




The chloroplast genome of *Cuphea hookeriana* Walp. (Lythraceae), a Mexico ornamental plant

Cuihua Gu^{a,b,c,*}, Yacheng Ye^{a,b,c,*}, Guozhe Zhang^{a,b,c,*}, Mengxin Yu^{a,b,c}, Mingzhu Bai^{a,b,c}, Qingqing Ma^{a,b,c}, Weili Shao^{a,b,c} , Yu Zhao^{a,b,c}  and Sidan Hong^{a,b,c} 

^aCollege of Landscape and Architecture, Zhejiang A&F University, Hangzhou, China; ^bZhejiang Provincial Key Laboratory of Germplasm Innovation and Utilization for Garden Plants, Zhejiang A&F University, Hangzhou, China; ^cKey Laboratory of National Forestry and Grassland Administration on Germplasm Innovation and Utilization for Southern Garden Plants, Zhejiang A&F University, Hangzhou, China

ABSTRACT

Cuphea hookeriana Walp. is an ornamental plant belonging to the Lythraceae. In this study, we reported the complete chloroplast (cp) genome sequence here and analyzed the phylogenetic relationship among Lythraceae plants. The length of the cp genome was 158,999 bp, including a large single-copy (LSC, 89,311 bp) region and a small single-copy (SSC, 18,436 bp) region separated by a pair of inverted repeats (IRs, 25,626 bp). There were 72 unique protein-coding genes (PCGs), 30 transfer RNA (tRNA) genes, and four ribosomal RNA (rRNA) genes in the cp genome of *C. hookeriana*. A total of 223 simple sequence repeats (SSRs) and 34 long repeat sequences were identified. Phylogenetic analyses using maximum-likelihood (ML) revealed that *C. hookeriana* was close to *C. hyssopifolia*. In addition, the two *Cuphea* species were the sister group of *Woodfordia fruticosa*.

ARTICLE HISTORY

Received 17 October 2022
Accepted 12 April 2023

KEYWORDS

Chloroplast genome;
phylogeny; *Cuphea hookeriana*

Introduction

Cuphea hookeriana Walp. 1897 is a member of the Lythraceae family (Figure 1) and originated in Mexico and distributed in tropical and south subtropical areas (Duss 1897). *C. hookeriana* is a small shrub with purple flowers that bloom all year. It is a popular ornamental plant that can be found in gardens, parks, and roadside green spaces and is now cultivated in Beijing, Tianjin, and Shaanxi, among other places in China. Besides, *C. hookeriana* is also identified as having economic value as an industrial oil plant, and the research about it is mainly focused on fatty acid synthases (Dehesh et al. 2001; Feng et al. 2018). Herein, in order to provide the basic genomic information for researching the evolution of Lythraceae, we described the entire chloroplast (cp) genome of *C. hookeriana* for the first time and carried out a phylogenetic analysis to resolve the relationship with close relatives.


Materials and methods

Fresh leaves samples of *C. hookeriana* were collected from the greenhouse of the College of Landscape and Architecture in Hangzhou (Hangzhou, China; 30°3' N; 120°21' E). The leaf of *C. hookeriana* was stored subsequently as a specimen at

the Herbarium of Zhejiang A&F University under the accession code ZAFU20220106 (Cuihua Gu, gucuihua@zafu.edu.cn). The total DNA was extracted following the method of previous reports (Wang et al. 2021). Subsequently, the pair-end raw reads were produced based on the Illumina HiSeq platform at Beijing Genomics Institute (Shenzhen, China). After being purified by removing the adapter and low-quality reads, 3 Gb clean reads of *C. hookeriana* were used to perform the de novo assembly process. To obtain the complete cp genome of *C. hookeriana*, first, the reads of *C. hookeriana* were filtered by SeqKit (Shen et al. 2016). Subsequently, the resulting reads were assembled by SPAdes (Bankevich et al. 2012) using the cp genome of *C. hyssopifolia* (NC046574) as a reference. To know the accuracy of the assembly, we mapped the clean reads to assemble cp genome by Geneious Prime version 11.1.5 (Figure S1) (Deng et al. 2020). Subsequently, the assembled sequence was annotated and visualized by GeSeq (Tillich et al. 2017) after Bandage (Wick et al. 2015) made the necessary adjustments. In addition, the CPGVIEW (www.1kmpg.cn/cpgview/) (Liu et al. 2023) was applied to structures to visualize the intron-containing genes. The completed annotation was uploaded to GenBank and assigned the accession number OM949050. MISA (Beier et al. 2017) was used to search for simple sequence repeats (SSRs) of one to six nucleotide units (mono-, bi-, tri-, tetra-, penta-, and

CONTACT Sidan Hong  2020105012006@stu.zafu.edu.cn; Yu Zhao  zhaoyu@stu.zafu.edu.cn; Weili Shao  70085055@qq.com  School of Landscape and Architecture, Zhejiang A&F University, Hangzhou 311300, China

*These authors contributed equally to this work.

 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2023.2203783>.

© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.



Figure 1. Plant materials of *C. hookeriana*. It is a shrub with little purple flowers which can bloom for 10–12 months a year. *C. hookeriana* in the photo was planted in Zhejiang A&F University and the photo itself was shot by Sidan Hong.

hexa-) across the cp genome. The minimal threshold of the unit size for searching was set as: 8, 5, 4, 3, 3, and 3. To find the long repeat sequences throughout the cp genome, REPuter (Kurtz et al. 2001) (<https://bibiserv.cebitec.uni-bielefeld.de/reputer>) was performed. The parameters are as follows: minimum size = 30, hamming distance = 3, and maximum computed repeats = 5000.

After aligning and trimming the cp genome by mafft v 7 (Kato et al. 2019) and Gblocks 0.91b (Talavera and Castresana 2007), respectively, the IQtree v 1.6 (Nguyen et al. 2015) was used for generating a maximum-likelihood tree based on sequence from 14 Lythraceae species, three sequences from Onagraceae species were selected as an out-group. The bootstrap was set to 1000 replicates, and the best-fit substitution model for the data (GTR + F + R2) was chosen by ModelFinder v 1.6.8 (Kalyaanamoorthy et al. 2017). The following sequences were used: NC037023 (Xue et al. 2017), MZ617461 (Fan et al. 2022), NC054309 (Lin et al. 2021), MN833212 (Wang et al. 2020), MK881637 (Gu et al. 2019), MK881626 (Gu et al. 2019), NC042891 (Gu et al. 2019), NC046574 (Ma et al. 2020), NC010358 (Gu et al. 2019), NC010361 (Chapman et al. 1999), NC029211 (Chapman et al. 1999), MK881638 (Gu et al. 2019), MG921615 (Gu et al. 2018), NC042897 (Xu et al. 2017), NC042896 (Xu et al. 2017), KF572028 (Wang et al. 2023), NC042890 (Wang et al. 2023), MH727532 (Jian and Ren 2019), and MK881631 (Gu et al. 2019).

Results

From the cp genome map of *C. hookeriana* (Figure 2), the total length of the cp genome of *C. hookeriana* was

158,999 bp. A pair of inverted repeats (IRs, 25,626 bp) separated the large single-copy (LSC, 89,311 bp) region and small single-copy (SSC, 18,436 bp) region. LSC, SSC, and IRs contained the GC content of 36.97%, 31.05%, and 42.72%, respectively. A total of 106 unigenes were identified totally (44 of which were duplicated in the IRs) including 72 unique protein-coding genes (PCGs), 30 transfer RNA (tRNA) genes, and four ribosomal RNA (rRNA) genes. In addition, *ycf 3*, *clpP*, and *ndhB* had two introns (Figure S2). There 223 SSRs were found (Supple 1), including 196 mono-nucleotide, nine bi-nucleotide, seven tri-nucleotide, nine tetra-nucleotide, and four penta-nucleotide. With the threshold of *e*-value <1e-5, 34 long repeat sequences (Supple 2) were identified, consisting of 15 forward matches, 18 palindromic matches, and one reverse repeat.

As shown in the phylogenetic tree (Figure 3), the relationship of *C. hookeriana* in Lythraceae was presented. The *C. hookeriana* was mostly related to *C. hyssopifolia* with high bootstrap support, and as the sister group to *Woodfordia fruticosa* with *C. hyssopifolia* together.

Discussion and conclusions

C. hookeriana belongs to the *Cuphea* genus, and is a member of Lythraceae. Up to now, the research on phylogenetic analysis of the Lythraceae family has attracted much attention. The cp genome has been performed from many species of *Lagerstroemia* (Gu et al. 2016; Gu, Tembrock, Wu 2017; Gu, Tembrock, Zhang, et al. 2017), *Heimia* (Gu et al. 2018), *Punica* (Wang et al. 2020), and *Trapa* (Gu et al. 2019; Lin et al. 2021). In addition, the phylogenetic information of *C. hyssopifolia* has been published (Ma et al. 2020). However, the lack of cp

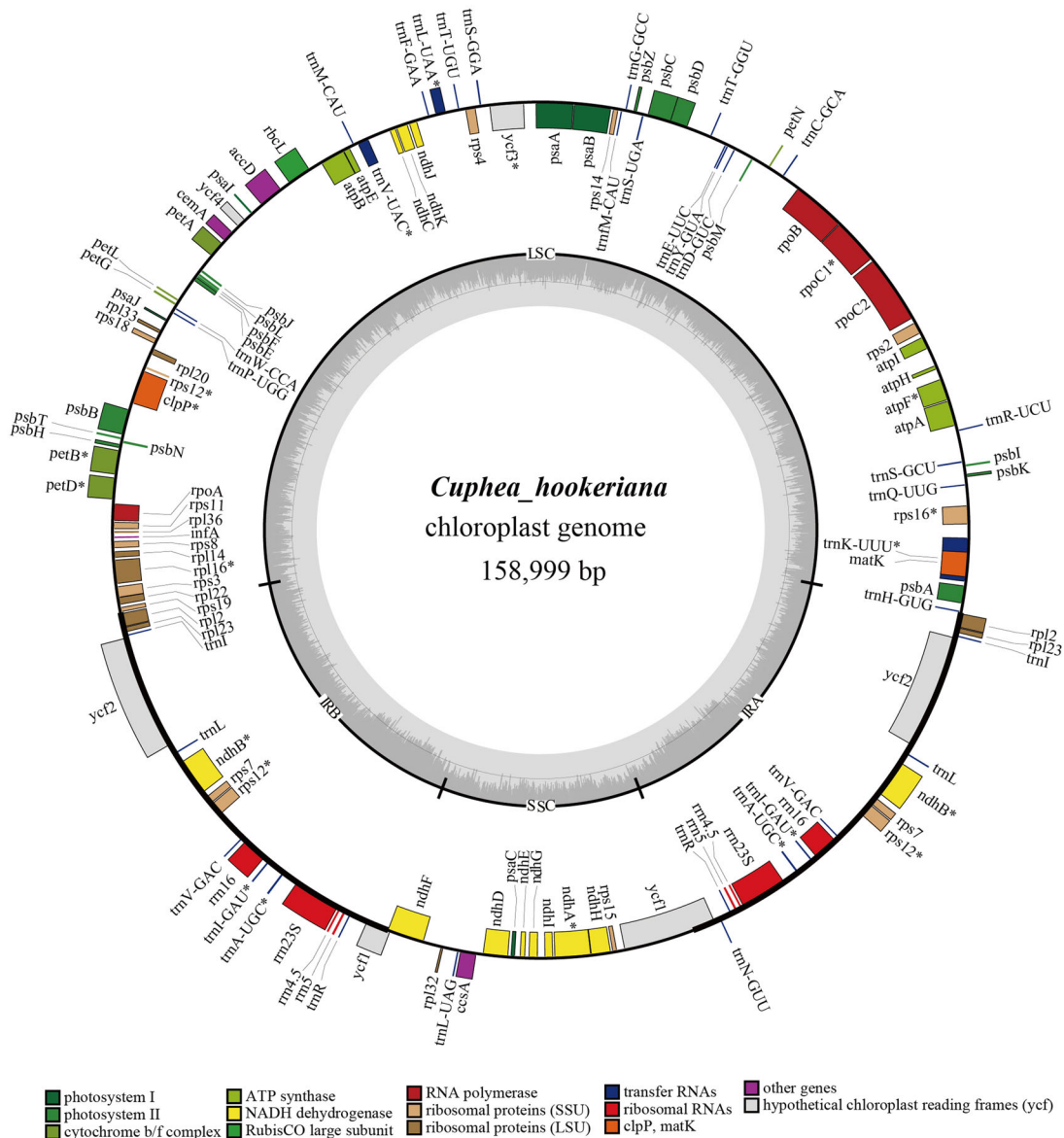


Figure 2. Complete and regional genome map of *C. hookeriana*. The different colored legend below the map presents the different types of unigenes.

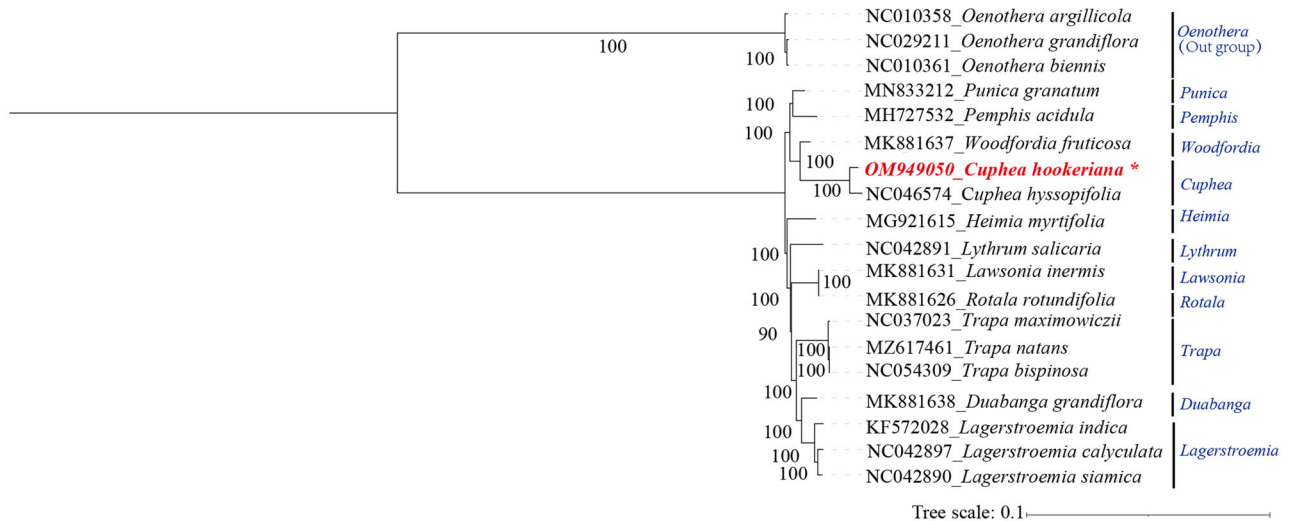


Figure 3. The maximum-likelihood tree indicates the position of *C. hookeriana* with other chloroplast sequences from 14 Lythraceae species and Onagraceae species as an outgroup. Number of the node represents the bootstrap value of ML methods.

genome sequence information from the tribe *Cuphea* makes it difficult to resolve the phylogenetic relationships of *Cuphea* species among the Lythraceae family. Herein, the cp genome of *C. hookeriana* was first sequenced and assembled to solve its relationship with other species in Lythraceae. As the result, *C. hookeriana* and *C. hyssopifolia* are most closely related, and they are the closest sister group to *W. fruticosa*.

Ethical approval

This research was carried out following the guidelines provided by the College of Landscape and Architecture, Zhejiang A&F University.

Author contributions

Sidan Hong, Yu Zhao, and Weili Shao were involved in the conception and design, Cuihua Gu, Yacheng Ye, and Guozhe Zhang participated in the analysis and drafting of the paper, as well as revised it critically for intellectual content; Mengxin Yu, Mingzhu Bai, and Qingqing Ma were involved in the interpretation of the data; and that all authors agree to be accountable for all aspects of the work.

Disclosure statement

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

Funding

This work was supported by grants from the Zhejiang Science and Technology Major Program on Agricultural New Variety Breeding [Grant No. 2021C02071-4] and Zhejiang Provincial Natural Science Foundation of China [Grant No. LY21C160001].

ORCID

Weili Shao  <http://orcid.org/0000-0001-8145-8512>
 Yu Zhao  <http://orcid.org/0000-0001-7671-6206>
 Sidan Hong  <http://orcid.org/0000-0001-5228-5149>

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 OM949050. The numbers of BioProject, Biosample, and SRA number are PRJNA814813, SAMN26567440, and SRR18298639, respectively.

References

- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 19(5):455–477.
- Beier S, Thiel T, Münch T, Scholz U, Mascher M. 2017. MISA-web: a web server for microsatellite prediction. *Bioinformatics.* 33(16):2583–2585.
- Chapman MJ, Mulcahy DL, Stein DB. 1999. Identification of plastid genotypes in *Oenothera* subsect. *Munzia* by restriction fragment length polymorphisms (RFLPs). *Theor Appl Genet.* 98(1):47–53.
- Dehesh K, Tai H, Edwards P, Byrne J, Jaworski JG. 2001. Overexpression of 3-ketoacyl-acyl-carrier protein synthase IIIs in plants reduces the rate of lipid synthesis. *Plant Physiol.* 125(2):1103–1114.
- Deng X, Gu W, Federman S, Du Plessis L, Pybus OG, Faria NR, Wang C, Yu G, Bushnell B, Pan C-Y, et al. 2020. Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California. *Science.* 369(6503):582–587.
- Duss R-PA. 1897. Flore phanérogamique des Antilles françaises.
- Fan X, Wang W, Wagutu GK, Li W, Li X, Chen Y. 2022. Fifteen complete chloroplast genomes of *Trapa* species (Trapaceae): insight into genome structure, comparative analysis and phylogenetic relationships. *BMC Plant Biol.* 22(1):230.
- Feng Y, Zhang Y, Wang Y, Liu J, Liu Y, Cao X, Xue S. 2018. Tuning of acyl-ACP thioesterase activity directed for tailored fatty acid synthesis. *Appl Microbiol Biotechnol.* 102(7):3173–3182.
- Gu C, Dong B, Xu L, Tembrock LR, Zheng S, Wu Z. 2018. The complete chloroplast genome of *Heimia myrtifolia* and comparative analysis within Myrtales. *Molecules.* 23(4):846.
- Gu C, Ma L, Wu Z, Chen K, Wang Y. 2019. Comparative analyses of chloroplast genomes from 22 Lythraceae species: inferences for phylogenetic relationships and genome evolution within Myrtales. *BMC Plant Biol.* 19(1):281.
- Gu C, Tembrock LR, Johnson NG, Simmons MP, Wu Z. 2016. The complete plastid genome of *Lagerstroemia fauriei* and loss of *rpl2* intron from *Lagerstroemia* (Lythraceae). *PLOS One.* 11(3):e0150752.
- Gu C, Tembrock LR, Wu Z. 2017. The complete chloroplast genome of *Lagerstroemia intermedia* (Lythraceae), a threatened species endemic to southwestern Yunnan province, China. *Conserv Genet Resour.* 9(3):357–360.
- Gu C, Tembrock LR, Zhang D, Wu Z. 2017. Characterize the complete chloroplast genome of *Lagerstroemia floribunda* (Lythraceae), a narrow endemic crape myrtle native to Southeast Asia. *Conserv Genet Resour.* 9(1):91–94.
- Jian S, Ren H. 2019. The complete chloroplast genome sequence of *Pemphis acidula* (Lythraceae). *Mitochondrial DNA B.* 4(1):912–913.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods.* 14(6):587–589.
- Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform.* 20(4):1160–1166.
- Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C, Stoye J, Giegerich R. 2001. REPuter: the manifold applications of repeat analysis on a genomic scale. *Nucleic Acids Res.* 29(22):4633–4642.
- Lin L, Wang J, Zhao YX, Ma L, Gu CH, Wu ZQ. 2021. Chloroplast genome of *Trapa bispinosa* Roxb. (*Trapa*, Lythraceae). *Mitochondrial DNA B Resour.* 6(2):333–334.
- Liu S, Ni Y, Li J, Zhang X, Yang H, Chen H, Liu C. 2023. CPGView: a package for visualizing detailed chloroplast genome structures. *Mol Ecol Resour.* 23(3):694–704.
- Ma L, He Y-Z, Yu J-Y, Gu C-H, Liao X-Z, Wu Z-Q. 2020. The complete chloroplast genome sequence of *Cuphea hyssopifolia*. *Mitochondrial DNA B.* 5(2):1415–1416.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 32(1):268–274.
- Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLOS One.* 11(10):e0163962.
- Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol.* 56(4):564–577.
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45(W1):W6–W11.
- Wang J, He W, Liao X, Ma J, Gao W, Wang H, Wu D, Tembrock LR, Wu Z, Gu C. 2023. Phylogeny, molecular evolution, and dating of divergences in *Lagerstroemia* using plastome sequences. *Hortic Plant J.* 9(2):345–355.
- Wang J, Wu Z, Ma L, Gu C. 2021. Characterization of the chloroplast genome of *Lagerstroemia villosa* Wall. ex Kurz. (*Lagerstroemia*, Lythraceae). *Mitochondrial DNA B Resour.* 6(1):19–20.

- Wang J, Wu ZQ, Ma L, Gu CH. 2020. Complete chloroplast genome sequence of *Punica granatum* 'Nana' (Lythraceae) and phylogenetic analysis. *Mitochondrial DNA B Resour.* 5(3):2070–2071.
- Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics.* 31(20):3350–3352.
- Xu C, Dong W, Li W, Lu Y, Xie X, Jin X, Shi J, He K, Suo Z. 2017. Comparative analysis of six *Lagerstroemia* complete chloroplast genomes. *Front Plant Sci.* 8:15.
- Xue Z-Q, Xue J-H, Victorovna KM, Ma K-P. 2017. The complete chloroplast DNA sequence of *Trapa maximowiczii* Korsh. (Trapaceae), and comparative analysis with other Myrtales species. *Aquat Bot.* 143:54–62.