

PLASTOME REPORT



The complete chloroplast genome of *Buxus sinica* var. *parvifolia* (Buxaceae) and its phylogenetic analysis

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ABSTRACT

Buxus sinica var. *parvifolia* is a shrub or small arbor of the Buxaceae family, rich in various medicinal alkaloids and of great horticultural value. In this study, we sequenced, assembled, and annotated the complete chloroplast genome of *B. sinica* var. *parvifolia* for the first time. The length of the chloroplast genome is 158,995 bp with 38.1% overall GC content. It includes a large single-copy (LSC) region of 88,140 bp, a small single-copy (SSC) region of 17,761 bp, and two inverted repeat regions of 26,547 bp. Additionally, 132 functional genes in the genome are identified, including 87 protein-coding genes, eight ribosomal RNA genes, and 37 transfer RNA genes. Phylogenetic analysis showed that *B. sinica* var. *parvifolia* is closely related to *Buxus microphylla*. The complete chloroplast genome sequence of *B. sinica* var. *parvifolia* and its phylogenetic analysis provides useful genomic information for the further study of *B. sinica* var. *parvifolia* and other *Buxus* species.

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Introduction

Buxus sinica var. *parvifolia* M. Cheng is a perennial medicinal shrub or small arbor belonging to the genus *Buxus* in the Buxaceae, which occurs naturally in cliff habitats in semi-tropics alpine areas of China, at altitudes between 1200 and 3000 m above sea level (Cheng 1979; Min and Brückner 2008). Chemical compositional analyses have shown that *B. sinica* var. *parvifolia* contains more than 200 alkaloids (Lv et al. 2013) including cyclovirobuxine D (CVB-D) (Bai and Pang 2021). Modern pharmacological studies have shown that these alkaloids exhibit good anti-arrhythmic, anti-myocardial ischemic, and cardiostimulant effects in animals and clinical trials (An and He 2015; Wang, Zhang, et al. 2022), and inhibit the proliferation of non-small cell lung cancer A549 (Ming et al. 2024). As an important horticultural plant, *B. sinica* var. *parvifolia* is hardy, drought-tolerant (Li et al. 2023; Jiang et al. 2024), and has strong particulate matter adsorption properties (Huang et al. 2023).

Since its narrow distribution and the large number of excavations for bonsai cultivation, the wild resources are on the verge of extinction. It has been listed as a second-grade protected plant in Anhui Province, China (Huang et al. 2005). In addition, the taxonomic status and phylogenetic relationships of *B. sinica* var. *parvifolia* in the genus *Buxus* have yet remained controversial. It was first published as a variety of *B. sinica* (Cheng 1979), while it was regarded as a synonym of *B. rugulosa* or a distinctive species with closer relationship to *B. henryi*, subsequently (Lin 2004; Wang et al. 2012).



Currently, although few molecular studies involving RAPD, ISSR markers, and ITS sequences have been conducted, the intrageneric relationships and species discrimination of *B. sinica* var. *parvifolia* were still uncertain (Huang et al. 2008; Jiang et al. 2008; Wang et al. 2012).


The chloroplast genome, with its small size, highly conserved structure, and uniparental inheritance, can provide well solution for species discrimination and delimitation in taxonomically difficult taxa, as far as to inferring phylogenetics of angiosperms (Dodsworth 2015; Nguyen et al. 2024). Therefore, this study reports the complete chloroplast genome sequence of *B. sinica* var. *parvifolia* and further explores the phylogenetic relationships among the genus *Buxus*, which will contribute to future studies on species identification, phylogenetic evolution, and species conservation of *B. sinica* var. *parvifolia*.

Materials and methods

Fresh leaves of *B. sinica* var. *parvifolia* (Figure 1) were collected from Chenggong County, Kunming (24°52′0.995″N, 102°51′16.668″E), Yunnan Province, China. The voucher specimen (LMF09) has been deposited in the Herbarium of Yunnan Normal University (YNUB, website: <https://life.ynnu.edu.cn/>, Contact: Jian-Lin Hang, Email: hjlynub@163.com).

The genomic DNA from fresh samples was extracted using a modified CTAB (cetyltrimethylammonium bromide) method (Allen et al. 2006) and the constructed library was sequenced

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Figure 1. Plant morphological characteristics of *B. sinica* var. *parvifolia*. Yue Yin took these photos in Chenggong County, Kunming, Yunnan Province, China. (A) The leaves of this species are small, broadly elliptic, or broadly ovate. (B) The capsules of this species are glabrous.

using the Illumina HiSeq 2500-PE150 platform (Illumina, San Diego, CA). All raw reads were filtered to obtain clean reads with default parameters using NGS QC Toolkit v2.3.3 (Patel and Jain 2012). These trimmed reads were assembled by GetOrganelle software (Jin et al. 2020). Geneious v2022.2.2 (Kearse et al. 2012) was used for annotation based on the *B. microphylla* (GenBank accession number: NC_009599) chloroplast genome (Li, Li, et al. 2021) and then manually corrected. The circular genome map was generated using CPGAVAS2 (Shi et al. 2019, <http://47.96.249.172:16019/analyzer/annotate>). The schematic map of the trans-splicing and cis-splicing genes was generated using the CPGView software (Liu et al. 2023, <http://www.1kmpg.cn/cpgview/>). The assembly was further evaluated using Trim Galore 0.6.4 and Samtools (Li et al. 2009), respectively. The assembled chloroplast genome sequence was submitted to the GenBank (accession number: OQ236088) of the National Center for Biotechnology Information (NCBI). For phylogenetic analysis, the complete chloroplast genome sequences of 25 reported species were downloaded from NCBI (*Paeonia*: four species, *Dillenia*: two species, *Liquidambar*: two species, *Exbucklandia*: two species, *Loropetalum*: two species, *Altingia*: two species, *Pachysandra*: two species, and one species was selected from *Daphniphyllum*, *Cercidiphyllum*, *Hamamelis*, *Tetracera*, *Semiliquidambar*, *Tetracentron*, *Trochodendron*, and *Buxus*, respectively). The outgroup was

Meliosma oldhamii. Sequences were aligned by MAFFT (Katoh and Toh 2010). The best nucleotide substitution model GTR + G + I was calculated by MEGA X (Kumar et al. 2018). Maximum-likelihood (ML) analysis was performed with RAXML v8.0 (Stamatakis 2014) using default parameters and 1000 bootstrap replicates.

Results

The complete chloroplast genome sequence of *B. sinica* var. *parvifolia* is 158,995 bp in length, containing a large single-copy (LSC) region of 88,140 bp, a small single-copy (SSC) region of 17,761 bp, and a pair of inverted repeat regions (IRa and IRb) of 26,547 bp (Figure 2). We annotated 132 genes, including 87 protein-coding genes, 37 tRNA genes, and eight rRNA genes. The average coverage depth ($1859.6\times$) of the *B. sinica* var. *parvifolia* chloroplast genome is shown in Figure S1. The base compositions of the chloroplast genome were uneven (A: 30.6%, T: 31.4%, G: 18.7%, C: 19.4%). The overall GC content of the chloroplast genome was 38.1%, and 43% for the IRs, which was higher than that in LSC and SSC regions (32.2%, and 36.3%, respectively). The GC content in the rRNA (55.4%) was higher than that in the tRNA (53.3%). In total, 19 genes replicate in the IR region, repeating inversely with each other, including eight protein-coding genes (*rpl2*, *rpl23*, *ycf2*, *ndhB*, *rps7*, *rps12*, *ycf15*, and *ycf1*), seven transfer RNA genes (*trnI*-CAU, *trnL*-CAA, *trnV*-GAC, *trnI*-GAU, *trnA*-UGC, *trnR*-ACG, and *trnN*-GUU), and four ribosomal RNA genes (*rrn16S*, *rrn23S*, *rrn4.5S*, and *rrn5S*). The *rps12* is a trans-splicing gene (Figure S2) and 13 genes including *rps16*, *atpF*, *rpoC1*, *ycf3*, *clpP*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, *ndhA*, *ndhB*, and *rpl2* are cis-splicing genes (Figure S3).

To understand the phylogenetic position of *B. sinica* var. *parvifolia*, we selected 25 reported chloroplast genomes, using *Meliosma oldhamii* as an outgroup. Our result indicated that *B. sinica* var. *parvifolia* was closely related to *B. microphylla* with 100% bootstrap support and was clustered with the clade of two *Pachysandra* species (BS = 100%) (Figure 3).

Discussion and conclusions

In this study, the chloroplast genome of *B. sinica* var. *parvifolia* is sequenced, assembled, and annotated for the first time. The chloroplast genome structures of *B. sinica* var. *parvifolia* contain one LSC region, one SSC region, and two IR regions, which is consistent with the basic quadripartite structure characteristics of angiosperm chloroplast genomes (Hansen et al. 2007). Meanwhile, it has a length of 158,995 bp, with 132 genes, which is not significantly different from the size reported in previous studies of *B. microphylla* (NC_009599, 159,010 bp with 139 genes, Hansen et al. 2007), indicating a close genetic relationship in the structural characteristics of the chloroplast genome.

B. sinica var. *parvifolia*, along with five other varieties, forms a subordinate taxonomic group of *B. sinica* (Min and Brückner 2008). Due to their similarity in leaf shape and capsule characteristics, it is difficult to identify their lineage relationship solely based on morphological features (Lin 2004). In

Buxus sinica var. *parvifolia*

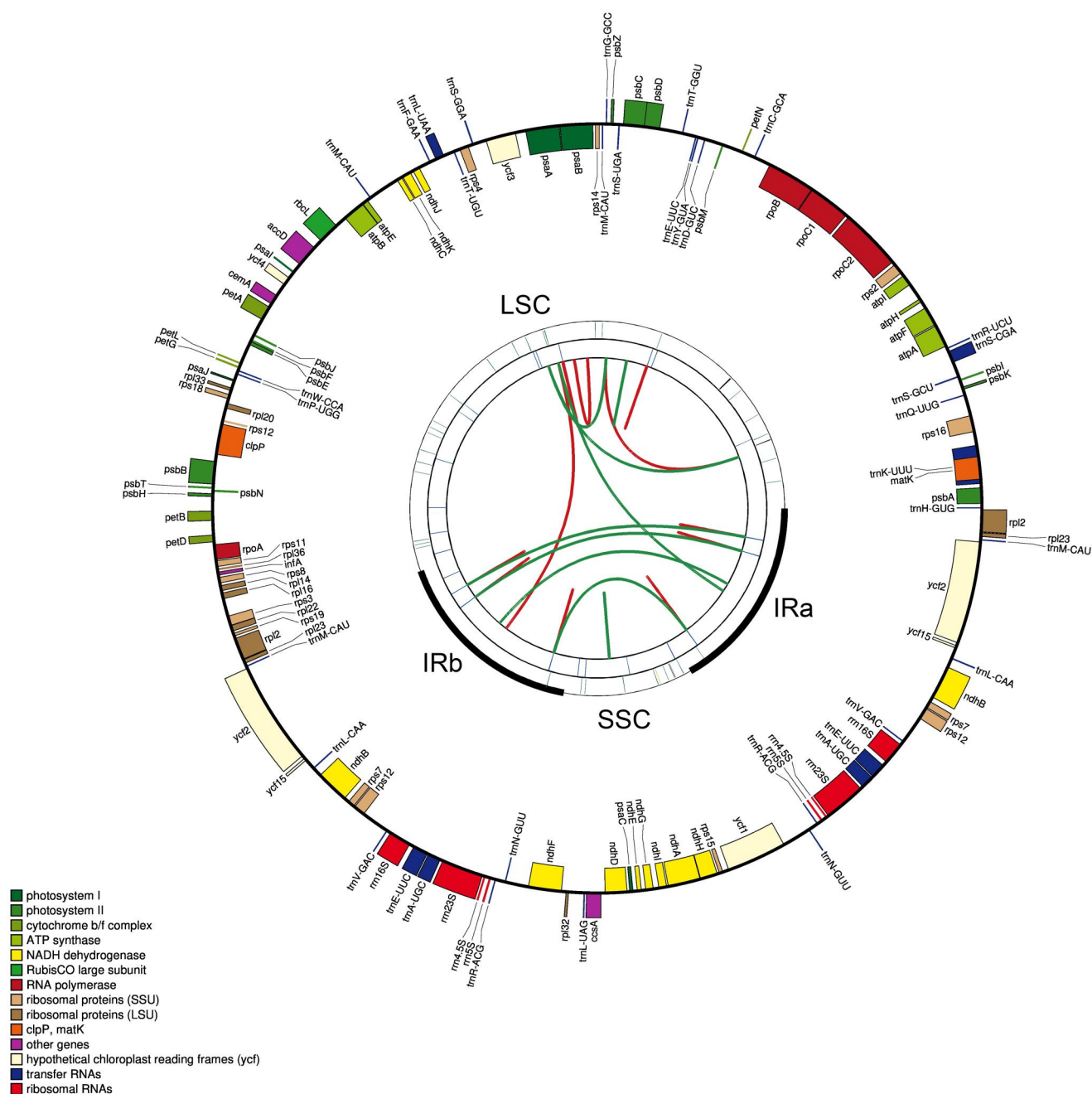


Figure 2. Gene map of the *B. sinica* var. *parvifolia* chloroplast genome. The map contains four rings. From the center going outward, the first circle shows the forward and reverse repeats connected with red and green arcs, respectively. The next circle shows the tandem repeats marked with short bars. The third circle shows the microsatellite sequences identified using MISA. The fourth circle shows the gene structure on the plastome. The genes were colored based on their functional categories.

this study, the results of phylogenetic analysis show that there is a sister relationship between *B. sinica* var. *parvifolia* and *B. microphylla*. In previous study, Wang et al. (2012) proposed that *B. sinica* var. *parvifolia* should be a sister species instead of a variant of *B. sinica*, with closer genetic relationship to *B. henryi*, utilizing ITS sequences. However, in both studies, it was inadequate with only one variant, *B. sinica* var. *parvifolia*, to explore the intraspecific phylogenetic

relationship. Therefore, to better understand the intragenetic and intraspecific phylogenetic relationships within the genus, it is necessary to further increase the samples of the chloroplast genome of *Buxus* in the future for in-depth research. This study has contributed to the enlargement of the chloroplast genome database for *Buxus* and has provided valuable insights into the evolutionary relationships among various *Buxus* species.

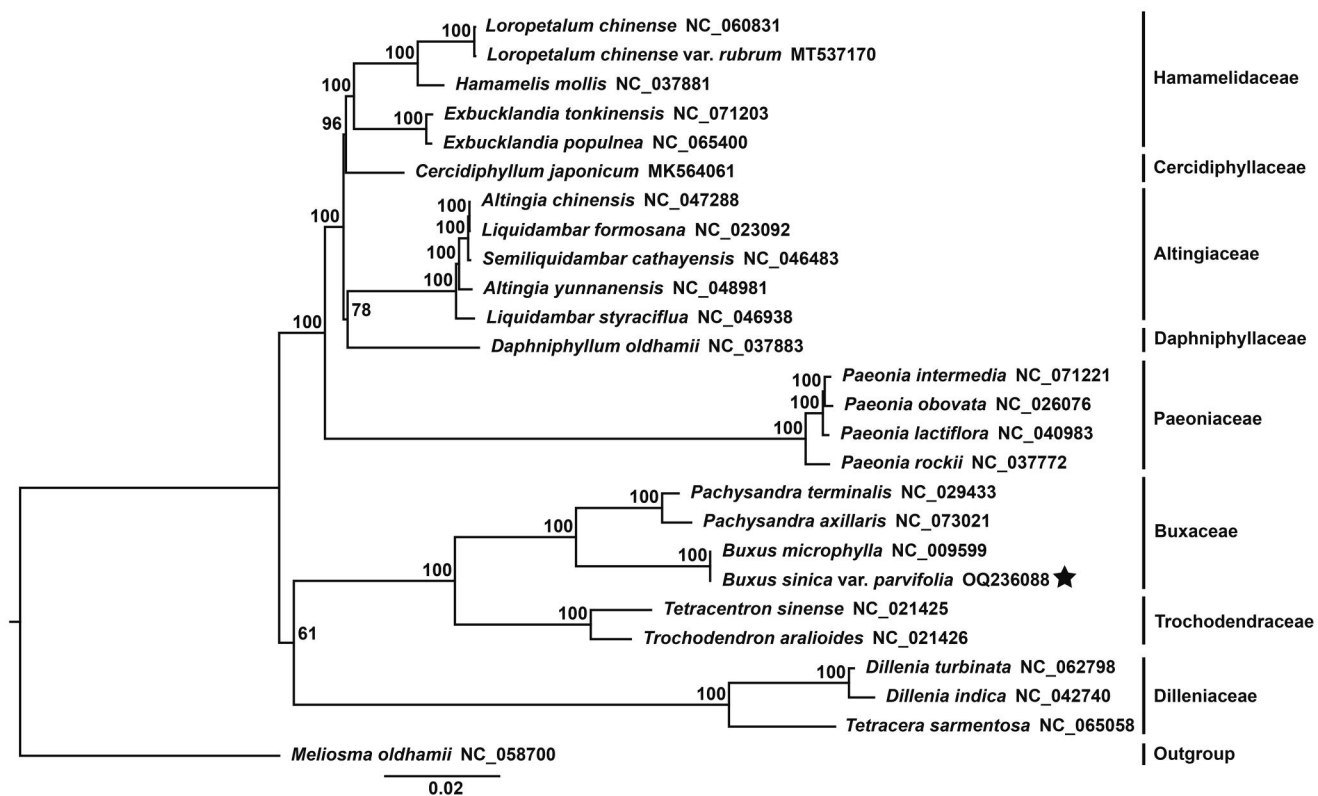


Figure 3. The phylogenetic position for *B. sinica* var. *parvifolia* according to the ML phylogenetic tree constructed based on 26 chloroplast genomes with *Meliosma oldhamii* as outgroup. The numbers above the nodes indicate the bootstrap values from 1000 replicates analysis. The scale bar in the lower left corner of the figure represents the evolutionary distance, with a unit length of 0.02. The following sequences were used: *Altingia chinensis* NC_047288, *Altingia yunnanensis* NC_048981 (Qiu et al. 2020), *Buxus microphylla* NC_009599 (Hansen et al. 2007), *Buxus sinica* var. *parvifolia* OQ236088 (this study), *Cercidiphyllum japonicum* MK564061 (Zhu et al. 2019), *Daphniphyllum oldhamii* NC_037883 (Dong et al. 2013), *Dillenia indica* NC_042740 (Tan et al. 2019), *Dillenia turbinata* NC_062798 (Li, Xie, et al. 2021), *Exbucklandia populnea* NC_065400, *Exbucklandia tonkinensis* NC_071203, *Hamamelis mollis* NC_037881 (Dong et al. 2018), *Liquidambar formosana* NC_023092 (Dong et al. 2013), *Liquidambar styraciflua* NC_046938, *Loropetalum chinense* NC_060831 (Wang, Chen, et al. 2022), *Loropetalum chinense* var. *rubrum* MT537170, *Meliosma oldhamii* NC_058700, *Pachysandra axillaris* NC_073021, *Pachysandra terminalis* NC_029433 (Sun et al. 2016), *Paeonia intermedia* NC_071221, *Paeonia lactiflora* NC_040983 (Samigullin et al. 2018), *Paeonia obovata* NC_026076, *Paeonia rockii* NC_037772 (Bai et al. 2018), *Semiliquidambar cathayensis* NC_046483 (Tang et al. 2020), *Tetracentron sinense* NC_021425 (Sun et al. 2013), *Tetracera sarmentosa* NC_065058, and *Trochodendron aralioides* NC_021426 (Sun et al. 2013).

Author contributions

Yonghong Zhang designed the research and revised the manuscript. Yue Yin analyzed data and prepared a preliminary manuscript. Tao Xiao analyzed the data and revised the manuscript. All authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI (<https://www.ncbi.nlm.nih.gov/>) with accession number OQ236088. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA945587, SRR23884465, and SAMN33789276, respectively.

Ethical approval

This research does not involve ethical issues. *Buxus sinica* var. *parvifolia* does not belong to the China Species Red List. The collection of plant material was carried out according to guidelines provided by the authors' institution (Yunnan Normal University) and national regulations.

Disclosure statement

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

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