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PLASTOME REPORT

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# The chloroplast genome of a subtropical tree *Sassafras randaiense* (Hayata) Rehder, 1920 (Lauraceae)

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**ABSTRACT**

The complete chloroplast genome sequence of *Sassafras randaiense* (Hayata) Rehder, 1920, a subtropical tree in the family Lauraceae, was determined. For a better understanding of the differences between *S. randaiense* and *S. tzumu*, the complete chloroplast genome of *S. randaiense* was sequenced and analyzed. The complete chloroplast genome is 151,781 bp in length, consisting of a pair of inverted repeat (IR) regions of 20,114 bp, one large single-copy (LSC) region of 92,740 bp, and one small single-copy (SSC) region of 18,813 bp. The overall GC content of the complete chloroplast genome is 39.2%. Further, maximum-likelihood phylogenetic analysis was conducted using 31 complete plastome sequences, which support that *S. randaiense* and *S. tzumu* are nested among the members of *Cinnamomum*, suggesting that *Sassafras* belongs to *Cinnamomum*.

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**KEYWORDS**
*Sassafras randaiense*; Lauraceae; chloroplast genome; phylogenetic analysis

## Introduction

*Sassafras* Nees belongs to the *Cinnamomum-Ocotea* clade in the tribe Laureae, the largest group in Lauraceae family (Song et al. 2020). Characters that set *Cinnamomum* apart morphologically include evergreen shrubs or trees, bisexual and trimerous flowers, triplinerved or pinninerved leaves, paniculate-cymose inflorescences, nine fertile stamens, well developed fourth whorl staminodes, and fruits with a cupule (Yang et al. 2022). The genus contains only three tree species distributed in eastern Asia and North America. *Sassafras randaiense* is a medium-sized deciduous tree species, recently assessed and listed as vulnerable following IUCN criteria (Lu and Pan 1998). The North American species *S. albidum* Nees is highly distinct from the eastern Asian taxa *S. tzumu* Hemsl and *S. randaiense* with their male or female flowers (Nie et al. 2007). At the molecular level, however, the reported nuclear ITS sequence and three plastid regions *psbA-trnH*, *rpl16*, and *trnL-F* failed to resolve the phylogenetic and species identification problems between *S. tzumu* and *S. randaiense*. Song et al. (2016) suggest that the extremely low genetic variation and short sequence length among species of Lauraceae could be improved by recent chloroplast genome data. For a better understanding of the differences between *S. tzumu* and *S. randaiense*, the complete plastid genome of *S. randaiense* was sequenced.

## Materials and methods

Dry leaves of *S. randaiense* (Figure 1) in Lushan Botanical Garden (Jiujiang, China; Long. 115.995618 E, Lat. 29.552275 N, 1106 m) were collected for DNA extraction (Doyle and Dickson 1987). The voucher was deposited at the Biodiversity Research Group in the herbarium of Xishuangbanna Tropical Botanical Garden (http://m.extbg.cas.cn/, Yu Song, songyu@gxnu.edu.cn) under the voucher number XTBG-BRG-SY36803. Following Zhang et al. (2016), we sequenced the whole plastid genome using their 15 universal primer pairs for long-range PCR for Illumina sequencing. According to the manufacturer's instructions (Illumina Nextera XT Library), the mixture was fragmented and used to construct 500-bp short-insert libraries. The clean data exhibited a GC content of 39.2%, a Q20 of 95.1%, and a Q30 of 88.1%, indicating high quality sequencing and assembly. The contigs were aligned using the publicly available plastid genome of *S. tzumu* (accession number LAU00020) (Song et al. 2017) and annotated in Geneious 4.8.

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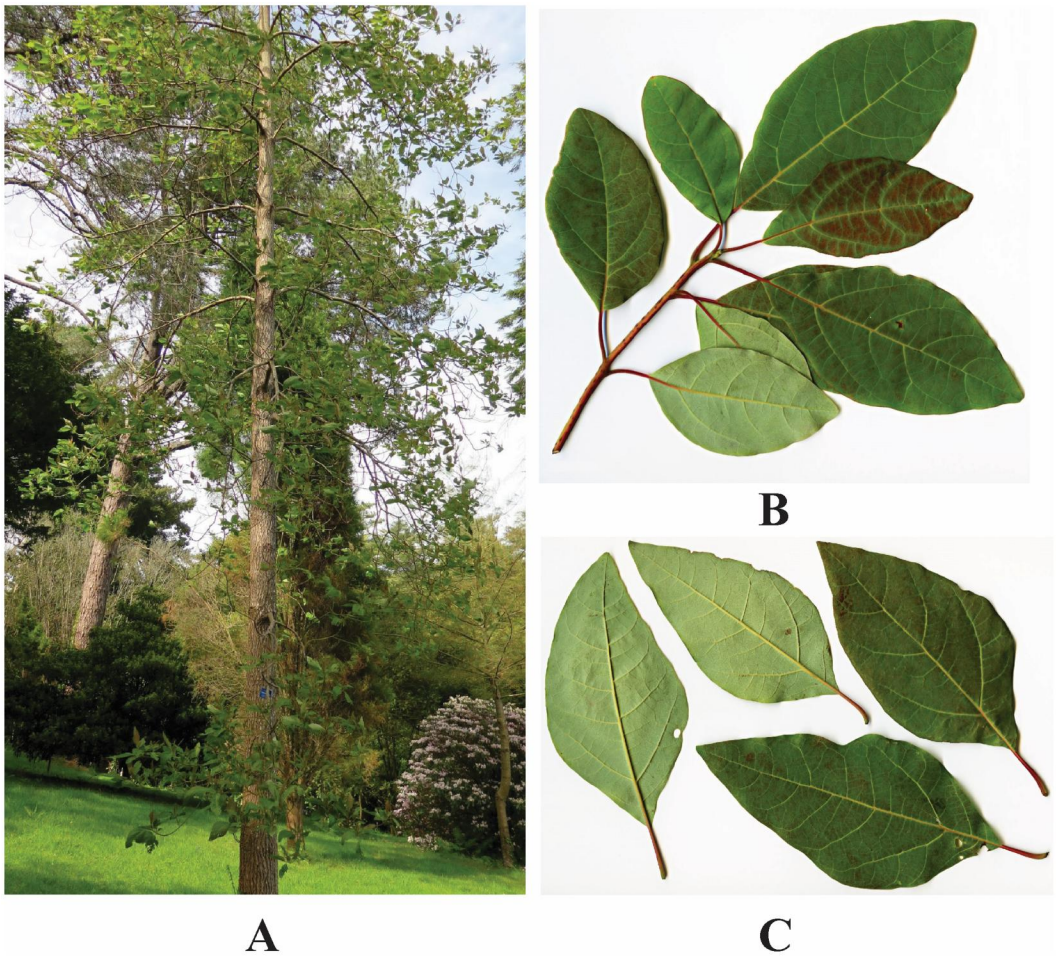


**Figure 1.** Morphology features of *Sassafras randaiense*. (A) Individual of *S. randaiense*; (B) young branch; (C) petiolate leaf. The photos of *S. randaiense* were taken from the website Trees and Shrubs Online (Trees and Shrubs Online 2024). *S. randaiense* is a deciduous tree with medium size (20–25 m tall). The leaves (10–15 cm long and 5–6 cm broad) have an acute apex, and are acute or obtuse at base. The flowers are bisexual, and their third-whorl anthers are extrorse, resembling a typical hermaphroditic flower. The fruits are globose (6–7 mm), with thick pedicel.

Phylogenetic analysis was conducted to confirm the evolutionary relationship between *S. randaiense*, *S. tzumu*, and other species within the *Cinnamomum-Ocotea* clade that have published plastid genomes. We obtained 30 complete chloroplast genomes sequences from GenBank and *Machilus-Persea* clade used as the out-group (Song et al. 2020). We aligned the complete chloroplast genome sequences of *S. randaiense* and other 30 species using MAFFT (Katoh and Standley 2013) and performed maximum-likelihood (ML) phylogenetic analyses based on GTR + F + R3 model in the iqtree version 1.6.7 (Nguyen et al. 2015), which was selected by ModelFinder (Kalyaanamoorthy et al. 2017).

## Results and discussion

The overall depths of coverage for the assembled genome are illustrated in Figure S1. The plastid genome of *S. randaiense* (accession number MW337246), is 151,781 bp in length (Figure 2), which is shorter by 17 bp compared to *S. tzumu* (151,798 bp, LAU00020) (Song et al. 2017), 934 bp shorter than *Cinnamomum glanduliferum* (152,715 bp, LAU00111) (Zhao et al. 2019), and 972 bp shorter than *C. chago* (152,753 bp, LAU00078) (Chen et al. 2019). *S. randaiense* chloroplast genome composed of a large single-copy (LSC) region of 92,740 bp, a small single-copy (SSC) region of 18,813 bp, and a pair of inverted repeats (IRs) of 20,114 bp (Supplemental Figures 2 and 3). The LSC, SSC, and IR regions have GC contents of 38.0%, 34.0%, and 44.4%, respectively, resulting in an overall GC content of 39.2% for the *S. randaiense* chloroplast genome.

To confirm the evolutionary relationship between *S. randaiense*, *S. tzumu*, and other species with published plastid genomes in the *Cinnamomum-Ocotea* clade, we obtained the chloroplast genomes of 30 other closely related species from NCBI GenBank database. Subsequently, a ML analysis was performed to reconstruct a phylogenetic tree (Figure 3). The ML phylogenetic tree, with 100% bootstrap support at the node, confirmed that *S. randaiense* is closely related to *S. tzumu* and both species are nested within the genus *Cinnamomum*. The phylogenetic tree suggested that *Sassafras* belongs to *Cinnamomum* lineage. However, further

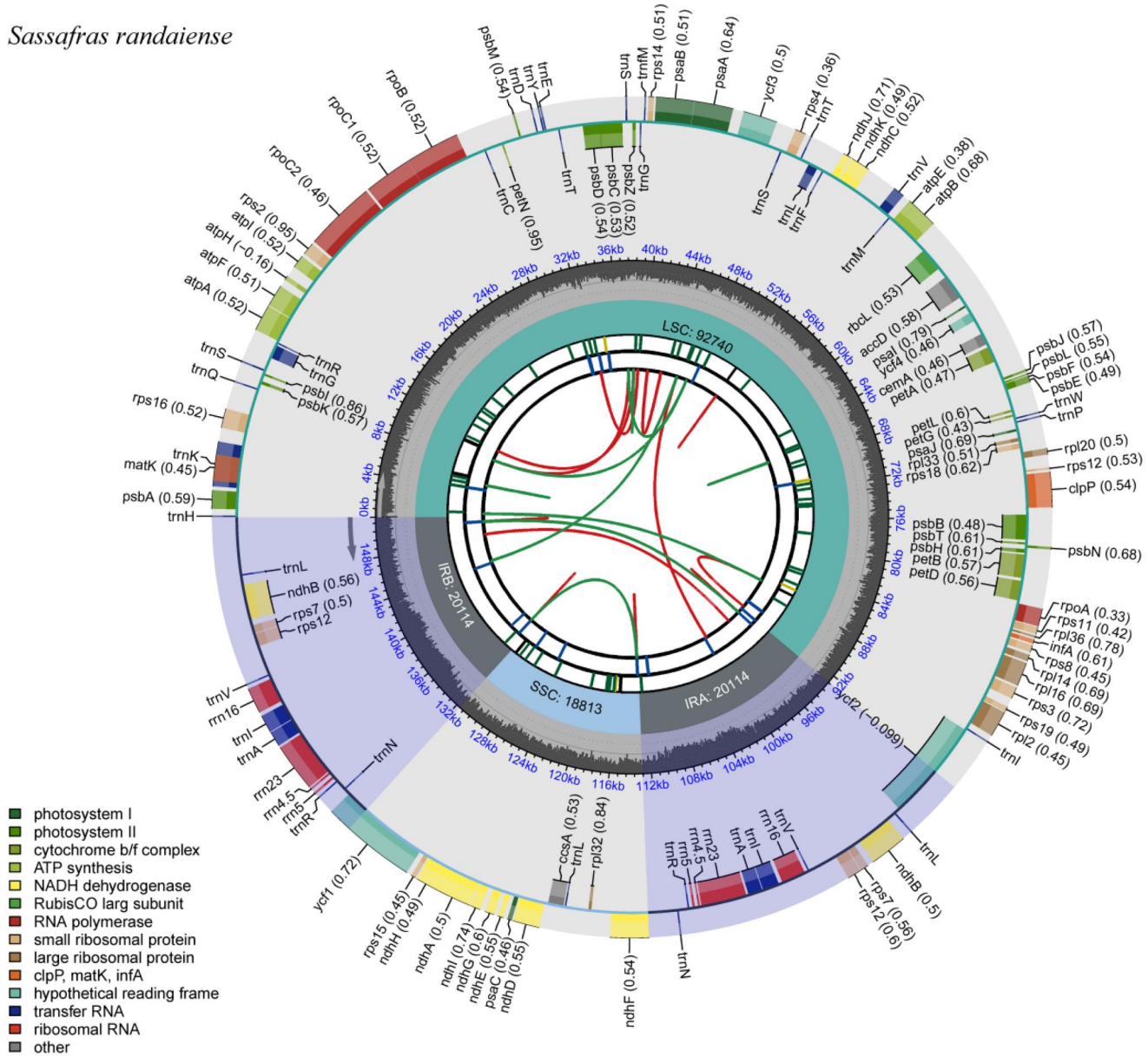


**Figure 2.** Chloroplast genome maps of *Sassafras randaiense*. Different functional groups of genes are signed according to the colored boxes. LSC: large single-copy; SSC: small single-copy; IRA: inverted repeat regions.

research is required to determine the phylogenetic relationship between *Sassafras* and *Cinnamomum*.

## Conclusions

The complete chloroplast genome of *Sassafras randaiense* is reported herein for the first time. It had a typical circular structure composed of 151,781 bp. Based on molecular phylogenetic analysis using 31 complete chloroplast genomes, we provided evidence that *S. randaiense* is a sister species to *S. tzumu*, and these two species are nested among the members of *Cinnamomum*. The published chloroplast genome of *S. randaiense* will provide genetic information for further phylogenetic studies of *Sassafras*.

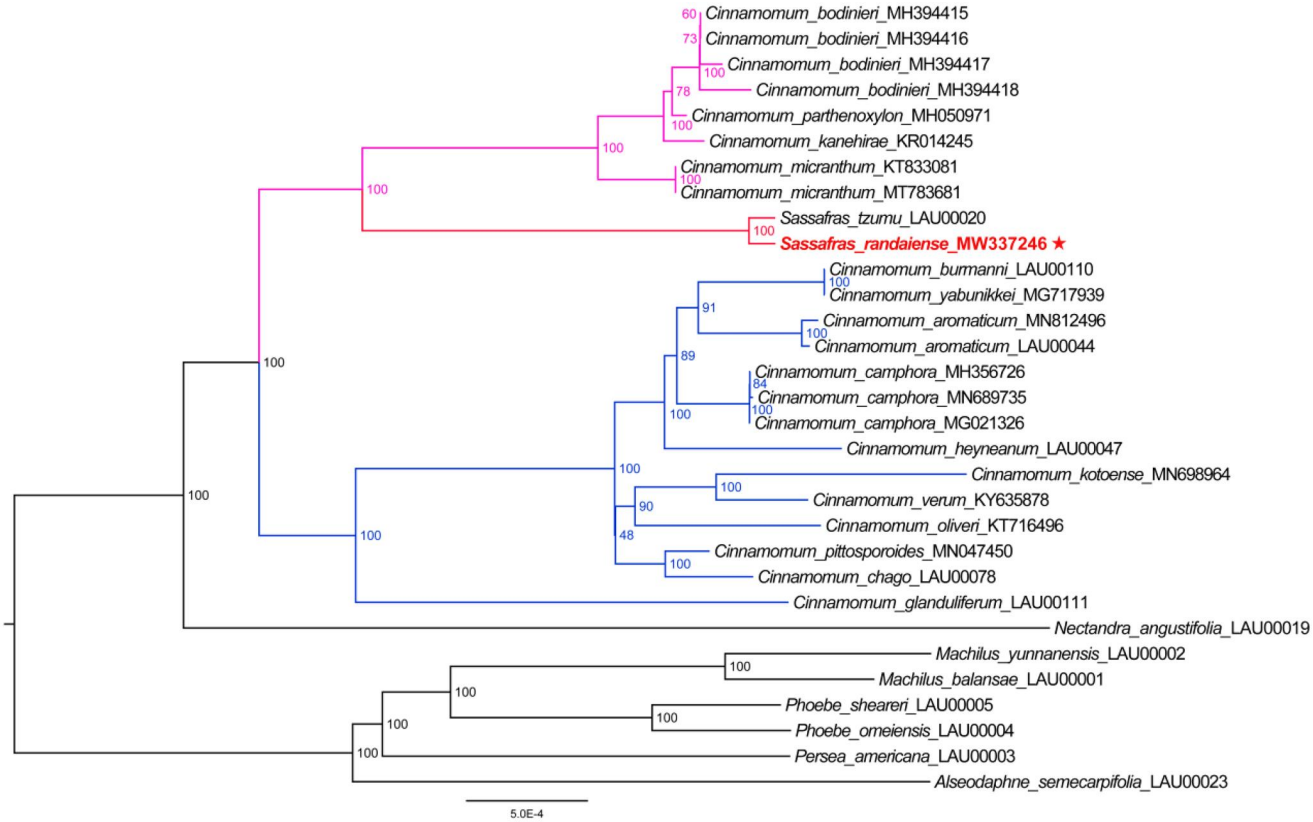


**Figure 3.** The ML phylogenetic tree for *Sassafras randaiense* based on 31 species plastid genomes, with the *Machilus-Persea* clade serving as the out-group. The following sequences were used: *Cinnamomum kanehirae* KR014245 (Wu et al. 2016), *Cinnamomum micranthum* KT833081 (Wu et al. 2017), *Cinnamomum burmanni* LAU00110 (Yang et al. 2019), *Cinnamomum glanduliferum* LAU00111 (Zhao et al. 2019), *Cinnamomum parthenoxylon* MH050971 (Wu et al. 2019), *Cinnamomum camphora* MH356726 (Li et al. 2019), *Cinnamomum camphora* MN689735 (Qiu et al. 2020), *Cinnamomum kotoense* MN698964 (Yuan et al. 2020), *Cinnamomum pittosporoides* MN047450 (Zhou et al. 2019), *Sassafras randaiense* MW337246 (this study), *Cinnamomum oliveri* KT716496, *Cinnamomum verum* KY635878, *Machilus balansae* LAU00001, *Machilus yunnanensis* LAU00002, *Persea americana* LAU00003, *Phoebe omeiensis* LAU00004, *Phoebe sheareri* LAU00005, *Nectandra angustifolia* LAU00019, *Sassafras tzumu* LAU00020, *Alseodaphne semecarpifolia* LAU00023, *Cinnamomum aromaticum* LAU00044, *Cinnamomum heyneanum* LAU00047, *Cinnamomum chago* LAU00078, *Cinnamomum camphora* MG021326, *Cinnamomum yabunikkei* MG717939, *Cinnamomum bodinieri* MH394415, *Cinnamomum bodinieri* MH394416, *Cinnamomum bodinieri* MH394417, *Cinnamomum bodinieri* MH394418, *Cinnamomum aromaticum* MN812496, and *Cinnamomum micranthum* MT783681 (unpublished).

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## Author contributions

NHB and WL: conceptualization, fieldwork, experiments, and modified the manuscript. ZW: methodology, phylogenetic analysis, and writing. YG and HC: design, assembly and annotating the chloroplast genome, performing the data acquisition, data analysis, data interpretation, and manuscript revision. All authors approved the manuscript, and agreed to be accountable for all aspects of the work.

## Ethical approval

This species is not endangered or collected in nature reserves, so it does not need any specific permission. The research involved *Sassafras randaiense*, which was a vulnerable species, so in this study we complied with the policies of the International Union for Conservation of Nature (IUCN), the Convention on Biological Diversity, and the Convention on the Trade in Endangered Species of Wild Fauna and Flora, and tried our best to protect the resources of *S. randaiense*. All acquisition and sequencing work was carried out in strict compliance with relevant local laws and laboratory regulations in order to preserve wild resources.

## Disclosure statement

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

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## Data availability statement

The assembled chloroplast genome sequence data that support the findings of this study are freely available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) under the accession number of MW337246. The

associated BioProject, SRA, and Bio-Sample numbers are PRJNA1103359, SRR28776521, and SAMN41051158, respectively.

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