

The complete chloroplast genome sequence of *Lycium barbarum* var. *implicatum* (Solanaceae), a new species of wolfberry from the Yellow River Basin in Ningxia, China

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ABSTRACT

Lycium barbarum var. *implicatum* (2010), is a new variety of *L. barbarum* in Solanaceae. Here, we sequenced, assembled, and annotated the complete chloroplast (cp) genome of *L. barbarum* var. *implicatum*. Results showed that the complete cp genome of *L. barbarum* var. *implicatum* was 154,888 bp in length, containing a large single copy (LSC) region of 85,894 bp, a pair of inverted repeats (IR) region of 25,393 bp, and a small single copy (SSC) region of 18,208 bp. Maximum-likelihood (ML) phylogenetic tree elucidated that *L. barbarum* var. *implicatum* was sister to *L. ruthenicum*. Our results provide useful information for future phylogenetic studies in the family of Solanaceae.

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1. Introduction


Lycium barbarum var. *implicatum* T. Y. Chen & Xu L. Jiang in 2010, is a new variety of *L. barbarum* in Solanaceae, which was found in the Qingshui River tributary of the Yellow River Basin in Ningxia, and the whole plant is characterized by many dense branches, narrow leaves, 2-fid calyx, ovoid or elliptic berries appear purple or white transparent, juicy and sweet (Chen et al. 2012). This species can not only grow well in saline-alkali land and arid land, but also its fruit contains more nutrients, such as amino acids, flavonoids, polyphenols, pigments, polysaccharides, potassium, and trace elements than *Lycium barbarum* (Zhu et al. 2016). In particular, the higher crude protein content (Liu et al. 2016) can be used in the development of fermented feed and the wolfberry industry. However, studies on the phylogeny of *L. barbarum* var. *implicatum* remain limited, and there is little submission for nucleic-acid sequences in NCBI Genbank.

2. Materials and methods

Fresh leaves of *L. barbarum* var. *implicatum* were collected from Qingshui River Basin, a branch of the Yellow River, Ningxia Hui Autonomous Region, China (38°57'53.28 N, 106°25'21.32 E, alt. 1069 m), and dried with silica gel. The specimen (No. 2023LBVI001LY) was deposited in the

Herbarium of Plant and Plant Physiology Laboratory, Ningxia Technical College of Wine and Desertification Prevention (<http://www.nxfszs.cn/>, Wangsuo Liu, email: liuwangsuo@sina.com) (Figure 1). Genomic DNAs were extracted by a modified CTAB method (Stefanova et al. 2013). The genome sequencing was performed by Illumina Hiseq 2500 at Biomarker Technologies Corporation. A total of 31,284,518 paired-end raw reads were obtained, and each of which comprised 50–151bp. The sequencing coverage achieved a remarkable 99.9%, effectively capturing the comprehensive information of the *L. barbarum* var. *implicatum* chloroplast (cp) genome. By removing low-quality reads, the trimmed reads, which comprised 25,010,894bp in read bases, were assembled via the program SPAdes 3.15 (Prijbelski et al. 2020), with that of *Lycium barbarum* (GenBank: MH032560) as the initial reference. The CPGView program (<http://www.1kmpg.cn/cpgview/>) was conducted to generate the cp genome maps (Liu et al. 2023). A comparison of the junction of LSC, SSC, and IR regions was performed using R (version 4.2.0) to activate the IRscope website (<https://irscope.shinyapps.io/irapp/>) (Amiryousefi et al. 2018). The sequence of *L. barbarum* var. *implicatum* complete cp genome has been submitted to GenBank (accession number OR551485). 18 of the most relevant *Lycium* cp genomes in Solanaceae were downloaded from the NCBI database, and aligned with four outgroups using MAFFT-7.037 (Katoh and Standley 2013). We constructed

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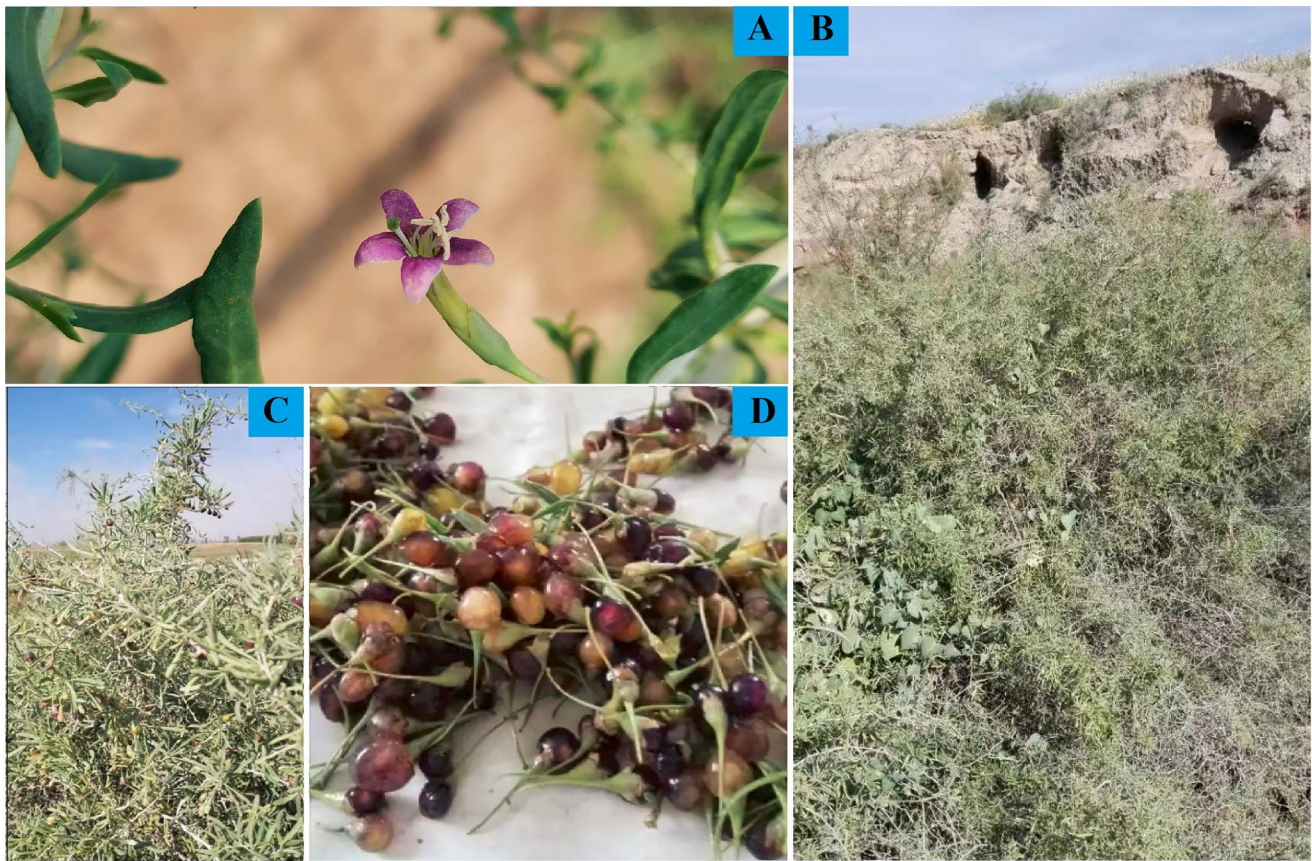


Figure 1. Morphological characteristics of *L. barbarum* var. *implicatum* (A: flower, bifid calyx; B: habitats, extremely arid and water-scarce; C: branches with thorns; D: berries, purple or white transparent). Photographs of *L. barbarum* var. *implicatum* were taken by Wangsuo Liu in the Qingshui River Basin (38°57'53.28 N, 106°25'21.32 E).

a molecular phylogenetic tree based on the already-aligned genomes, a maximum likelihood tree was generated by MEGA-X with the nucleotide model and 1000 bootstrap replicates (Kumar et al. 2018).

3. Results

The *L. barbarum* var. *implicatum* chloroplast genome is 154,888 bp in length, including a pair of inverted repeats (IRa and IRb) region of 25,393 bp, a large single copy (LSC) region of 85,894 bp, and a small single copy (SSC) region of 18,208 bp (Figure 2). Additionally, the GC content of the *L. barbarum* var. *implicatum* cp genome accounted for 37.9%, and the GC content in IR (43.2%) regions was higher than that of LSC (36%) and SSC (32.3%) regions. The results showed that the maximum and average read mapping depths of the assembled genomes were $\times 8000$ and $\times 4505$ (Figure S1), while the structures of cis- and trans-splicing genes are illustrated in Figure S2. As shown in Figure 2, the cp genome of *L. barbarum* var. *implicatum* contains 132 genes, including 87 protein-coding genes, 37 tRNA genes, and eight rRNA genes. In the IR region, there were 19 duplicated genes identified including 8 protein-coding genes (*ndhB*, *rpl2*, *rpl23*, *rps12*, *rps7*, *ycf1*, *ycf15*, *ycf2*), 4 rRNA genes (*rrn16S*, *rrn23S*, *rrn4.5S*, *rrn5S*), and 7 tRNA genes (*trnI-CAU*, *trnA-UGC*, *trnL-CAA*, *trnI-GAU*, *trnN-GUU*, *trnV-GAC*, *trnR-ACG*). There were also identified 15 genes with one intron (*ndhB*,

ndhA, *petD*, *petB*, *atpF*, *rpl2*, *rpl16*, *rps16*, *rpoC1*, *trnA-UGC*, *trnG-UCC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, *trnV-UAC*) and 3 genes with two introns (*rps12*, *clpP*, *ycf3*).

As shown in Figure 3, the IR-LSC and IR-SSC boundaries of *L. barbarum* var. *implicatum* were similar to the related species, such as *L. dasystemum* (NC067978), *L. chinense* (MN102357), *L. ruthenicum* (MK994530), *L. ferocissimum* (MN866909), and *L. barbarum* (MH032560) except for *L. barbarum* var. *auranticarpum* (OP846044). By comprehensive comparison, the boundary of *L. barbarum* var. *implicatum* was similar to that of *L. ruthenicum*, but it is different from that of *L. barbarum* var. *auranticarpum* with the same variety. The phylogenetic analysis was performed based on the 22 complete cp genomes (including 4 outgroups) by maximum likelihood (ML), it suggested that *L. barbarum* var. *implicatum* was sister to *L. ruthenicum* with strong support (Figure 4).

4. Discussion and conclusions

Our study was the first to report the complete cp genomes of *L. barbarum* var. *implicatum*, which is a typical circular form with 154,888 bp in length. A total of 131 genes were found, including 86 protein-coding genes, 37 tRNA genes, and 8 rRNA genes, and the GC content accounted for 37.9%. *L. barbarum* var. *implicatum* is a new species of *Lycium* found in Qingshui River Basin, Zhongning County, Ningxia, China (Chen et al. 2012). Early taxonomists identified this species as

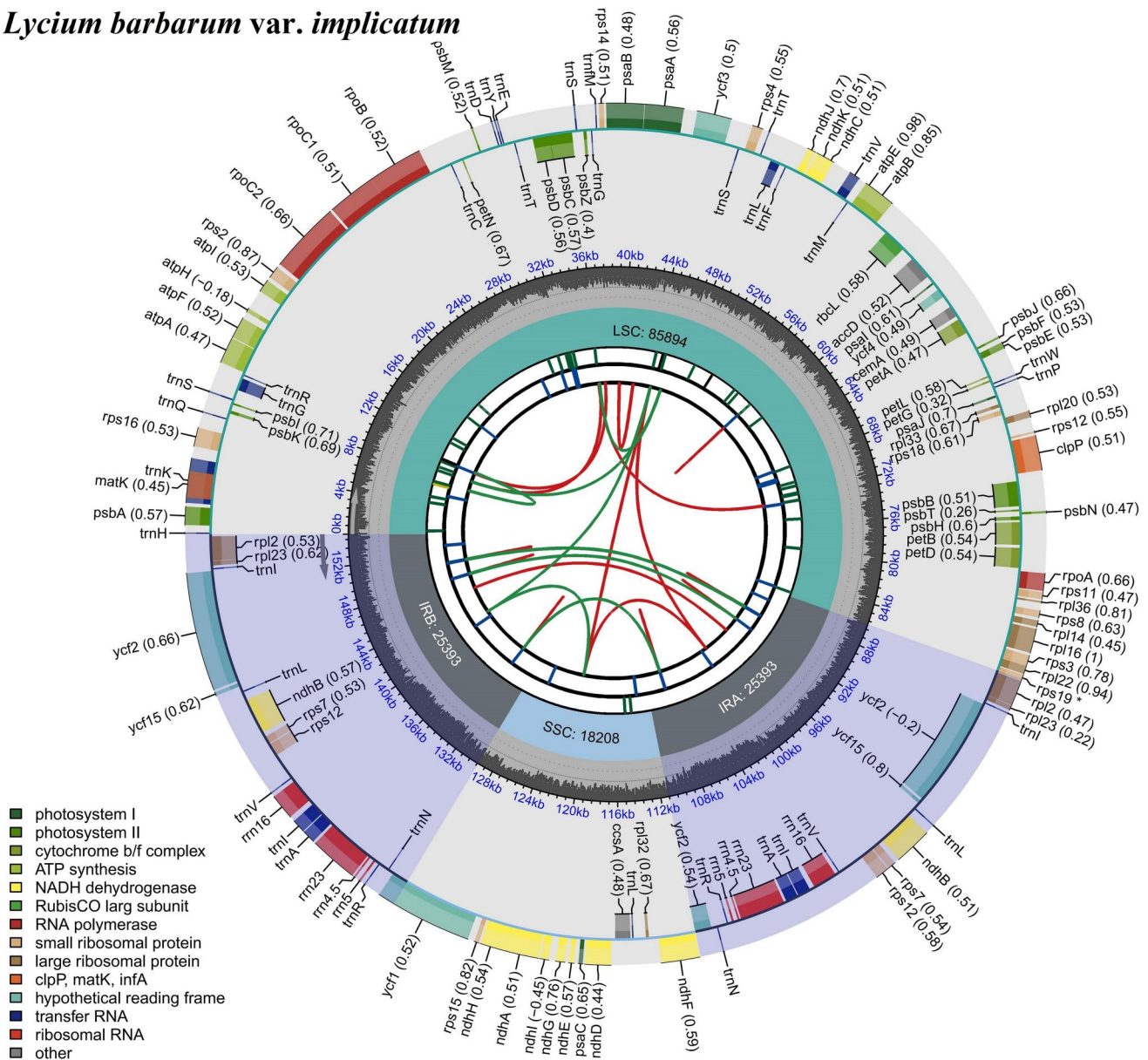
Lycium barbarum var. *implicatum*

Figure 2. Genomic map of overall features of the cp genome of *L. barbarum* var. *implicatum* was visualized using CPGview. This map consists of six tracks arranged from the center outward. The first track represents dispersed repeats, while the red arcs depict direct repeats and the green areas represent palindromic repeats. In the second track, a long tandem repeat is indicated by short blue bars. The third track displays short tandem repeats or microsatellite sequences as colored bars. The fourth track illustrates the regions of small single-copy (SSC), inverted repeat (IRs), and large single-copy (LSC). Additionally, the fifth track showcases the GC content of the cp genome, while in the last track coding genes are categorized based on their functions. Notably, transcription directions for inner and outer genes are depicted as clockwise and anticlockwise, respectively.

a variety of *Lycium barbarum* (Chen et al. 2012), but according to the results of our study, we speculate that it is more closely related to the phylogenetic evolution of *L. ruthenicum*, and may be its mutant hybrid offspring. In addition, IRs are a conserved region of the cp genome for the plant, and the contraction and expansion of its boundaries often reflect the evolutionary relationship of cp genomes among species (Amiryousefi et al. 2018; Konhar et al. 2019), our study also confirmed the close relationship between *L. barbarum* var. *implicatum* and *L. ruthenicum*. However, according to the unique characteristics of rich flesh and high crude protein

content of *L. barbarum* var. *implicatum* (Liu et al. 2016), it is in sharp contrast to the characteristics of proanthocyanidins and no flesh of *L. ruthenicum* (Li et al. 2022). Although Miller (2002) performed a comprehensive systematic phylogenetic analysis of 25 species of *Lycium*, this species was not included. Therefore, it is necessary to conduct a comprehensive analysis of all *Lycium* species in the future. The result provides a phylogenetic relationship of the genus *Lycium* with the other related species, which would provide useful information for the study of plant evolution in Solanaceae and the further study of DNA barcoding.

Inverted Repeats

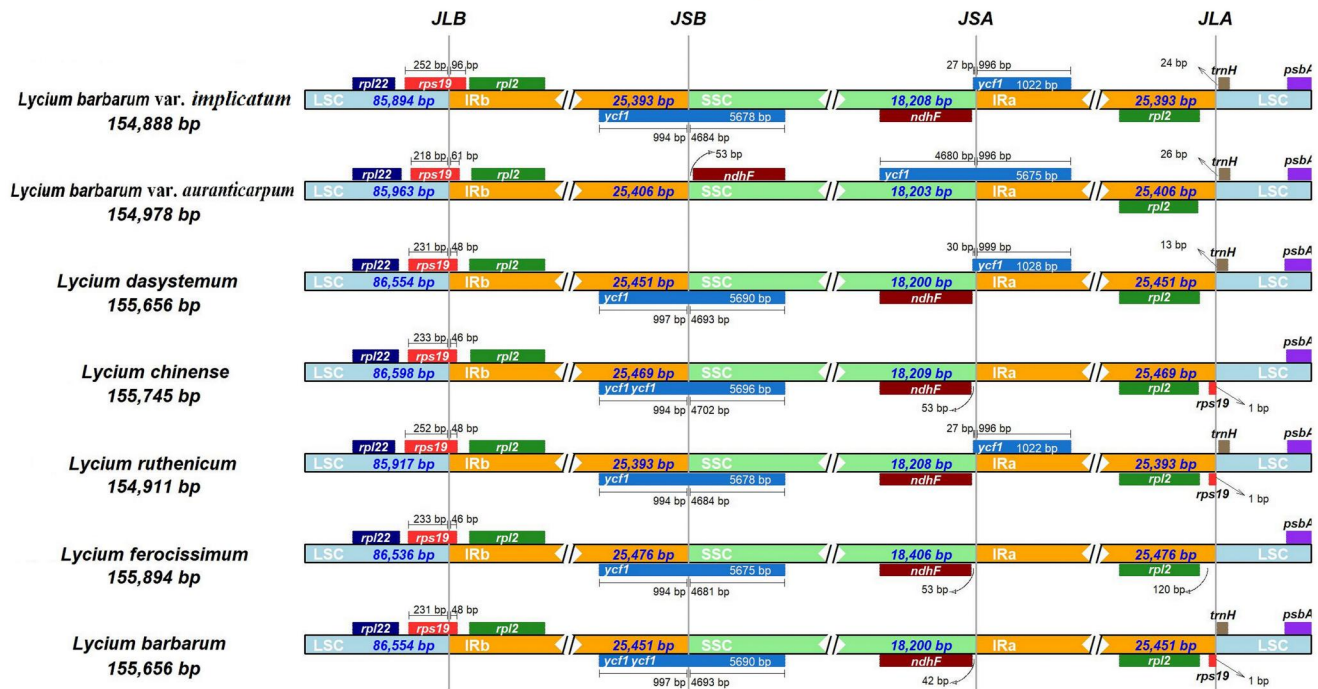


Figure 3. Comparison of borders of LSC, IR, and SSC regions in the complete cp genomes of *L. barbarum* var. *implicatum* (OR551485), *L. barbarum* var. *auranticarpum* (OP846044), *L. dasystemum* (NC067978), *L. chinense* (MN102357) (He et al. 2020), *L. ruthenicum* (MK994503) (Wang et al. 2019), *L. ferocissimum* (MN866909) (Li et al. 2020), and *L. barbarum* (MH032560) (Jia et al. 2018). The junction line between LSC and IRb is abbreviated as JLB; the junction line between SSC and IRb is abbreviated as JSB; the junction line between SSC and IRa is abbreviated as JSA; the junction line between LSC and IRa is abbreviated as JLA.

Ethical approval

The collection and research process of *L. barbarum* var. *implicatum* was carried out in strict accordance with the guidelines provided by the herbarium of Ningxia Technical College of Wine and Desertification Prevention and Chinese regulations.

Authors' contributions

WL and DB planned and designed the research. BZ and ZJ sampled the plant materials, DB and WL analyzed the data. BZ and WL wrote the manuscript.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in the NCBI database at <https://www.ncbi.nlm.nih.gov/>, reference number OR551485. The associated BioProject, Biosample, and SRA numbers are PRJNA1016474, SAMN37386561, and SRR26058098, respectively.

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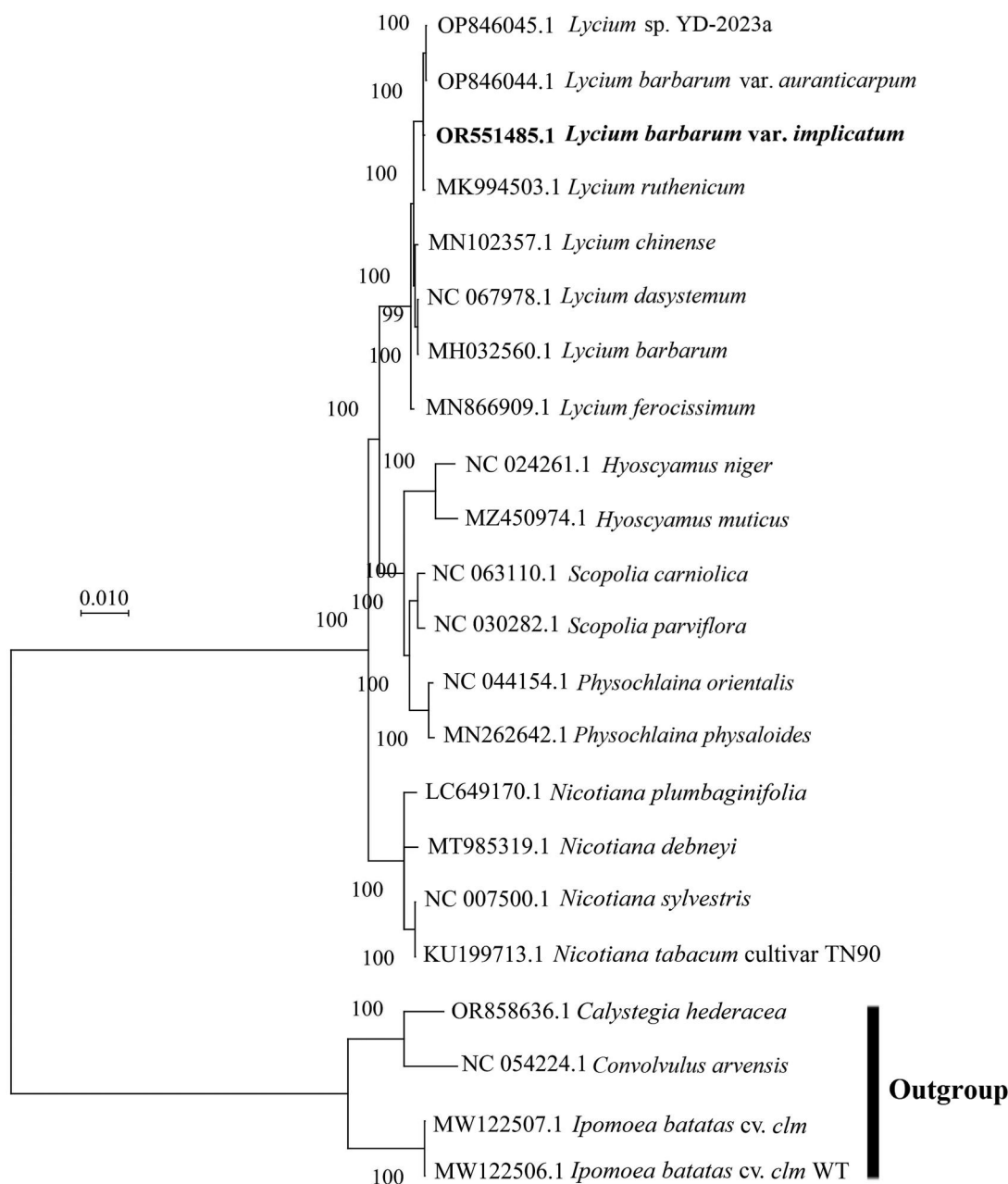


Figure 4. Maximum-likelihood (ML) tree generated with 17 complete cp genomes related to *L. barbarum* var. *implicatum* and four outgroups. Bootstrap values are shown near the nodes, and the bold font represents the cp genome of *L. barbarum* var. *implicatum* in this study. The following sequences, some of which are stored in the NCBI database and most of which remain unpublished, were used: *L. dasystemum* (NC 067978), *Hyoscyamus niger* (NC 024261), *Scopolia carniolica* (NC 063110), *S. parviflora* (NC 030282), *Physochlaina orientalis* (NC 044154), *Nicotiana sylvestris* (NC 007500), *Convolvulus arvensis* (NC 054224) (Wang et al. 2021), *N. tabacum* (KU 199713) (Gao et al. 2016), *N. plumbaginifolia* (LC 649170) (Tong et al. 2022), *L. barbarum* (MH032560) (Jia et al. 2018), *L. ruthenicum* (MK994503) (Wang et al. 2019), *L. chinense* (MN102357) (He et al. 2020), *L. ferocissimum* (MN866909) (Li et al. 2020), *P. physaloides* (MN 262642) (Tong et al. 2019), *N. debneyi* (MT985319) (Zeng et al. 2021), *Ipomoea batatas* (MW122506, MW122507) (Zou et al. 2021), *H. muticus* (MZ 450974), *Lycium* sp. (OP846045), *L. barbarum* var. *auranticarpum* (OP846044) and *Calystegia hederacea* (or 858636).

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