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PLASTOME REPORT

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Characterization of the complete chloroplast genome sequence of *Bassia scoparia* (L.) A. J. Scott 1978 (Amaranthaceae) and its phylogenetic analysis

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ABSTRACT

Bassia scoparia, an annual potherb belonging to the family Amaranthaceae, has been widely used in traditional Chinese and Japanese medicine for over 2000 years. Herein, we presented its complete chloroplast. The chloroplast genome sequence was 151,278 bp in length with a 36.6% content of GC. The genome showed the typical quadripartite structure, comprising a pair of inverted repeat (IR) regions (24,353 bp) separated by a large single-copy (LSC) region (84,067 bp) and a small single-copy (SSC) region (18,505 bp). This chloroplast genome harbored 133 predicted genes, including 88 protein-coding genes, 37 transfer RNA (tRNA) genes, and eight ribosomal RNA (rRNA) genes. The phylogenetic analysis indicated that *B. scoparia* was closely related to *B. littorea*. This newly sequenced chloroplast genome not only enhances our understanding of the genome of *Bassia* but also provides valuable insights for the evolutionary study of the family Amaranthaceae.

1. Introduction

Bassia scoparia (L.) A. J. Scott 1978, also called *Kochia scoparia* (Linn.) Schrad, is a large annual potherb native to Eurasia in the family Amaranthaceae (Beckie et al. 2013). Due to its low seed persistence, aggressive growth, tolerance to various stresses, and tumble mechanism of seed dispersal, *B. scoparia* has been widely distributed in Europe, Asia, Africa, Australia, and America (Friesen et al. 2009; Beckie et al. 2013; Dille et al. 2017; Kumar et al. 2017). In China, Korea, and Japan, the mature fruit of *B. scoparia*, Kochia Fructus, has been traditionally used as an edible and topical drug in the treatment of skin, urinary, inflammation, and eye diseases for more than 2000 years (Matsuda et al. 1999; Han et al. 2010; Shao and Shao 2015). With the deepening and development of pharmacology research, researchers have demonstrated that *B. scoparia* mainly contains saponins, momordin Ic, triterpenoid glycosides, flavone glycosides, alkaloids, essential oils, trace elements, and other ingredients (Zou et al. 2021), and has anti-bacterial, anti-inflammatory, anti-allergic, anti-gastric mucosal damage, hypoglycemic, and immunity effects (Yan et al. 2015). Although there were many studies on the constituents and pharmacological activities of *B. scoparia*, the genomic information that offers critical information for management plans is limited. Chloroplast is maternally inherited in angiosperm plant species, whose genome sequences have been widely used in the delimitation and phylogeny of species (Yang et al. 2013; Ruhsam et al. 2015). With the

development of high-throughput sequencing technology, the chloroplast genomes of many plants have been completely sequenced and available on NCBI.

In this study, we sequenced the first complete chloroplast genome of *B. scoparia* using high-throughput sequencing technology and further reconstructed the phylogenetic relationships of the Amaranthaceae family, which would provide vital information on the genetic resources, phylogenetic relationships, and the species identification of the genus *Bassia*.

2. Materials

Samples of *B. scoparia* were collected from the germplasm resource nursery of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences, Hubei Province, China (N 30°31′53″, E 109°48′11″) (Figure 1). A specimen was deposited at the Herbarium of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences (Contact: Wei Fu, fuwei5@mail2.sysu.edu.cn) under the voucher number DF-202309.

3. Methods

For whole-genome DNA sequencing, the total genomic DNA was extracted from the fresh leaves using the modified CTAB method (Doyle and Doyle 1987). After DNA quality and quantity testing, a paired-end library with an insert size of 350 bp

Figure 1. Species reference image of *B. scoparia*. The photos were taken by Miss Lin Li at the germplasm resource nursery of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences, Hubei Province, China, in September 2023.

was constructed and sequenced on the DNBSEQ-T7 platform (Beijing Biomics Tech Co., Ltd., Beijing, China), producing approximately four million high-quality 150 bp paired-end reads. After removing the low-quality sequences and adapter sequences using Trimmomatic software (Version 0.39) (Bolger et al. 2014), we performed *de novo* assembly using GetOrganelle (version 1.7.1) (Jin et al. 2020) with the k-mer length: 21, 35, 45, 65, 85, and 105. CPGAVAS2 (Shi et al. 2019) was used to annotate the assembled chloroplast genome with the *Bassia littorea* chloroplast genome (OK539756) annotation as the reference, and CPGview (http://www.1kmpg.cn/cpgview) was employed to improve annotation, visualize the structure of chloroplast genome, and identify cis-splicing genes and trans-splicing genes (Liu et al. 2023). We identified simple sequence repeat (SSR) loci in the chloroplast genome were identified using MISA (https://webblast.ipk-gatersleben.de/misa/) (Beier et al. 2017) with 10, 6, 5, 5, 5, and 5 as the minimum number of repeats for mono-nucleotide, di-, tri-, tetra-, penta-, and hexa-, respectively. Finally, the annotated chloroplast genome of *B. scoparia* was deposited into the NCBI GenBank (PP048887).

To clarify the phylogenetic position of *B. scoparia*, the chloroplast genome sequence of 32 representative species was obtained from the GenBank database to reconstruct the chloroplast genome phylogenetic tree, with *Panax ginseng* (MH049735) (Wang et al. 2018) as an outgroup. A total of 69 common protein-coding genes were extracted from the genome annotation files using PhyloSuite (Version 1.2.2) (Zhang et al. 2020), and multiple sequence alignment was performed using the MAFFT (Version 7.407) (Katoh and Standley 2013). The aligned sequences were further concatenated into a supermatrix using PhyloSuite (Version 1.2.2) (Zhang et al. 2020), and the best-fit model (GTR + F + I + G4) of nucleotide substitution was estimated by Modeltest (Version 3.7)

(Posada and Crandall 1998). The concatenation matrix was used to construct a phylogenetic tree using a maximum-likelihood (ML) method implemented in IQtree (Version 1.7) (Nguyen et al. 2015) with 1000 bootstrap replicates.

4. Results

The complete chloroplast genome sequence of *B. scoparia* had a circular quadripartite structure with a total length of 151,278 bp, including a large single-copy (LSC) region of 84,067 bp, a small single-copy (SSC) region of 18,505 bp, and a pair of inverted repeat (IR) regions of 24,353 bp (Figure 2). The read depth map and coverage map are shown in Figure S1, and they yielded an average coverage of 983.25× (Figure S1). This sequenced chloroplast genome contained 133 predicted genes, including 88 protein-coding genes, 37 transfer RNA (tRNA) genes, and eight ribosomal RNA (rRNA) genes. Among them, nine protein-coding genes (*atpF*, *ndhA*, *ndhB*(x2), *petB*, *petD*, *rpl16*, *rpoC1*, and *rps16*) and eight tRNA genes (*trnA-UGC*(x2), *trnG-GCC*, *trnI-GAU*(x2), *trnK-UUU*, *trnL-UAA*, and *trnV-UAC*) had a single intron, and two protein-coding genes (*ycf3* and *clpP*) had two introns. Meanwhile, 11 cis-splicing genes and one trans-splicing gene were identified, and their structures are shown in Figure S2. The total GC content of this chloroplast genome was 36.6%, with the GC content in the IR region (42.90%) being significantly higher than that in the LSC region (34.48%) and the SSC region (29.94%). We also identified 48 SSRs, including 47 mononucleotides and one dinucleotide (Tables S1–S3).

To further study the phylogenetic position of *B. scoparia*, a phylogenetic analysis was conducted. The phylogenetic tree (Figure 3) showed that Amaranthaceae species formed a monophyletic group diverging from the outgroup, and *B. scoparia* was grouped with a clade containing *B. littorea*, *Suaeda*
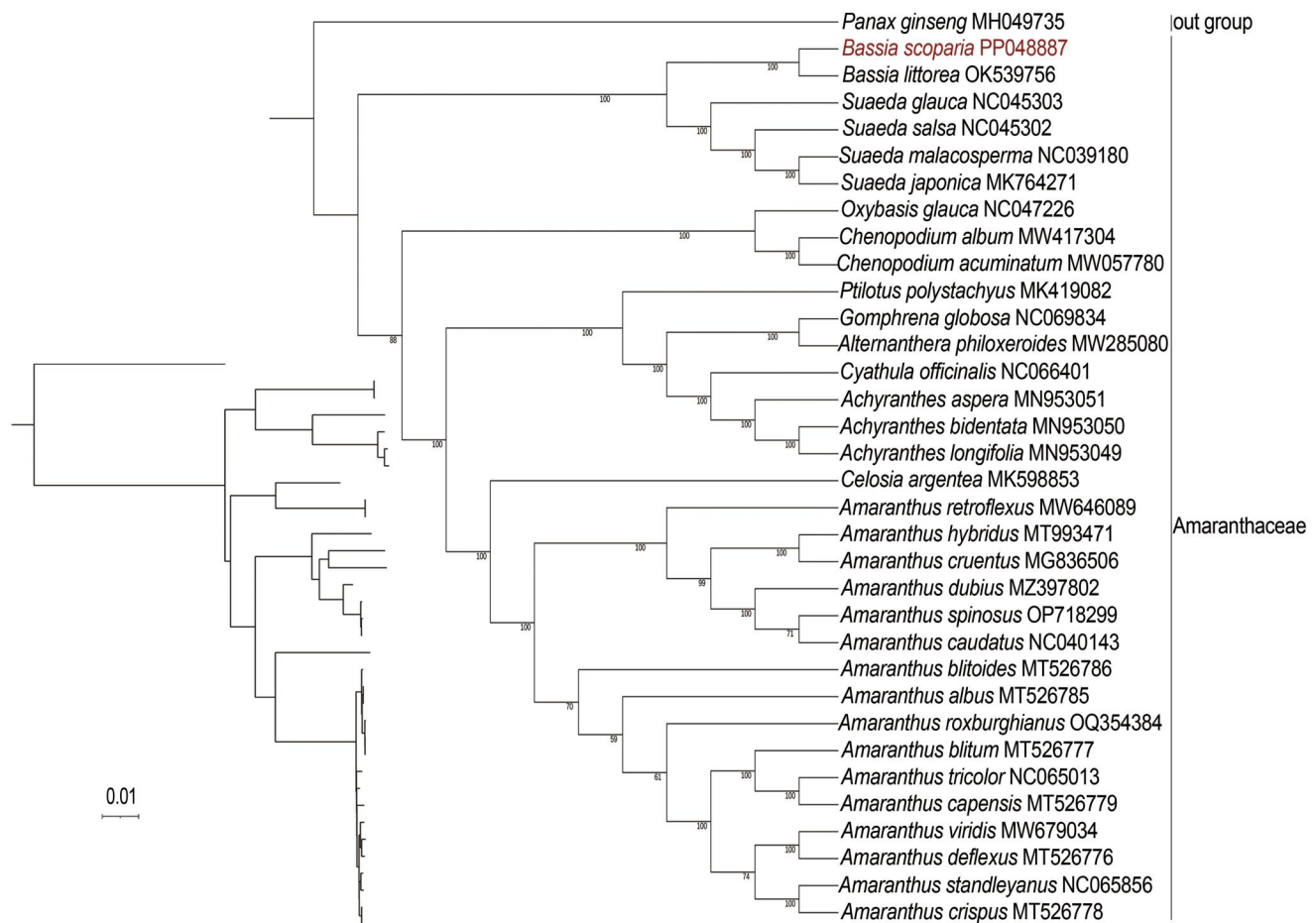
Figure 2. The map of *B. scoparia* chloroplast genome by CPGview. The map consists of six tracks. From the center to the outer, the first track shows dispersed repeats connected by red and green arcs indicating the direction (forward and reverse, respectively). The second track shows long tandem repeats as blue bands, and the third track shows short tandem repeats or microsatellites as green bands. The fourth track represents the GC content along the plastome. Finally, the sixth track represents the genes as colored boxes, the inner boxes present clockwise transcription, and the outer boxes present counterclockwise transcribed genes.

glauca, *Suaeda salsa*, *Suaeda malacosperma*, and *Suaeda japonica*. Moreover, *B. scoparia* exhibited the closest relationship with *B. littorea*, which is generally consistent with previous studies. These results based on the chloroplast genome reported here would facilitate phylogenetic and population genetic diversity of *B. scoparia*.

5. Discussion and conclusions

The chloroplast genome of land plants was highly conserved, which can provide information for the evolutionary relationship of species (Daniell et al. 2016; Mehmood et al. 2019). Many herbaceous plants of the Amaranthaceae family are highly similar morphologically, and the genomic information is limited. Thus, we reported the first complete chloroplast genome of *B. scoparia*. In the current study, the complete

chloroplast genome sequence was 151,278 bp in length and exhibited a typical quadripartite structure, including an LSC region (84,067 bp), an SSC region (18,505 bp), and a pair of IR regions (24,353 bp). In total, 133 genes were annotated, including 88 PCG genes, 37 tRNA genes, and eight rRNA genes. The total GC content of the chloroplast genome was 36.6%. These features are comparable to the published chloroplast genome of *B. littorea*. The phylogenetic results indicate that *B. scoparia* exhibits the closest relationship with *B. littorea*, which is consistent with the traditional taxonomy.

Amaranthaceae is a large family containing about 65 genera and 850 species (Hundiwale et al. 2012; Chandrashekhar 2020). Analysis of the chloroplast genome helps understand their relationships and study the phylogeny. The complete chloroplast genome presented in this study would provide a genetic resource for further genetic and genomic studies of

Figure 3. Phylogenetic relationships of *B. scoparia* based on the maximum-likelihood (ML) analysis of protein-coding genes in chloroplast genomes. Bootstrap values next to the nodes are based on 1000 replications. *Panax ginseng* was set as the outgroup. GenBank accession numbers: *Panax ginseng* MH049735 (Wang et al. 2018), *Bassia littorea* OK539756, *Suaeda glauca* NC045303 (Qu et al. 2019a), *Suaeda salsa* NC045302 (Qu et al. 2019b), *Suaeda malacosperma* NC039180 (Park et al. 2018), *Suaeda japonica* MK764271 (Park et al. 2018), *Oxybasis glauca* NC047226, *Chenopodium album* MW417304 (Li et al. 2021), *Chenopodium acuminatum* MW057780 (Wariss and Qu 2021), *Celosia argentea* MK598853 (Qian et al. 2019), *Ptilotus polystachyus* MK419082 (Hammer et al. 2019), *Gomphrena globosa* NC069834, *Alternanthera philoxeroides* MW285080 (Jiang et al. 2021), *Cyathula officinalis* NC066401 (Guo et al. 2022), *Achyranthes aspera* MN953051 (Xu et al. 2020), *Achyranthes bidentata* MN953050 (Xu et al. 2020), *Achyranthes longifolia* MN953049 (Xu et al. 2020), *Celosia argentea* MK598853(Qian et al. 2019), *Amaranthus retroflexus* MW646089 (Lou and Fan 2021), *Amaranthus hybridus* MT993471 (Bai et al. 2021), *Amaranthus cruentus* MG836506 (Hong et al. 2019), *Amaranthus dubius* MZ397802 (Xu et al. 2021), *Amaranthus spinosus* OP718299, *Amaranthus caudatus* NC040143 (Hong et al. 2019), *Amaranthus blitoides* MT526786 (Xu et al. 2022), *Amaranthus albus* MT526785 (Xu et al. 2022), *Amaranthus roxburghianus* OQ354384, *Amaranthus blitum* MT526777 (Xu et al. 2022), *Amaranthus tricolor* NC065013, *Amaranthus capensis* MT526779 (Xu et al. 2022), *Amaranthus viridis* MW679034 (Ding et al. 2021), *Amaranthus deflexus* MT526776 (Xu et al. 2022), *Amaranthus standleyanus* NC065856 (Xu et al. 2022), and *Amaranthus crispus* MT526778 (Xu et al. 2022).

the genus *Bassia*. In the future, increasing the number of chloroplast genomes of family species will provide deeper insights into the evolution of this important family.

Author contributions

Lin Li (L.L.) and Haiying Wan (H.W.) conceived the research; Wei Fu (W.F.) conducted the bioinformatics analysis; Zhenxing Zhu (Z.Z.), Qiaohui Zhang (Q.Z.), Yajie Li (Y.L.), and Lan Long (L.L.) contributed reagents/materials and performed the experiments; Wei Fu (W.F.) drafted the paper. Lin Li (L.L.) and Haiying Wan (H.W.) revised the paper critically for intellectual content. All of the authors have read and approved the final manuscript and have agreed to be accountable for all aspects of the work.

Ethical approval

No ethical approval/permission is required in this study. This study includes no human, animal, or endangered plant samples, and the sample was legally collected in accordance with guidelines provided by the authors' institution and national or international regulations.

Disclosure statement

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

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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 the accession no. PP048887. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1063496, SRR27534540, and SAMN39397596, respectively.

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