

## Characteristics and phylogenetic analysis of the complete chloroplast genome of *Rubus swinhoei* Hance 1866 from the family Rosaceae

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### ABSTRACT

*Rubus swinhoei* Hance is an important plant owing to its medicinal root and edible fruit, and extensively distributed in China. In this study, we reported the complete chloroplast genome of *R. swinhoei*. The chloroplast genome was 156,335 bp in size with the overall GC content of 37.15%, having a circular and quadripartite structure, which contained a large single-copy (LSC) and a small single-copy (SSC) regions of 85,897 bp and 18,858 bp separated by a pair of 25,790 bp inverted repeat (IR) regions. The complete chloroplast genome comprised 131 unique genes of which 86 and 37 were protein-coding genes and tRNA genes, respectively, and also eight rRNA genes. The phylogenetic analysis revealed that *R. swinhoei* was closely related to *R. kawakamii*. The genome information reported in this paper will be beneficial for further investigation on the evolution of this species.

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### Introduction

*Rubus swinhoei* Hance (1866, [Figure 1](#)), an important plant containing medicinal root and edible fruit, is extensively distributed in China (mainly in provinces of Guangdong, Guangxi, Yunnan, Hunan, Anhui, Zhejiang, Jiangsu, Fujian, Sichuan, and Taiwan) (Lin and Zhang 1985; Zhao, Ding, Wang 2001). It is a perennial shrub that grows in thickets under the altitude from 400 m to 1000 m (Lin and Zhang 1985). The root of *R. swinhoei* was reported to have effects on stopping bleeding, regulating menstruation, promoting blood circulation and so on (Zhao, Ding, Wang 2001). Phytochemical researches have shown that the root of *R. swinhoei* mainly includes triterpenoids, sterols, tannins, etc. (Zhao, Ding, Wang 2001; Zhao, Ding, Zhang, et al. 2001).

Blackberry and raspberry, which belong to genus *Rubus*, have high health value with multiple biological activities (Goodman et al. 2021). They are rich in sugars, vitamin, ellagic acid, flavones, anthocyanins, etc. (Huang et al. 2022). As well as the fruit of other plants from *Rubus*, the fruit of *R. swinhoei* with high vitamin content has the very high nutritional value (Lin et al. 2000; Chen et al. 2001). Its fruit rich in hematochrome with the ripening period from June to August, which can be used as an excellent raw material for extracting natural edible hematochrome (Chen et al. 2001).


There are more than 700 species of *Rubus* in the world-wide, while 208 species grow in China, including 139



**Figure 1.** *Rubus swinhoei* reference image. Photographed by Wei Wang. It is a vine like shrub with thin and round stems, dark purplish brown. The leaves are single leaf, leaf shape varies greatly, from broad ovate to oblong-lanceolate, apex acuminate, base truncate to shallowly cordate. The petioles are grey-white villous, sometimes with hook-like prickles. Stipules ovate-lanceolate, slightly pilose. The flowers are white, and the fruit is red and nearly spherical, with a diameter of 1–1.5 cm.

endemic species (Graham and Brennan 2018; Foster et al. 2019; Wu et al. 2003). To better understand the phylogenetic position of *R. swinhoei* within Rosaceae, its complete chloroplast genome (cpDNA) was assembled and characterized in this study. The result of this study will be useful for evolutionary studies on *R. swinhoei* and the inference of phylogenetic relationships with other species.

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## Materials and methods

### Sampling, cpDNA extraction, and sequencing

The fresh leaf materials of the *R. swinhoei* were collected from the medicinal botanical garden of West Anhui University (latitude: 31°77' N, longitude: 115°93' E), Anhui Province, China. A voucher specimen was deposited at Changchun Sci-Tech University (contact Bing Li, [lb18643018937@163.com](mailto:lb18643018937@163.com)) under the voucher number: MM-20220716-1. The modified CTAB method was used to extract the total genomic DNA from leaves of *R. swinhoei* CTAB method (Doyle and Doyle 1987). The whole genome sequencing was performed using the BGISEQ-500 platform (Hefei Biodata Biotechnologies Inc., Hefei, China).

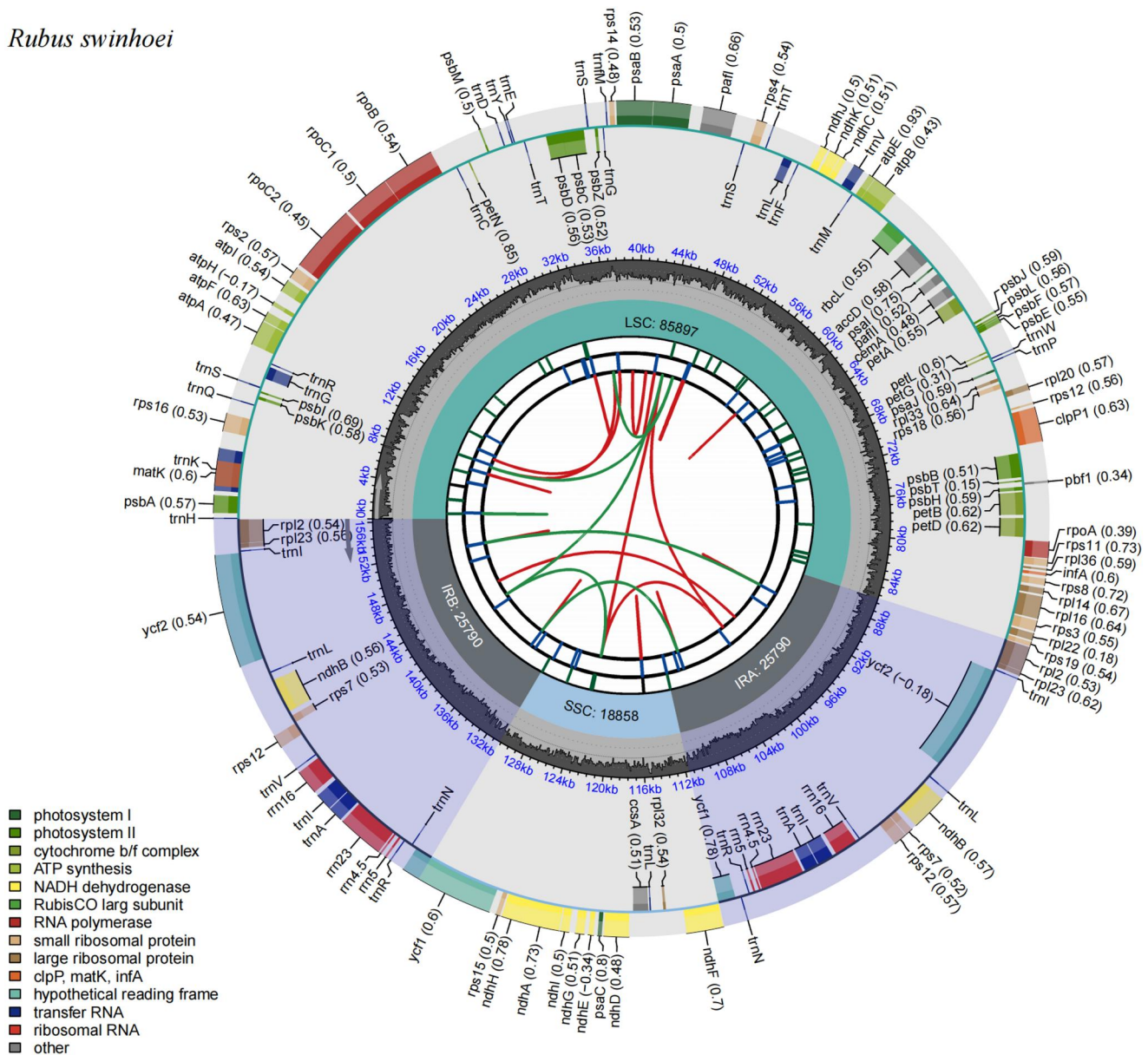
### CpDNA assembly and annotation

The chloroplast genome of *R. swinhoei* was filtered and assembled by the program fastp (Chen et al. 2018) and SPAdes assembler 3.10.0 (Bankevich et al. 2012), respectively. Annotation of the *R. swinhoei* chloroplast genome was performed using the GeSeq (Tillich et al. 2017) and BLASTx (Gish and States 1993). The complete chloroplast genome sequence of *R. swinhoei* was submitted to GenBank (Accession number OQ411240).

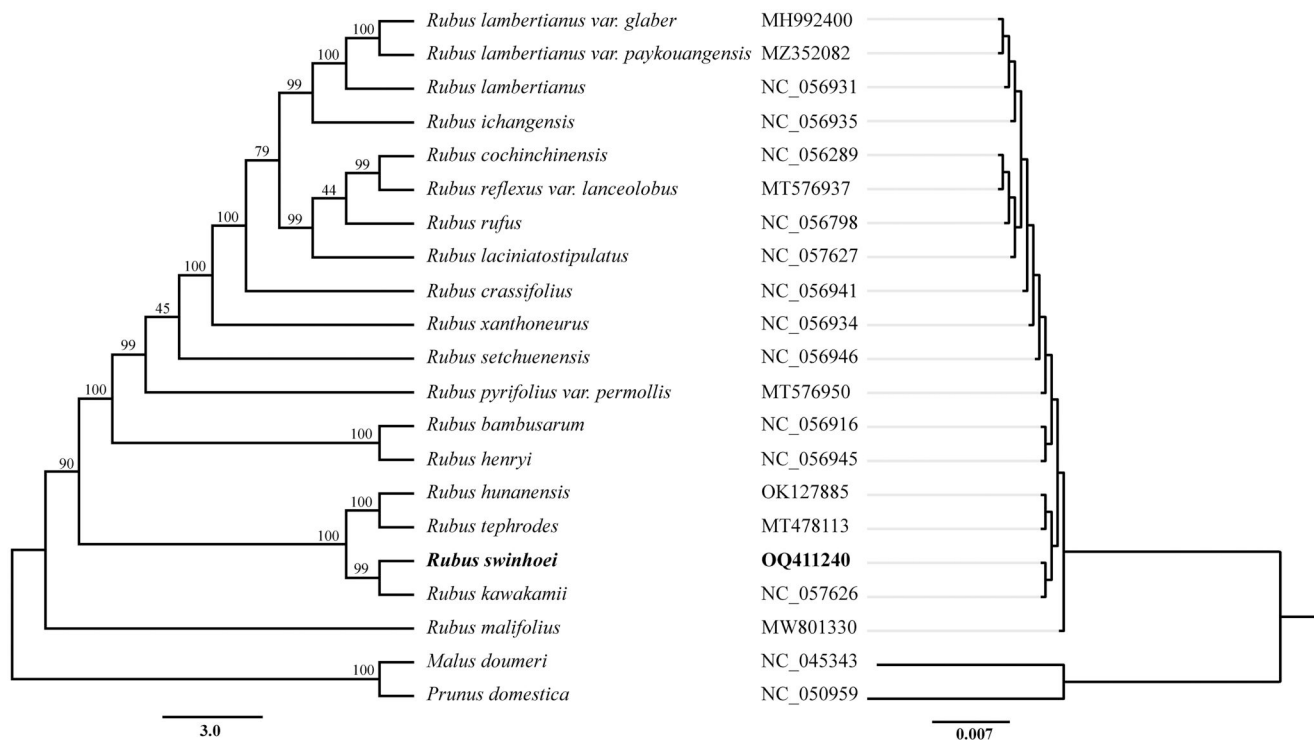
### Phylogenetic analysis

Phylogenetic analysis was conducted based on the CpDNA of *R. swinhoei* and 20 other related species. Among them,

#### *Rubus swinhoei*



**Figure 2.** The complete chloroplast genome map of *R. swinhoei*. Graphic representation of features identified in the complete chloroplast genome *R. swinhoei* using CPGview online tool. The map contains seven tracks. The first track shows the dispersed repeats connected with red (forward direction) and green (reverse direction) arcs from the center going outward, respectively. The second track shows the long tandem repeats marked with short blue bars. The third track displays the short tandem repeats or microsatellite sequences as short bars with different colors. The fourth track depicts the sizes of the small single-copy (SSC) and large single-copy (LSC). The fifth track shows the inverted repeats (IRA and IRB). The sixth track plots the distribution of GC contents along the plastome. The seventh track shows the genes belonging to different functional groups, with each differently colored.



**Figure 3.** Phylogenetic tree inferred by maximum-likelihood (ML) method based on 20 representative species showed with the cladogram (left) and phylogram (right). *Prunus domestica* and *Malus doumeri* were used as outgroup taxa. A total of 1000 bootstrap replicates were computed and the bootstrap support values are shown at the branches. The following 20 sequences were used: *Rubus lambertianus* var. *glaber* MH992400 (Chen et al. 2020), *Rubus lambertianus* var. *paykouangensis* MZ352082 (Zhu et al. 2022), *Rubus lambertianus* NC\_056931, *Rubus ichangensis* NC\_056935, *Rubus cochinchinensis* NC\_056289 (Chen et al. 2020), *Rubus reflexus* var. *lanceolobus* MT576937, *Rubus rufus* NC\_056798, *Rubus laciniatostipulatus* NC\_057627, *Rubus kawakamii* NC\_057626 (Yang et al. 2021), *Rubus crassifolius* NC\_056941, *Rubus xanthoneurus* NC\_056934, *Rubus setchuenensis* NC\_056946 (Zhu et al. 2022), *Rubus pyrifolius* var. *permollis* MT576950, *Rubus bambusarum* NC\_056916 (Zhu et al. 2023), *Rubus henryi* NC\_056945 (Zhu et al. 2023), *Rubus hunanensis* OK127885, *Rubus tephrodes* MT478113 (Yu et al. 2022), *Rubus malifolius* MW801330, *Malus doumeri* NC\_045343 (Wang et al. 2019), and *Prunus domestica* NC\_050959 (Geng et al. 2020).

*Prunus domestica* and *Malus doumeri* were used as outgroups. The cpDNAs of other related species were downloaded from the NCBI GenBank database. Maximum-likelihood (ML) analysis was carried out using the FastTree version 2.1.10 with the generalized time-reversible (GTR) model and Shimodaira–Hasegawa (SH) test, and the support values based on the SH test were shown at the branches (Price et al. 2010).

## Results

### Characteristics of *R. swinhoiei* CpDNA

The chloroplast genome of *R. swinhoiei* was a 156,335 bp in size with a circular and quadripartite structure identified by CPGview tool (Liu et al. 2023). It contained a pair of 25,790 bp inverted repeat (IR) regions that divided the genome into two single-copy regions (a large single-copy (LSC) and a small single-copy (SSC) regions of 85,897 bp and 18,858 bp, respectively) (Figure 2). The percentage of GC in the gene regions was 37.15%, and the corresponding values in LSC, SSC, and IR regions were 35.10%, 31.16%, and 42.76%, respectively. The chloroplast genome of *R. swinhoiei* had a total of 131 genes of which 86 and 37 were protein-coding genes and tRNA genes, respectively, and also eight rRNA genes. Within the *R. swinhoiei* chloroplast genome, 18 genes duplicating in IR regions contained six protein-coding genes, eight tRNA genes and four rRNA genes. There were 18 genes with two exons and four genes (*psaI*, *clpP1*, and two

*rps12*) with three exons. The read coverage map also showed that the read coverage was relatively uniform (Figure S1), indicating that the data quality is reliable in this study. Moreover, the *rps16*, *rpoC1*, *psaI*, *clpP1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, and *ndhA* genes were cis-splicing genes (Figures S2 and S3).

### Phylogenetic analysis

To investigate the taxonomic status of *R. swinhoiei*, we aligned the complete chloroplast genome of *R. swinhoiei* and 20 other related species to reveal the phylogenetic position of *R. swinhoiei* using MAFFT v7.307 (Katoh and Standley 2013). As expected, the ML analysis produced a phylogenetic tree which supported *R. swinhoiei* to be closely related with *R. kawakamii* with a 99% bootstrap value (Figure 3), whereas *R. hunanensis* was a sister to *R. tephrodes*. In addition, *R. swinhoiei* and *R. kawakamii* were most closely related to *R. hunanensis* and *R. tephrodes*, which forms a monophyletic group with a 100% bootstrap value of the two clades.

## Discussion and conclusions

In this study, we successfully sequenced and definitively identified the entire chloroplast genome of *R. swinhoiei*. We verified the accuracy of the sequence assembly by obtaining consistent results using different software tools. Specifically, we reassembled the genome using SPAdes assembler 3.10.0



and NOVOplasty 4.3 (Dierckxsens et al. 2017), and both approaches yielded identical assembly outcomes.

The analysis results of phylogenetic relationship showed that *R. swinhoei* was closely related to *R. kawakamii*. Although these two species are closely related, there are several distinct morphological features that differ significantly. Specifically, *R. swinhoei* has hairy leaves, raceme flowers and glandular hairs. *R. kawakamii* is only slightly hairy along the vein below, and the flowers are corymbose raceme without glandular hair (Hsieh and Ohashi 1993). The chloroplast genome of *R. swinhoei* was assembled for the first time in this study. The genome information will provide a valuable genetic source for the *Rubus* species, and also be beneficial for further investigation on the evolution of *R. swinhoei*. This study establishes a robust foundation for providing pivotal information that will facilitate species identification, further evolutionary analyses, and genetic enhancement of this notable species.

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## Author contributions

Conception and design: Li B and Wang L; data analysis and interpretation: Li B, Wang W, and Liu Y; manuscript writing and revising: Li B, Wang W, and Liu Y; all authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

## Ethical approval

The sample collection of *R. swinhoei* is legal, and it is not an endangered or a protected species in China. Only a few leaves of *R. swinhoei* were sampled for the assay.

## Disclosure statement

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

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## Data availability statement

The genome sequence data of *R. swinhoei* that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. OQ411240. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA938064, SRR23599434, and SAMN33424482, respectively.

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