

## The complete chloroplast genome sequence of *Nostolachma jenkinsii* (Hook.f.) Deb & J.Lahiri, an endangered coffeeae plant

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### ABSTRACT

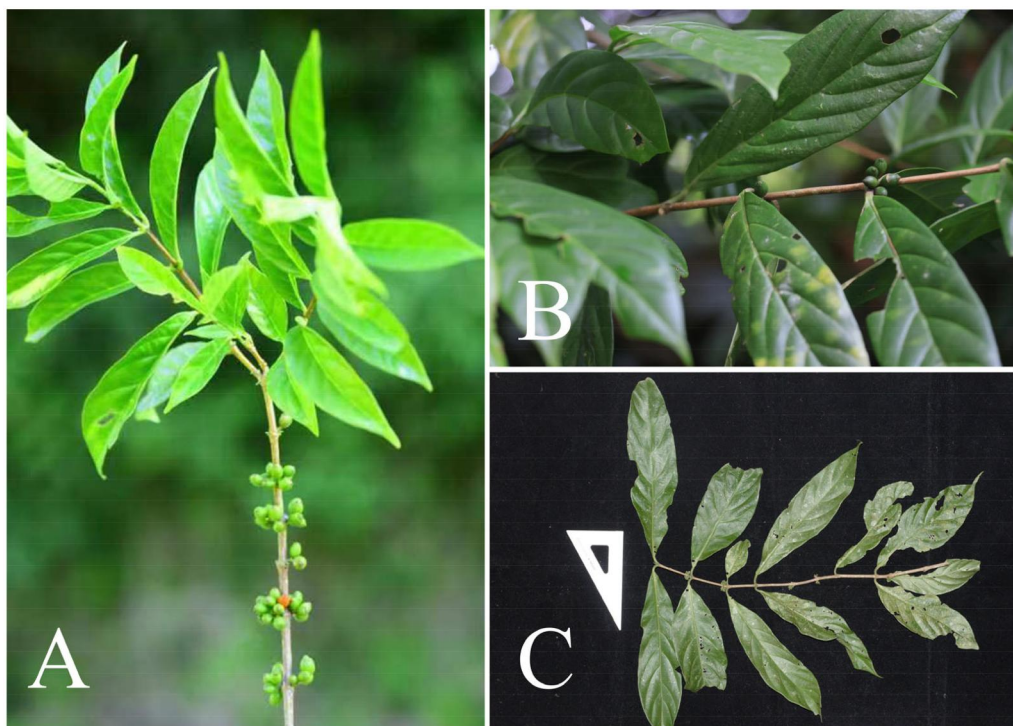
*Nostolachma jenkinsii* (Hook.f.) Deb & J.Lahiri, a member of the *Rubiaceae* family, is an endangered wild plant species with potential economic value. In this research, the complete chloroplast genome of *N. jenkinsii* was sequenced to gain insight into its genome feature and better understand the phylogenetic relationships among the *Rubiaceae* species. The chloroplast genome, with a total length of 155,036 bp, comprises two inverted repeats (IR) regions spanning 25,692 bp each, a large single-copy (LSC) region measuring 85,437 bp, and a short single-copy (SSC) region measuring 18,215 bp. There is an overall 37% GC content in the chloroplast genome. By annotation analysis, 54 tRNA genes, 10 rRNA genes, and 107 protein-coding genes were all annotated in *N. jenkinsii*. Furthermore, we applied phylogenetic analysis that revealed a close relationship between *N. jenkinsii*, *D. fruticosa* and *D. dubia*, placing them together within the *Rubiaceae* family.

### ARTICLE HISTORY



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Genome feature;  
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phylogenetic analysis;  
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**Figure 1.** A Drupe of *N. jenkinsii*. B-C leaves of *N. jenkinsii*. *Nostolachma jenkinsii* is shrubs, to ca. 3 m tall; leaf blade drying papery, elliptic-oblanceolate, 12–24 × 4.5–6 cm, margins flat or occasionally crisped-undulate; inflorescences with cymes 1–3 in each axil, each cyme subcapitate to fasciculate, 3–6-flowered, subsessile to pedunculate with peduncles to 7 mm; drupe red, ellipsoid to subglobose. Photo by Rongjie Zhu in Motuo County.

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## Introduction

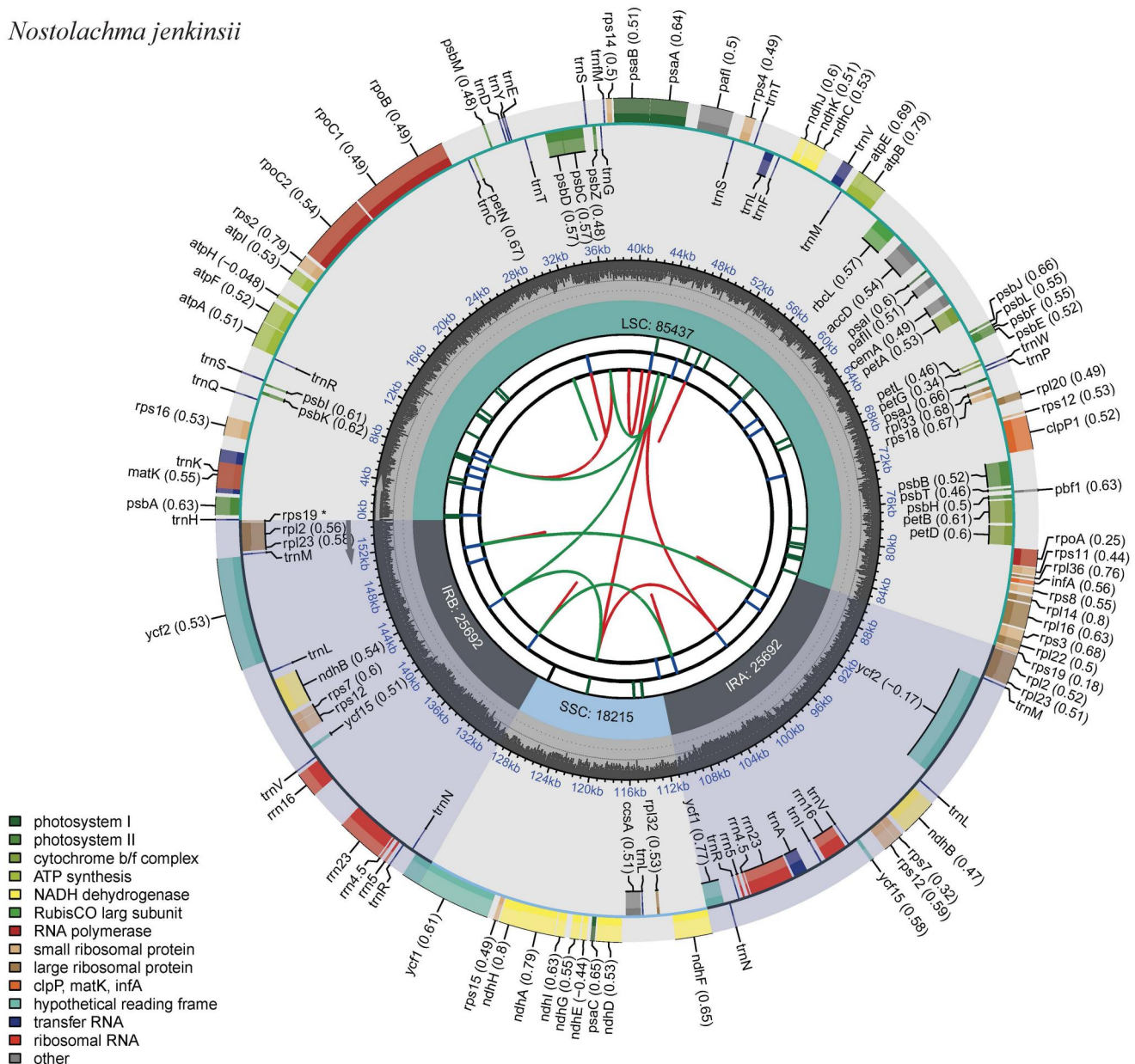
*Nostolachma jenkinsii* (Hook.f.) Deb & J. Lahiri 1978 (IPNI 2022) is an endangered plant in the genus of *Nostolachma* (Family Rubiaceae), which is listed on the International Union for Conservation of Nature Red List (IUCN) with a protection level of Endangered (EN). *Coffea jenkinsii* J. D. Hooker was included in the Fl. Xizang and India (Wu 1985). Davis et al. (2006) treated *C. jenkinsii* as a species of *Nostolachma*, under Coffeae. *N. jenkinsii* is shrubs, to ca. 3 m tall; leaf blade drying papery, elliptic-oblong, 12–24 × 4.5–6 cm, margins flat or occasionally crisped-undulate; Inflorescences with cymes 1–3 in each axil, each cyme subcapitate to fasciculate, 3–6-flowered, subsessile to pedunculate with peduncles to 7 mm; Drupe red, ellipsoid to subglobose (Figure 1). Presently, there are 32 species of Rubiaceae family listed in NCBI (<https://www.ncbi.nlm.nih.gov/>). However, the complete chloroplast

genome sequence of *N. jenkinsii*'s has not been reported yet. In order to provide a genomic resource and shed light on the phylogenetic relationship between *N. jenkinsii* and related taxa, we present here the complete chloroplast genome sequence of those species.

## Materials and methods

*Nostolachma jenkinsii* samples were procured in the Xizang Autonomous Region of Southeast China's Motuo County (e.g. latitude 29.2984 N and longitude 95.3242 E). We collected leaf samples for total genomic DNA extraction, and branches for voucher specimen preparation, which was deposited at the Herbarium of Tibet Plateau Institute of Biology (XZ) (Xilong Wang, [wangxilong@hotmail.com](mailto:wangxilong@hotmail.com)) under voucher number ZRJ20201979. Genomic DNA extraction, library building, and

### *Nostolachma jenkinsii*



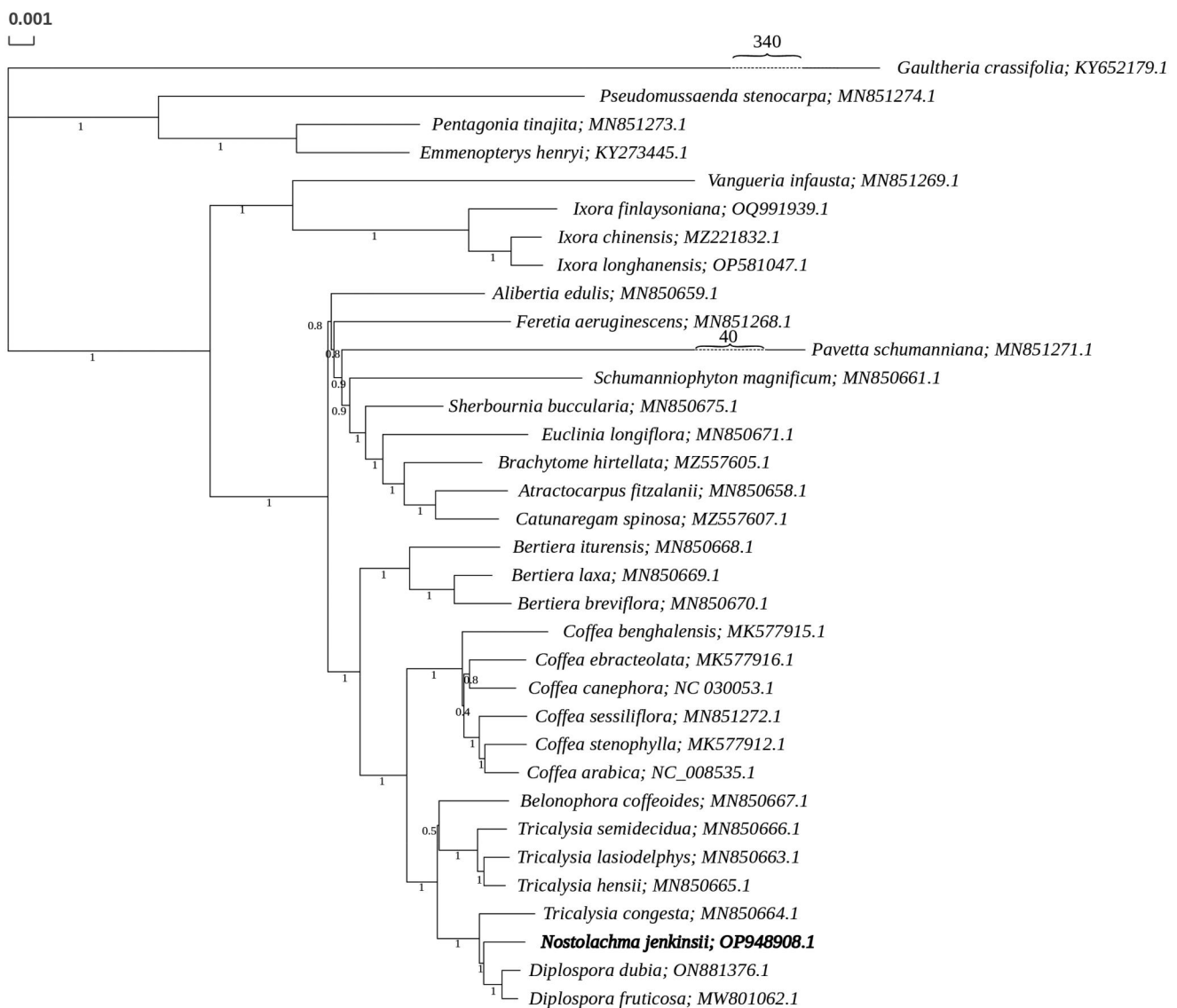
**Figure 2.** The genome map of the *N. jenkinsii* chloroplast. The map was circular with a typical four-segment, composed of two inverted repeats (IR) regions, one large single-copy (LSC) region, and one small single-copy (SSC) region.

Illumina HiSeq 2500 sequencing were performed by Annoroad (Beijing, China). Isolated genomic DNA was processed to construct a shotgun library with an average length of 400 bp, which was then sequenced using the Illumina NovaSeq platform with a paired-end mode of 2150 bp. The generated reads were subsequently assembled using GetOrganelle v1.6.2e (Jin et al. 2020), and annotated through the online annotation tool CPGView (<http://www.1kmpg.cn/cpgview/>) (Liu et al. 2023). In order to reveal the phylogenetic relationship, we downloaded 32 species in the family Rubiaceae from NCBI (<https://www.ncbi.nlm.nih.gov/>), using *Gaultheria crassifolia* (*Ericaceae*) as an outgroup (Ly et al. 2020). Multiple alignments were performed using MAFFT version 7 software through the online analysis website (<https://mafft.cbrc.jp/alignment/server/>) (Standley and

Katoh 2013), followed by trimming with MEGA X (Sudhir et al. 2018). The phylogenetic tree was generated in MAGA X utilizing, the Maximum-Likelihood statistical method and 1000 bootstrap replicates.

## Results

The results demonstrated that the *N. jenkinsii* chloroplast genome sequence is circular with a typical four-segment length of 155036 bp (Figure 2). The reads average depth at 4000x and 100% genome sequencing coverage (Supplementary Figure 1). It comprises two inverted repeats (IR) regions of 25,692 bp, one large single-copy (LSC) region of 85,437 bp, and one small single-copy (SSC) region of 18,215 bp. The entire chloroplast



**Figure 3.** The ML phylogenetic tree for *Nostolachma jenkinsii* and other species of Rubiaceae, based on the complete plastid genomes catenated dataset. Numbers in each node indicated the bootstrap support values. The following sequences were used: *Gaultheria crassifolia* KY652179.1 (Zhang et al. 2017), *Pseudomussaenda stenocarpa* MN851274.1, *Pentagonia tinajita* MN851273.1, *Vangueria infausta* MN851269.1, *Pavetta schumanniana* MN851271.1, *Feretia aeruginescens* MN851268.1, *Coffea sessiliflora* MN851272.1 (Ly et al. 2020), *Emmenopterys henryi* KY273445.1 (Duan et al. 2017), *Coffea benghalensis* MK577915.1, *Coffea ebracteolata* MK577916.1, *Coffea stenophylla* MK577912.1 (Guyeux et al. 2019), *Ixora chinensis* MZ221832.1, *Schumanniophyton magnificum* MN850661.1, *Euclinia longiflora* MN850671.1, *Atractocarpus fitzalanii* MN850658.1, *Sherbournia buccularia* MN850675.1, *Sherbournia buccularia* MN850675.1, *Alibertia edulis* MN850659.1, *Bertiera iturenensis* MN850668.1, *Bertiera laxa* MN850669.1, *Bertiera breviflora* MN850670.1, *Belonophora coffeoides* MN850667.1, *Tricalysia semidecidua* MN850666.1, *Tricalysia lasiodelphys* MN850663.1, *Tricalysia hensii* MN850665.1, *Tricalysia congesta* MN850664.1, *Coffea arabica* NC\_008535.1, *Coffea canephora* NC\_030053.1, *Ixora longhanensis* OP581047.1, *Catunaregam spinosa* MZ557607.1, *Brachytome hirtellata* MZ557605.1, *Diplospora fruticosa* MW801062.1, *Diplospora dubia* ON881376.1, *Ixora finlaysoniana* OQ991939.1.



genome contains 37% GC overall. In addition, *N. jenkinsii* complete chloroplast genome includes 54 tRNA genes, 10 rRNA genes, and 107 protein-coding genes. The genome sequence includes 13 Cis-splicing genes (*rps16*, *atpF*, *rpoC1*, *paf1*, *clpP1*, *petB*, *petD*, *rpl16*, *ndhB*, *ndhA*, *rpl2*), the *rpl2* and *ndhB* were duplicates, eleven of them have one intron and two exons, and two have two introns and three exons (Supplementary Figure 2). The trans-splicing gene *rps12* has three unique exons, two of which are duplicated as they are located in the IR regions (Supplementary Figure 3). The ML phylogenetic tree was constructed in *Rubiaceae* family (Figure 3). According to phylogenetic tree, *N. jenkinsii* is closely related to *D. fruticosa* and *N. jenkinsii* are clustered with *T. congesta*, *D. dubia* and *D. ruticosa* (Figure 3).

## Discussion and conclusion

*Rubiaceae* is the fourth-largest angiosperm family has suggested three subfamilies *Rubioideae*, *Cinchonoideae*, and *Ixoroideae*. Subfamily *Ixoroideae* includes many well-known economically important genera used for horticultural, medicine and beverages e.g., *Coffea* L. two informal alliances are recognized within the *Ixoroideae*: the *Vanguerieae* alliance and the *Coffeae* alliance. (Amenu et al. 2022). Recently reports have shed light on the evolutionary circumstance of the *Coffeae* alliance but dose not *N. jenkinsii*. In this research, we firstly provided genomic resources of *N. jenkinsii*, an endangered species in *Rubiaceae* family. The complete chloroplast genome characteristics of *N. jenkinsii* will allow a better understanding of *Rubiaceae* biology, diversity, and evolution. The phylogenetic tree showed that *T. congesta*, *D. dubia* and *D. ruticosa*, and *N. jenkinsii* formed a monophyletic clade within *Rubiaceae* with 100% bootstrap values. It will thus be valuable in elucidating the evolutionary relationships within *Rubiaceae* in the future. In the phylogenetic tree, several species of the genus *Coffea* cluster in the same branch, and the branch of the target species is closely related to that of *Coffea*. Coffee is known to have high economic value, but cultivation areas are limited. As coffee cultivation is centered between 25° north and south latitudes, while *N. jenkinsii* is found at 29° north latitude in Xizang, hybridization between them may result in new varieties, which will expand coffee cultivation area.

## Ethics statement

The Science and Technology Plan Project of the Xizang Autonomous Region of China permitted and approved the study (SWS201801, XZ2019ZRG-74(Z), XZ202001YD0008C, XZ202201ZY0010G) to comply with the International Union for Conservation of Nature (IUCN) policies for research involving species at risk of extinction.

## Author contributions

Rongjie Zhu provided the conceptualization and methodology. Rongjie Zhu and Guanfei Zhao took samples and made specimens. Guanfei Zhao extracted DNA and sequenced the complete chloroplast genome. Wenshuai Chen and Ming Lei completed the data assembly annotation analysis, as well as the writing of the article. All authors agreed to be accountable for all aspects of the work and approved the final draught

to be published. The final draft of the work was accepted for publication and all writers agreed to accept responsibility for all parts of the work.

## Disclosure statement

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

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## 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>] (<https://www.ncbi.nlm.nih.gov/>) under accession no. OP948908.1. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1012112, SRP458099, and SAMN37235689 respectively.

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