


## The complete chloroplast genome of *Pyankovia brachiata* (amaranthaceae), an annual desert plant in China

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### ABSTRACT

*Pyankovia brachiata* (Pall.) Akhani & Roalson 2007, is an annual plant belonging to the genus *Pyankovia*, family Amaranthaceae, which is widely distributed in the inland deserts of Northwest China. *P. brachiata* was previously categorized under the genus *Salsola* in Salsoleae and has been a long-standing topic of debate. Therefore, the complete chloroplast genome of *P. brachiata* must be studied to provide a theoretical reference for species classification. In this study, we sequenced *P. brachiata* samples and determined the species' complete chloroplast genome. The complete chloroplast genome was 149,922 bp in length, with one large single copy (LSC: 83,565 bp), one small single copy (SSC: 18,535 bp), and two inverted repeat regions (IRa and IRb, 23,911 bp each). It contains 132 genes, including 87 protein-coding, eight rRNA, and 37 tRNA genes. The phylogenetic position showed that *P. brachiata* has the closest relationship with *Caroxylon passerinum* (accession number: NC057191.1). This study will provide genetic information and be beneficial to understanding the systematic position of *P. brachiata* within the Amaranthaceae.

### ARTICLE HISTORY

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### Introduction

As a member of the family Amaranthaceae, *Pyankovia brachiata* (Pall.) Akhani & Roalson 2007 (Figure 1), is a dominant inland desert annual plant with drought and salinity resistance and is mainly distributed in the gravel desert and piedmont of the Xinjiang Uygur Autonomous Region, China (Commissione Redactorum Florae Xinjiangensis 1994). This species exhibits a positive effect on soil and water conservation, thereby serving vital ecological functions in sand fixation (Huang 2005).

At present, chloroplast (cp) DNA is widely used for plant classification, phylogeny, and species identification because of its conserved genome structure and highly variable intergenic spacers (Mohanta et al. 2019; Ramundo et al. 2020; Daniell et al. 2021). However, information regarding the chloroplast genome of *P. brachiata* remains unreported, and only a limited number of studies have explored seed polymorphism, seed germination characteristics, and population dynamics of the species (Wei et al. 2008; Luo et al. 2015; Liu et al. 2018). This scarcity of research impedes the accurate determination of its systematic position through molecular methods. The classification of Salsoleae has been a long-standing topic of debate. *P. brachiata* was initially classified under the genus *Salsola* in 1803. Then, it was treated as a member of *Climacoptera* (Botschantzev 1956). Recently, the

application of molecular techniques in plant systematics has resulted in significant changes in the classification of the genus *Salsola*. In 2007, *P. brachiata* was reclassified from the genus *Salsola* to *Pyankovia* based on the first comprehensive phylogenetic analysis of Salsoleae using cp psbB-psbH DNA sequences (Akhani et al. 2007). However, the previous classification remains doubtful due to support from partial chloroplast data. Therefore, the study of the cp genome of *P. brachiata* is needed to provide a more accurate theoretical reference for species classification and phylogenetic position.


In this study, we sequenced the complete chloroplast genome of *P. brachiata*, with the aim of further exploring the phylogenetic relationships within the Salsoleae tribe and the Amaranthaceae family.

### Materials and methods

The fresh leaves of *P. brachiata* were collected from the Tianshan Mountains, Xinjiang Uygur Autonomous Region of China (43°47'39.9"N, 87°28'41.74"E). The voucher specimens (contact person: Huafeng Liu, email: [dtliu@qq.com](mailto:dtliu@qq.com), accession number: CWNU-LHF-202102) were deposited at the herbarium of the College of Life Sciences, China West Normal University.

Total genomic DNA was isolated using the CTAB method from the fresh leaves of *P. brachiata* (Doyle and Doyle 1987).

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**Figure 1.** Species reference image of *Pyankovia brachiata*. This image was taken from the piedmont of tianshan mountains, Xinjiang Uygur autonomous region of China (43°47'39.9"N, 87°28'41.74"E) by kaiqing xie (October 2021).

Subsequently, the genomic DNA was processed according to the standard protocol provided by Illumina Company. The sequencing of the total genomic DNA was performed on the Illumina HiSeq X-Ten platform (Illumina, San Diego, USA), with 150 bp paired-end (PE) reads. A total of 14.8 GB of clean data was checked using FastQC (v.0.12.0, <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and the sequencing depth and coverage map for *P. brachiata* was assessed using the Draw\_SequencingDepth.py script provided by Ni et al. (2023). The cp genome sequence was assembled using GetOrganelle v. 1.7.5.3 (Jin et al. 2020), and the K-mer length was selected as 39 bp. The complete cp genome sequence was annotated using GeSeq software v. 2.03 (Jin et al. 2020), with *Salsola abrotanoides* (accession number: NC057096) as a reference, and adjusted manually in Geneious v. 7.19 (Biomatters, Inc., Auckland, New Zealand). CPGView (Liu et al. 2023) was used to visualize the structure of cis- and trans-splicing. Finally, the cp genome structure map was constructed using OGDRAW v. 1.3.1 (Greiner et al. 2019). The results have been submitted to the chloroplast genome database (GenBank number: ON651428.1).

To determine the phylogenetic position of *P. brachiata*, we conducted a phylogenetic analysis using its complete cp genome alongside those of 22 other related species within the Amaranthaceae family retrieved from the NCBI database. All of the cp genome sequences were aligned using MAFFT v. 7 (Katoh and Standley 2013). A maximum likelihood tree was constructed with the RAxML v. 8 program (Alexandros 2014) using 1000 bootstraps under the GTR+G model with default parameters. *Arabidopsis thaliana* (accession number: NC000932.1) and *Cistanche deserticola* (accession number: KC128846.1) served as outgroups.

## Results

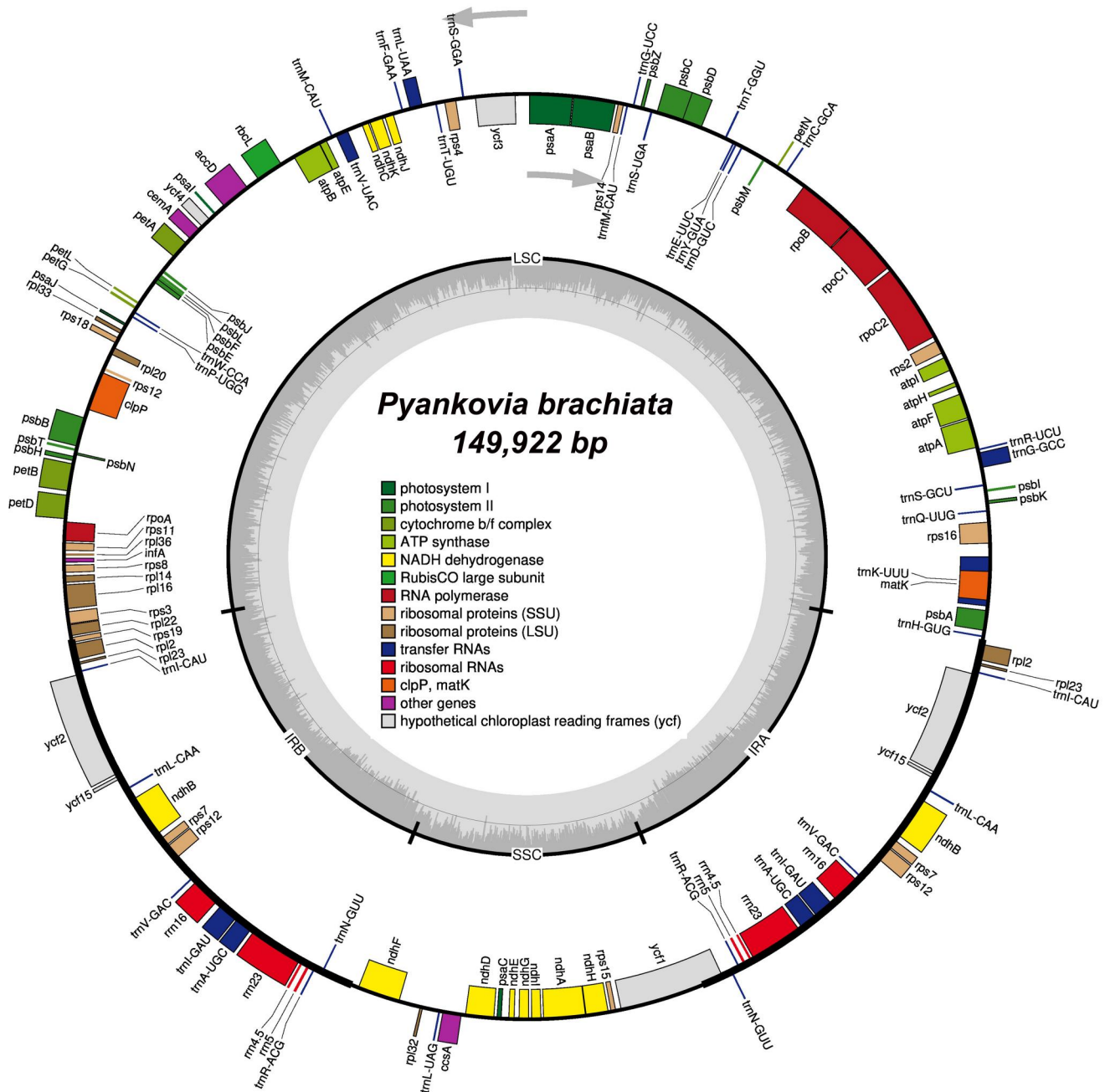
The complete cp genome of *P. brachiata* was 149,922 bp, with a large single-copy (LSC) region of 83,565 bp, a small single-copy (SSC) region of 18,535 bp, and two inverted repeat (IRa and IRb) regions of 23,911 bp each (Figure 2). The overall GC content was 36.2%, which is lower than that of IR regions (42.7%) and higher than that of the LSC (34.1%) and SSC regions (28.7%). The minimum and maximal read mapping depths for *P. brachiata* assembled chloroplast genomes were 53× and 3434× (average 1705× depth) (Figure S1).

The genome contained 132 genes, of which 114 were unique, including 87 protein-coding genes (80 unique), eight rRNA (4 unique), and 37 tRNA genes (30 unique). Among these genes, 17 genes possess one intron each (including rps16, atpF, rpoC1, petB, petD, rpl16, ndhB × 2, ndhA, trnK-UUU, trnG-GCC, trnL-UAA, trnV-UAC, trnA-UGC × 2, trnI-GAU × 2), and 4 genes contain two introns each (namely rps12 × 2, ycf3, and clpP). Furthermore, 11 cis-splicing genes (rps16 × 2, atpF, rpoC1, ycf3, clpP, petB, petD, rpl16, rpl2, ndhB × 2, ndhA) and one trans-splicing gene (rps12) were recognized in this analysis (Figures S2 and S3).

The phylogenetic relationships between *P. brachiata* and other members of the Amaranthaceae family were explored. Based on existing complete cp genome data, the phylogenetic position showed that *P. brachiata* aggregates with *Caroxylon passerinum* (accession number: NC057191.1) as a branch, showing that they have the closest relationship with bootstrap support values of 100% (Figure 3).

## Discussion and conclusion

The previous classification is still doubted in terms of partial chloroplast support and highly similar morphology,

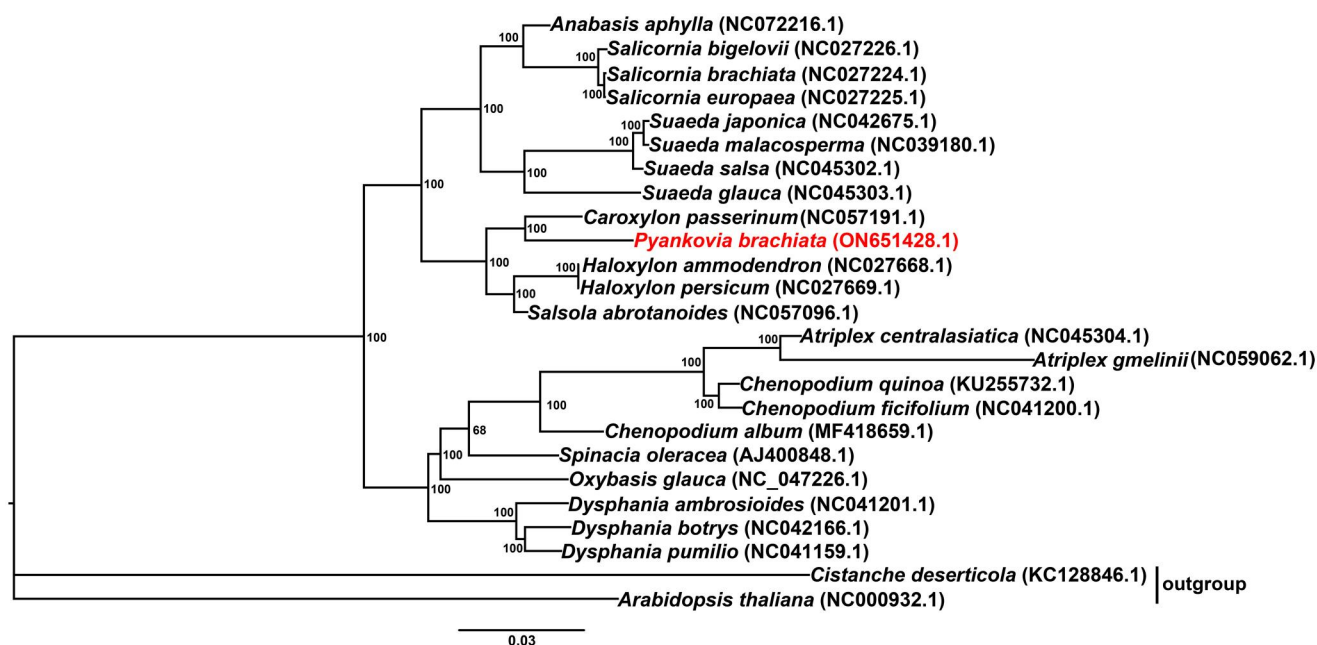


**Figure 2.** The chloroplast genome structure of *Pyankovia brachiata*. Genes on the inside map are transcribed clockwise and those on the outside map are transcribed counter clockwise. Genes are color-coded based on their functional categories. Large single-copy (LSC) region, small single-copy (SSC) region, large single copy (LSC), and a pair of inverted repeat regions (IRa and IRb) are indicated.

which have significantly hindered phylogenetic investigations of Salsoleae. Currently, the complete chloroplast genome is increasingly and widely utilized to resolve outstanding questions in plant taxonomy (Ruhsam et al. 2015). In this study, the complete cp genome of *P. brachiata* was sequenced, assembled, and annotated for the first time. The genomic characterization and the phylogenetic relationship of *P. brachiata* among Amaranthaceae species were further analyzed. The phylogenetic analysis revealed that based on available data, *P. brachiata* is most closely related to *Caroxylon passerinum*. This finding

supports the grouping of the *Caroxylon* clade and *Climacoptera* clade within the tribe Caroxyloneae as sister groups. (Akhani et al. 2007). Notably, the complete chloroplast genomes of Salsoleae in NCBI remain relatively limited, up to on June 12, 2024. Therefore, to solve and improve the disputes and deficiencies in the traditional morphological classification, additional complete chloroplast genome sequences of Salsoleae species are required for further study. In summary, this study enriched and provided new information for the phylogenetic relationship within the Amaranthaceae family.





**Figure 3.** The phylogenetic tree of 23 amaranthaceae species and two outgroups. The phylogenetic position of *Pyankovia brachiata* (shown in red) was based on the complete chloroplast genome by Maximum-Likelihood method. The following sequences were used: *Anabasis aphylla* NC072216.1, *Atriplex gmelinii* NC059062.1 (Park et al. 2022), *Atriplex centralasiatica* NC045304.1 (Zhang et al. 2019), *Chenopodium quinoa* KU255732.1, *Chenopodium ficifolium* NC041200.1 (Kim et al. 2019a), *Chenopodium album* MF418659.1 (Devi and Thongam 2017), *Spinacia oleracea* AJ400848.1, *Oxybasis glauca* NC\_047226.1, *Dysphania ambrosioides* NC041201.1 (Kim and Park 2019), *Dysphania pumilio* NC041159.1 (Kim et al. 2019b), *Dysphania botrys* NC042166.1 (Chen and Yang 2018), *Haloxylon persicum* NC027669.1 (Dong et al. 2016), *Haloxylon ammodendron* NC027668.1 (Dong et al. 2016), *Salsola abrotanoides* NC057096.1 (Li et al. 2021), *Caroxylon passerinum* NC057191.1 (Xie et al. 2022), *Suaeda malacosperma* NC039180.1 (Park et al. 2018), *Suaeda japonica* NC042675.1 (Kim et al. 2019c), *Suaeda salsa* NC045302.1, *Suaeda glauca* NC045303.1 (Qu et al. 2019), *Salicornia europaea* NC027225.1, *Salicornia bigelovii* NC027226.1, *Arabidopsis thaliana* NC000932.1 and *Cistanche deserticola* KC128846.1 (Li et al. 2013) (outgroup). The genbank accession numbers are listed right to their scientific names, and numbers near the nodes sequentially indicate ML support values from 1000 replicates. The ML tree was analyzed based on the GTR + GAMMA model.

## Ethics statement

This study was approved by the authors' institution and national. This article does not contain any research on endangered plants or animal species by any of the authors. All research was conducted in accordance with ethical guidelines and the legal requirements of the study country. The collection of specimens conformed to the Regulations on the Protection of Wild Plants of the People's Republic of China, which does not damage the local environment, and the study species is not included in the List of National Key Protected Wild Plants in China.

## Authors' contributions

HFL, DLH, and KQX conceived and designed the work. KQX collected the samples. HFL and DLH analyzed the data. HFL wrote the manuscript. DLH and KQX revised the manuscript. All the authors have read and agreed to the published version of the manuscript.

## 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 NCBI, GenBank accession number for this study: ON651428 (<https://www.ncbi.nlm.nih.gov/nuccore/ON651428.1/>). The associated BioProject, Bio-Sample, and SRA numbers are PRJNA842837, SAMN28688634, and SRR19426909, respectively. Please note data should only be shared if it is ethically correct to do so, where this does not violate the protection of human subjects, or other valid ethical, privacy, or security concerns.

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