


The complete plastome of *Campsis radicans* (L.) Bureau 1864 and its phylogenetic analysis

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ABSTRACT

Campsis radicans (L.) Bureau 1864, a species of Bignoniaceae, has a widespread paleotropical distribution and is utilized for horticultural and traditional Chinese medicinal purposes. Despite the plant's significance, its genetic diversity must be better understood. In this study, we have successfully assembled and characterized the complete plastome of *C. radicans*, marking a significant advancement toward comprehending its genetic composition. The plastome is 153,630 bp long and harbors 130 genes, including 86 protein-coding genes, 36 tRNA genes, and eight rRNA genes. Our phylogenomic analysis of the representative species of Bignoniaceae indicated that *C. radicans* formed a monophyletic sister clade of *Campsis* with *C. grandiflora*. These findings are crucial for conserving and utilizing this important plant species. They also highlight the potential for future research into the evolution and preservation of *C. radicans*, which could be advantageous in pharmaceutical applications.

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Introduction



Campsis, a genus within the Bignoniaceae family, comprises two species of woody vines with a climbing tendency found in North America, China, and Japan. The model species of *Campsis* is *Campsis grandiflora* (Thunb.) K. Schum., characterized by features such as aerial roots, odd-pinnate compound leaves, large red or orange-red flowers, and capsules containing numerous seeds with semi-transparent membranous


wings. These plants exhibit a climbing growth habit, are typically deciduous, and tend to bloom during the summer. They are well-adapted to harsh climates, thrive in full sunlight, and employ aerial roots for climbing support (Zhang and Thawatchai 1998).

Campsis radicans (L.) Bureau (1864), another member of the *Campsis* genus, is a widely distributed paleotropical species used as a horticultural plant and in traditional Chinese medicine (TCM). Its flowers are employed in TCM under the



Figure 1. Field photos of *Campsis radicans*. The author Liqiang Wang shot the photo at the position of 35° 15' 9.27" N, 115° 29' 44.44" E. Main identifying traits: 9~11 leaflets, calyx five isolobes, shallow in the division, lobed to about one-third. The corolla is slender funnel-shaped, with distinct brownish-red longitudinal stripes inside, and the tube is three times the size of the calyx.

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name *Campsis Flos*, prescribed for conditions such as irregular menstruation, dysmenorrhea, postpartum breast enlargement, redness of rubella, itchy skin, and acne (Chinese Pharmacopeia, 2020 edition). This beautiful liana is typically cultivated in courtyards to enhance the esthetic appeal of the surrounding environment. *Campsis radicans* stands out from common fruits and vegetables due to its high concentration of phenolic compounds (Cai et al. 2004). These compounds have demonstrated potent antioxidant and anticancer properties. For instance, *C. radicans* flowers are a rich source of liver-protective compounds, exhibiting similar properties to other hepatoprotective TCMs (He et al. 2022). Despite its pharmaceutical potential, the genetic diversity of

C. radicans remains relatively unexplored. Therefore, this study aimed to assemble and characterize the plant's complete plastome, which could serve as a foundation for future research on the evolution, conservation, and potential medicinal applications of *C. radicans*.

Materials

The fresh leaves used for sequencing were obtained from Peony District, Heze City, Shandong Province, China (35°15'9.27"N, 115°29'44.44"E) (Figure 1), and a specimen has been deposited in the Heze University Herbarium (contact person: Hongqin Li, machh1111@126.com) under the

Campsis radicans

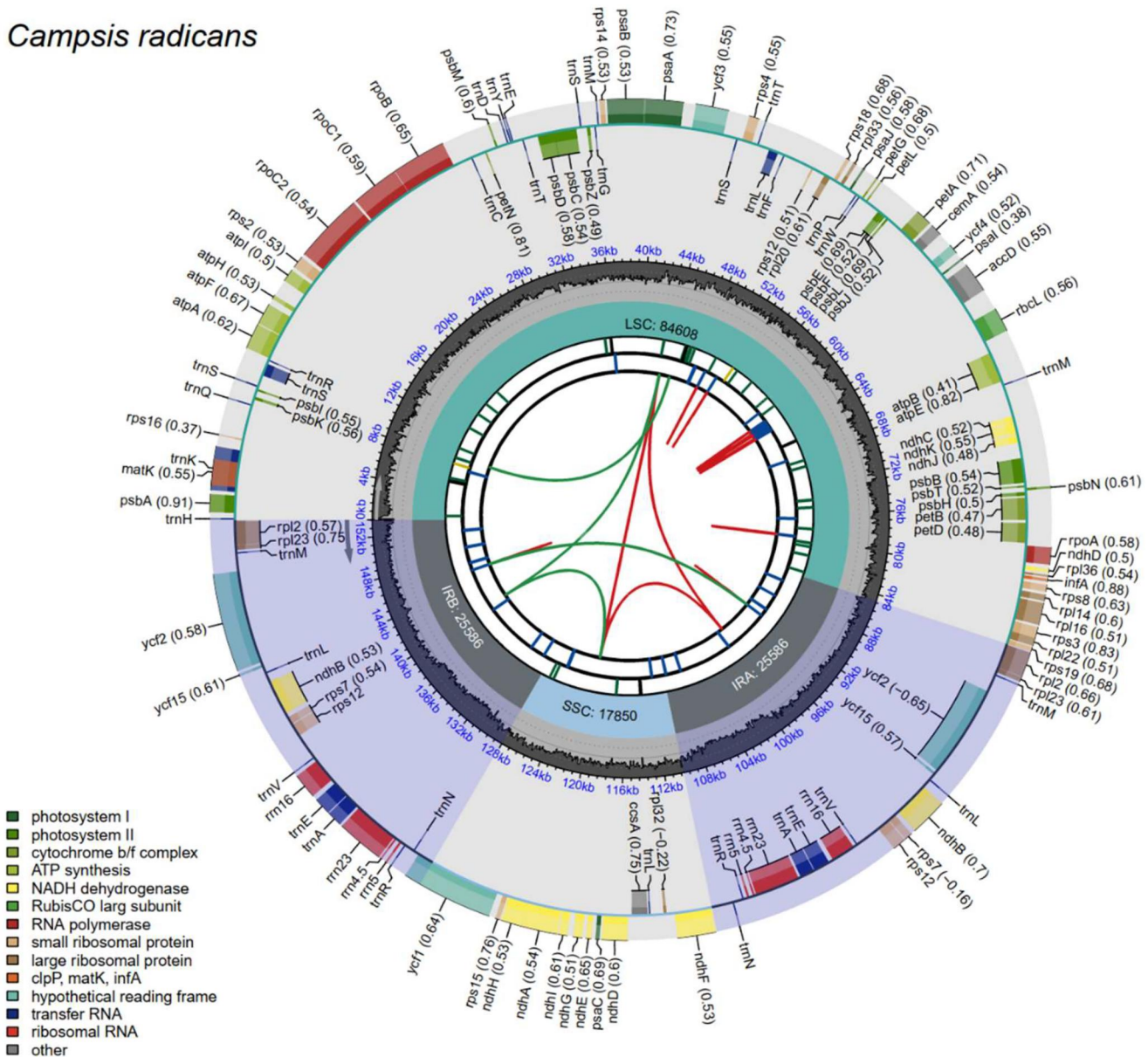


Figure 2. Gene map of the complete chloroplast genome of *Campsis radicans*. The species name is shown in the top left corner. The map contains six tracks by default. From the center outward, the first track shows dispersed repeats, including direct and palindromic repeats connected by red and green arcs. The second track displays long tandem repeats as blue bars, while the third displays short tandem repeats or microsatellite sequences as differently colored short bars. These colors correspond to the type and description of each repeat, with black representing complex repeats, green for repeat unit size 1, yellow for size 2, purple for size 3, blue for size 4, orange for size 5, and red for size 6. The fourth track displays the SSC, IRa, IRb, and LSC regions. The fifth track shows the GC content along the genome, while the sixth track sounds the genes. The gene names are followed by optional information about codon usage bias and color-coded based on their functional classification. The inner genes are transcribed clockwise, and the outer genes are transcribed anticlockwise. The functional type of the genes is shown in the bottom left corner.

specimen number HZ20220801. Genomic DNA was extracted using the plant genomic DNA kit (Tiangen Biotech, Beijing, China).

Methods

The total genomic DNA extracts were fragmented into approximately 300 bp short-insert fragments to generate libraries, which were then sequenced using Illumina NovaSeq 6000 technology platforms at Wuhan Benagen Technology Company Limited in Wuhan, China. Trimmomatic (v0.35) (Bolger et al. 2014) was implemented to filter the raw reads

by eliminating adapters and low-quality bases. Following the filtering process, roughly 24 GB of clean reads were assembled using GetOrganelle (v1.7.1) (Jin et al. 2020). The finished plastome was annotated using CPGAVAS2 (Shi et al. 2019) and manually adjusted through Apollo (Pontius 2018). Eventually, the annotated plastome was submitted to GenBank using Bankit and assigned the accession number OQ198454. The CPGview tool was utilized to exhibit the circular genome map of the novel plastome.

To establish the phylogenetic relationship of *C. radicans*, 19 species plastomes of Bignoniaceae were downloaded from GenBank that exhibited the highest degree of similarity to

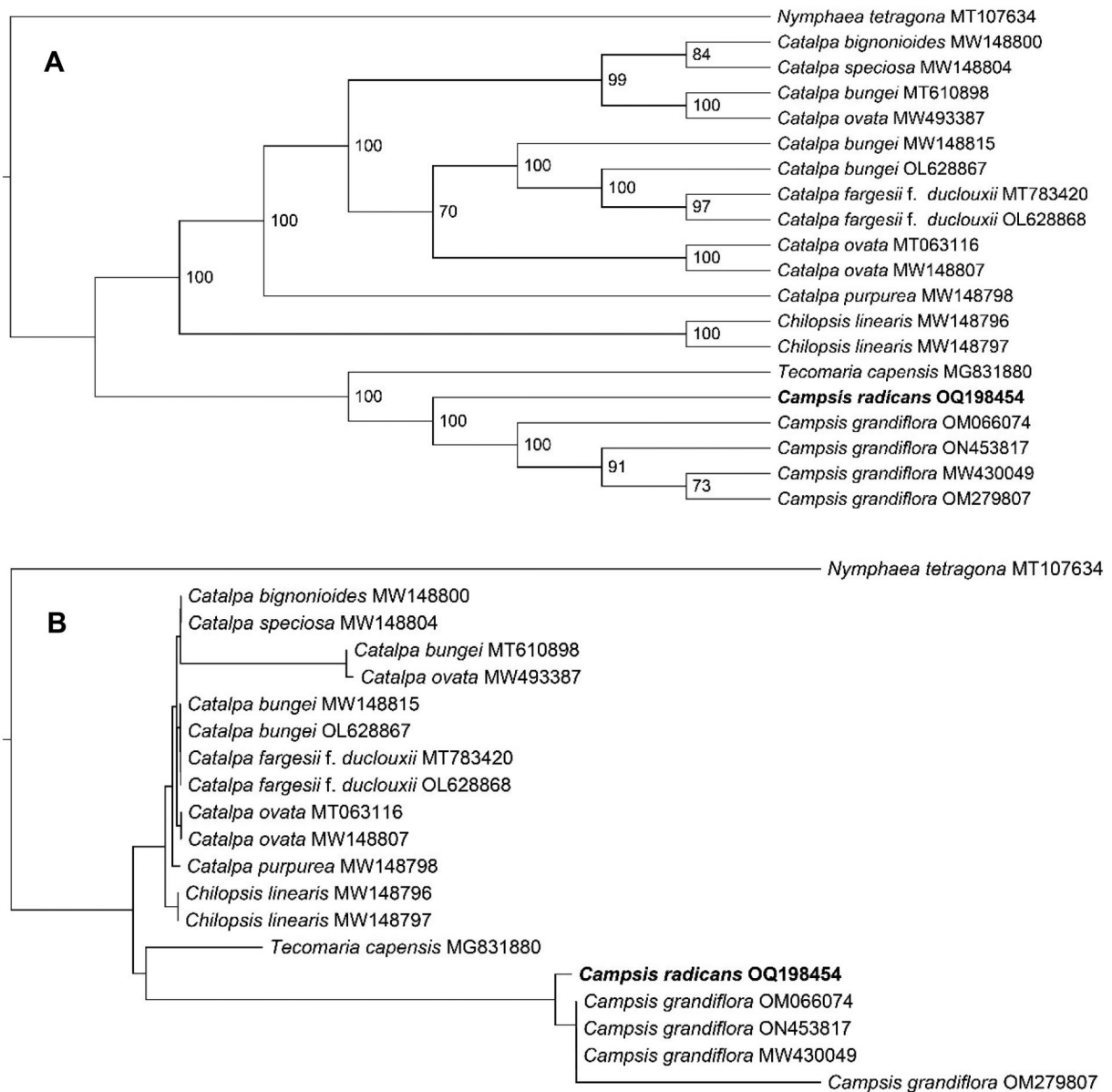


Figure 3. The Maximum-Likelihood phylogeny of *Campsis radicans* and its close relatives using whole genome sequences. The bootstrap values based on 1000 replicates were shown on each node in the cladogram tree (A). The corresponding phylogram tree is shown in panel B. We downloaded 19 *Campsis* species plastomes from GenBank, *C. grandiflora* (Thunb.) K.Schum. (MW430049) (Chen et al. 2022), *C. grandiflora* (OM066074) (Ngai et al. 2023), *C. grandiflora* (OM279807), *C. grandiflora* (ON453817), *Catalpa bignonioides* walter (MW148800) (Dong et al. 2022), *Catalpa bungei* C.A.Mey. (MT610898), *Catalpa bungei* (MW148815) (Dong et al. 2022), *Catalpa bungei* (OL628867) (Li et al. 2022), *Catalpa fargesii* f. *duclouxii* (dode) Gilmour (MT783420) (Ma et al. 2020), *Catalpa fargesii* f. *duclouxii* (OL628868) (Li et al. 2022), *Catalpa ovata* G.Don (MT063116) (Wang et al. 2020), *Catalpa ovata* (MW148807) (Dong et al. 2022), *Catalpa ovata* (MW493387), *Catalpa purpurea* griseb. (MW148798) (Dong et al. 2022), *Catalpa speciosa* teas (MW148804) (Dong et al. 2022), *chilopsis linearis* (cav.) sweet (MW148796) (Dong et al. 2022), *Catalpa linearis* (MW148797) (Dong et al. 2022), *Tecomaria capensis* (Thunb.) spach (MG831880) (Fonseca and Lohmann 2018), *Nymphaea tetragona* Georgi (MT107634) (Sun et al. 2021), from the nymphaeaceae, served as the outgroup. The new *C. radicans* plastome in this study were labeled in bold font.

C. radicans based on plastome blast results. In addition, the outgroup is *Nymphaea tetragona* Georgi. The entire plastome sequences with default parameters were aligned using MAFFT software (<https://mafft.cbrc.jp/alignment/software/>) (Kato and Standley 2016). Following this, a maximum-likelihood (ML) phylogenetic tree was constructed using IQ-TREE (v2.0) (Nguyen et al. 2015) with the Best-fit model of TVM + F + G4 along with 1000 bootstrap replicates. To check the reliability of the tree, the researchers used the Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa 1999) and proximately unbiased (AU) (Shimodaira 2002) methods embedded in IQ-TREE. The primary test script used was `iqtree -s input_mafft.phy -m TVM + F + G4 -z genome.unconstrain.constrain.trees -zb 10000 -zw -au`.

Results and discussion

The *C. radicans* plastome sequence measures 153,630 bp long and presents a typical quadripartite structure. It consists of two IR regions, each 25,586 bp in length, separated by a large LSC region of 84,608 bp and a small SSC region of 17,850 bp (Figure 2). Mapping experiments demonstrate the high reliability of the genome assembly (Figure S1). The plastome exhibits a varying GC content distribution, with an overall GC content of 38.2%. The highest GC content is found in the IR regions (43.2%), whereas the corresponding values are 36.2% and 32.8% for the LSC and SSC regions, respectively. Notably, the structure of *C. radicans* plastome bears similarities to that of *C. grandiflora* (Chen et al. 2022). Upon comparative analysis with the *Arabidopsis thaliana* plastome, we observed that the LSC of *C. radicans* underwent rearrangement (Figure S2), a phenomenon also seen in *C. grandiflora* (Chen et al. 2022).

The *C. radicans* plastome was predicted 130 genes, including 86 protein-coding genes (PCGs), eight rRNA genes, and 36 tRNA genes. Seven unique PCGs (*rps12*, *rps7*, *rpl2*, *rpl23*, *ndhB*, *ycf15* and *ycf2*), seven unique tRNA genes (*trnA*, *trnE*, *trnL*, *trnM*, *trnN*, *trnR* and *trnV*) and four unique rRNA genes (*rnn16S*, *rnn23S*, *rnn4.5S*, *rnn5S*) were located at the IR regions. In the whole plastome, ten PCGs (*rps16*, *atpF*, *rpoC1*, *accD*, *petD*, *petB*, *rpl16*, *rpl2*, *ndhB*, and *ndhA*) comprise one intron each, while two PCGs (*ycf3*, *rps12*) contain two introns; in addition, five tRNA genes (*trnK-UUU*, *trnS-CGA*, *trnL-UAA*, *trnE-UUC*, and *trnA-UGC*) contain one intron. The *rps12* gene was subject to trans-splicing. The structures of the cis-splicing and trans-splicing PCG genes are shown in Figure S3. The length of the protein-coding genes, tRNA genes, and rRNA genes in the plastome are 2,7701bp, 3,923bp, and 9,276bp, respectively, making up 18.0%, 2.6%, and 6.0% of the total genome length. Notably, although the size of *C. radicans* plastome is only 673 bp shorter than that of *C. grandiflora*, the number of predicted genes in *C. radicans* plastome was higher than that of *C. grandiflora* (Chen et al. 2022).

The ML phylogenetic tree demonstrated that the *C. radicans* and all four *C. grandiflora* plants formed a monophyletic clade, the sister clade of *Tecomaria capensis*, with 100% bootstrap values (Figure 3). This result corresponded

with a prior study (Chen et al. 2022) indicating a close relationship between *Campsis* and *Tecomaria* species. Despite some nodes having comparatively low bootstrap values, the topological structure of the ML tree was significantly included, with a p-SH value of 1.000 and a p-AU value of 0.536, respectively. Consequently, the phylogenetic analysis has provided a dependable account of *C. radicans*' evolution.

Conclusions

This study presents the primary characterization of the genome of *C. radicans* for the first time, which exhibits a typical annular tetrad structure with a size of 153,630 bp and 130 predicted genes. A rearrangement event has occurred in the plastome structure. Based on the phylogenetic analysis, *C. radicans* and *C. grandiflora* cluster within Bignoniaceae.

Ethical approval

The *C. radicans* specimen is not an endangered species. It requires no specific permissions or licenses. Research on this species, including the collection of plant material, was conducted following the guidelines provided by Heze University in this study.

Author contributions

The manuscript includes the contributions of all authors. Liqiang Wang was mainly responsible for the study design. Hongqin Li identified the species and collected samples of the species. Liqiang Wang assembled and annotated the plastome. Hongqin Li and Changhao Ma analyzed the structure of the plastomes and drafted the manuscript. All authors approved the version to be published and agreed to be accountable for all aspects of the work.

Disclosure statement

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

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Data availability statement

The complete chloroplast genome sequence of *C. radicans* in this study has been submitted to the NCBI database under the accession number OQ198454. <https://www.ncbi.nlm.nih.gov>. The associated BioProject, BioSample, and SRA numbers are PRJNA928567, SAMN32935701, and SRR23250959.

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