; Yang et al. 2022). The phylogenetic relationship revealed that both *Bergenia* species were closely related, which is same with the other findings (Bai et al. 2017; Chen et al. 2022). Our results provide fundamental information for comparative chloroplast genomics and further phylogenetic studies of *Bergenia*.

Authors contributions

Samples were collected by Ao L and Li S. Zhao TJ; Li N and Wang JL analyzed the sequenced data and drafted this paper. Zou YC, Chen SX, and Azam FMS designed this work and accomplished the revision of this paper.

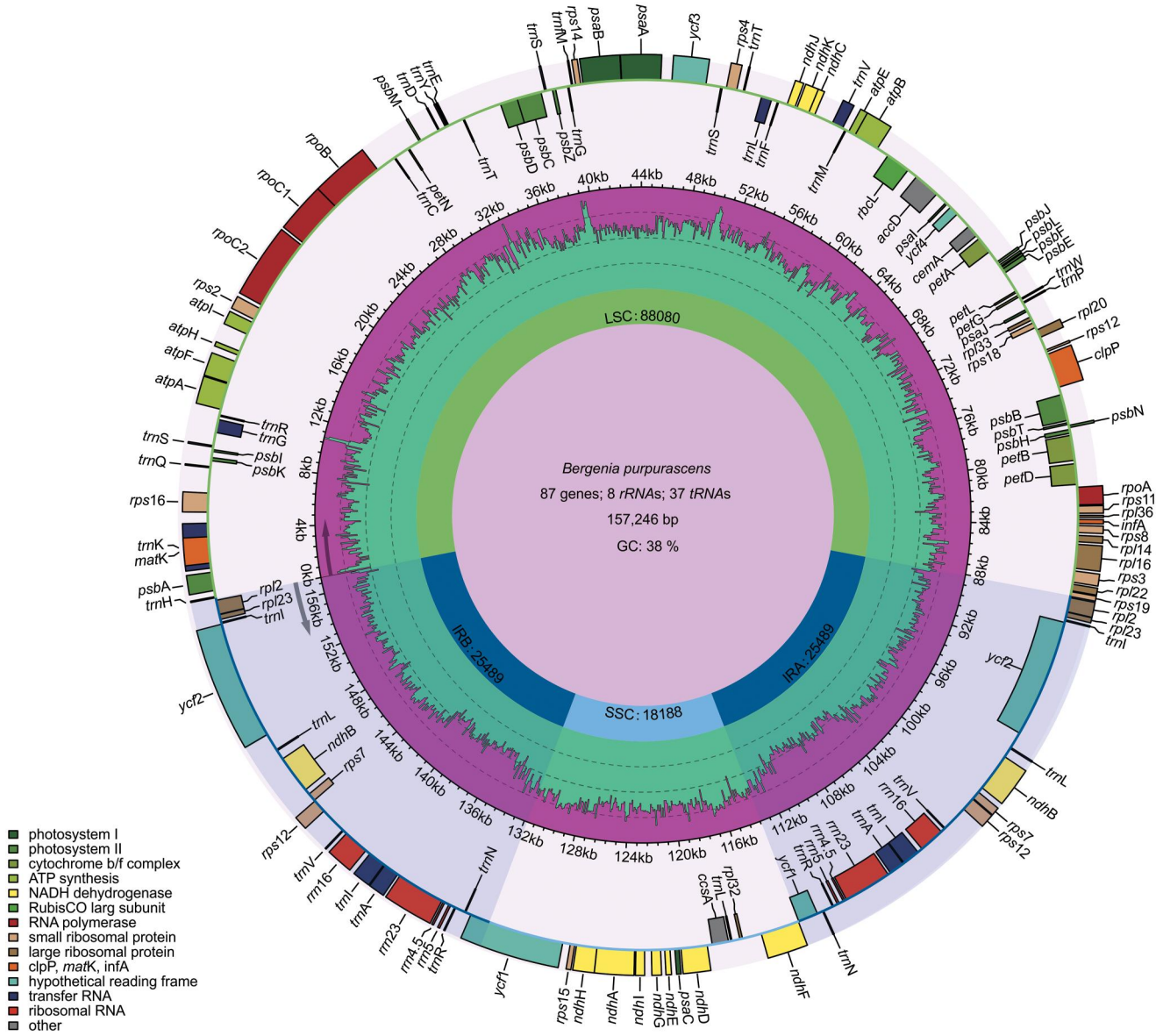

Figure 2. Graphic representation of features identified in the cp genome of *B. purpurascens*. Genes inside the circle are transcribed clockwise, while those outside are transcribed counterclockwise. Genes are color-coded according to functional groups. The dark pink region inside the inner circle indicates the GC content, while the green color indicates the at content of the cp genome. Boundaries of the small single copy (SSC) and large single copy (LSC) regions and the inverted repeat (IRa and IRb) regions are denoted in the inner circle.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article. The authors alone are responsible for the content and writing of the paper.

Ethics statement

No ethical issues were involved in this study. The collection of plant samples was legal and reasonable. Information on the voucher specimen, who identified it, the depositor, and the herbarium were included in the manuscript.

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Data availability statement

The chloroplast genome sequence data in this study are openly available in the GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ with the accession number OR004350. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1004063, SRR25609650, and SAMN36922130, respectively.

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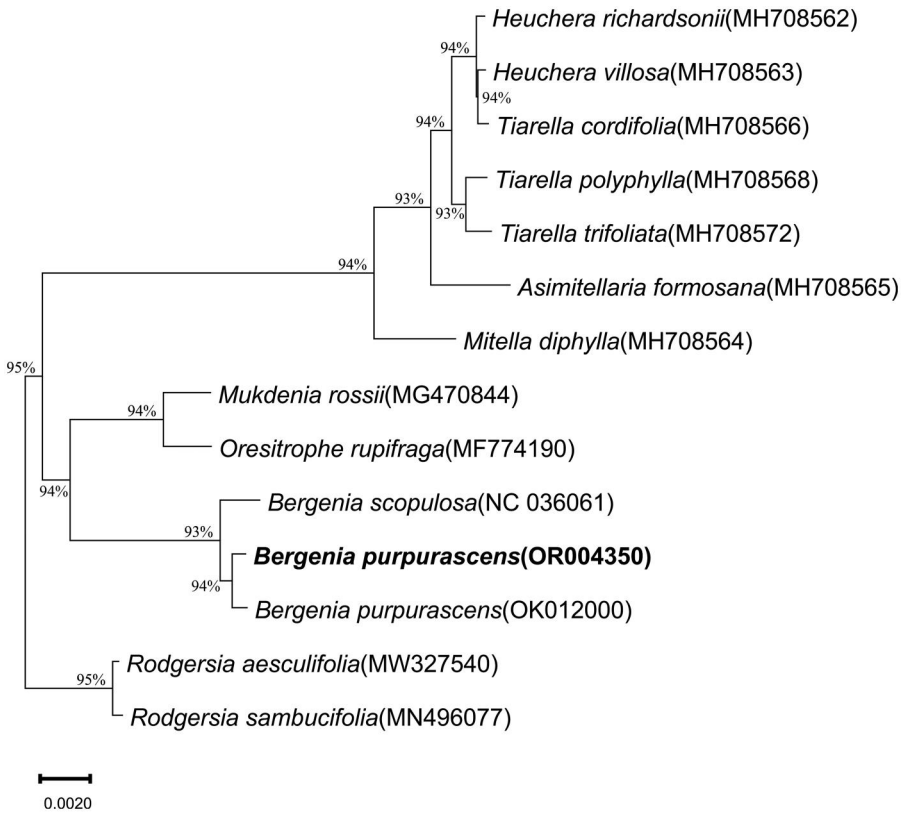

Figure 3. Phylogenetic relationships of *Bergenia purpurascens* inferred from maximum likelihood (ML) analysis. Numbers beside the node indicate bootstrap support values. The following sequences were used: *B. purpurascens* OR004350 (this study), OK012000 (Chen et al. 2022), *B. scopulosa* NC036061 (Bai et al. 2017), *Heuchera richardsonii* R.Br. 1823 MH708562, *Heuchera villosa* Michx. 1803 MH708563, *Tiarella cordifolia* L. 1753 MH708566, *Tiarella polyphylla* D. Don. 1825 MH708568, *Tiarella trifoliata* L. 1753 MH708572, *Asimitellaria formosana* R.A. Folk & Y. Okuyama. 2021 MH708565, *Mitella diphylla* L. 1753 MH708564, *Mukdenia rossii* Koidz. 1935 MG470844, *Oresitrophe rupifraga* Bunge 1835 MF774190, *R. aesculifolia* MW327540, *R. sambucifolia* MN496077 (Yang et al. 2022).

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