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PLASTOME REPORT

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The plastid genome characters and phylogenetic status of the endemic species *Trichosanthes sunhangii* D. G. Zhang, Z. M. Li, Qun Liu & T. Deng 2021 (Cucurbitaceae) in the Shennongjia forestry district of China

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ABSTRACT

Trichosanthes sunhangii (Cucurbitaceae) is an endemic species native to the Shennongjia forestry district of China, whose plastid genome was reported in this study. The whole genome exhibits the typical quadripartite structure with 156,906 bp in size. A total of 130 genes were identified, containing 85 protein-coding genes (CDS), 37 tRNA, and 8 rRNA genes. Phylogenetic reconstruction based on 83 shared CDS sequences reaffirmed the status of *T. sunhangii* within the Sect. *Foliobracteola*, revealing close relationships with morphologically similar species, *T. kirilowii* and *T. rosthornii*. Our findings will provide a significant foundation for future investigations into the evolution, conservation, and potential utilization of this species.

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Introduction

Trichosanthes Linnaeus is one of the largest genera in the family Cucurbitaceae Juss., comprising approximately 100 species of climbing herbs distributed from India and China to Australia and the eastern Pacific, but with a diversity center in Southeast Asia (Huang et al. 2011; Liu et al. 2021). The members within the genus are characterized by simple, unlobed, or palmately lobed leaves, rarely compound and 3-5-foliolate, with usually long fimbriate corolla, and ovoid to globose or elongated fusiform pepos, while the shape and structure of the seeds, the color of the fruit pulp, and size of the bracts of the staminate flowers can be employed to distinguish them (Huang et al. 2011; de Boer and Thulin 2012; Liu et al. 2021). In classification and systematics studies, Schaefer et al. (2009) combined with a phylogenetic analysis of plastid gene, spacer, and intron sequences based on limited sequencing of *Trichosanthes* species, suggested that the genus might be paraphyletic and closely related to *Gymnopetalum* Arn. and *Hodgsonia* Hook. f. & Thomson. However, molecular results by de Boer et al. (2012) based on 4,759 nucleotides of nuclear and plastid DNA sequences from about 60% of *Trichosanthes* species worldwide indicated that *Gymnopetalum* was nested inside the *Trichosanthes* lineage and that *Hodgsonia* was distantly related to *Trichosanthes*. Later, de Boer and Thulin (2012) merged *Gymnopetalum* into *Trichosanthes* and proposed a revised infrageneric classification: the division of *Trichosanthes* into two subgenera, namely subgen. *Trichosanthes* and *Scotanthus*, with a total of 11 sections. Park et al. (2021) evaluated the taxonomy of *Trichosanthes* based on plastid phylogenomic data, suggesting that *T. kirilowii* var. *japonica* (Miq.) Kitam. should once again be treated as an independent species *T. japonica* (Miq.) Regel. Besides, the roots, fruits, skins, and seeds of some species are used in traditional Chinese medicine. For instance, trichosanthin extracted from the tuber of *T. kirilowii* Maxim. possesses anti-pregnancy and anti-AIDS activities (Ng et al. 1992).

Biodiversity determines the evolutionary and ecological adaptation range of species to specific environments. The endemic plants are taxonomic groups that exclusively grow in specific regions and enhance biodiversity in these areas, serving as key models for studying plants' evolutionary history (Myers et al. 2000; Jung et al. 2024). During a field investigation of plant diversity in the Shennongjia National Nature Reserve (SNNR) (Hubei, China), our research group discovered and described a new species of *Trichosanthes*—*Trichosanthes sunhangii* D. G. Zhang, Z. M. Li, Qun Liu & T. Deng 2021. Morphological and molecular evidence confirmed the close relationships among *T. sunhangii*, *T. kirilowii*, and *T. rosthornii* Harms (Liu et al. 2021). It is understood that *T. sunhangii* is

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only distributed in SNNR, making it an endemic species of that region. Unfortunately, the plastid genome of this species has not been reported. In this study, we described the *T. sunhangii* plastome and once again verified its phylogenetic status, which will provide valuable genomic information for exploring the potential medicinal value of this endemic species and protecting regional biodiversity.

Materials and methods

The plant sample of *T. sunhangii* (Figure 1) was collected from Yangri town in the Shennongjia forestry district (110°51′43.97″, 31°42′13.85″). The voucher specimen (KUN1498882; appraiser: Qun Liu) was deposited in the herbarium of the Kunming Institute of Botany (KIB, Kunming, China) (contact: Tao Deng, dengtao@mail.kib.ac.cn). Total genomic DNA was extracted from the silica-dried leaf using a modified CTAB method, and the genome was sequenced by Novogene Technologies Co. Ltd. (Beijing, China) on the Illumina Hiseq platform. We obtained a total of 1.08 GB of clean data for assembling the plastid genome sequence on the GetOrganelle v1.7.4.1 (Jin et al. 2020) and used the bowtie2 to calculate the coverage depth by mapping readings onto the genome sequence (Langmead and Salzberg 2012). We annotated the assembled plastid genome by running the website Geseq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al. 2017) and checked our annotation results in Geneious v.9.0.2 (Kearse et al. 2012) using the genome of *Trichosanthes homophylla* Hayata (Genbank accession: MN542394) as a reference. Subsequently, the complete and correct plastid genome of *T. sunhangii* was uploaded to NCBI (National Center for Biotechnology Information) and assigned the corresponding Genbank accession (PQ473692). We used the the Chloroplot (https://irscope.shinyapps.io/Chloroplot/) (Zheng et al. 2020) and the Chloroplast Genome Viewer (http://www.1kmpg.cn/cpgview) (Liu et al. 2023) to draw circular maps of the plastid genome and splicing genes, respectively. For phylogenetic reconstruction, we obtained plastome sequences from NCBI for 11 *Trichosanthes* taxa and one outgroup (*Cucumis melo* var. momordica (Roxb.) Cogn. MF536701) to test the phylogenetic status of *T. sunhangii* based on data published in previous studies (Bellot et al. 2020; Park et al. 2021). We extracted 83 shared protein-coding sequences (CDS) from each plastid genome in the Geneious v.9.0.2 and then aligned them using the MAFFT method (Katoh and Standley 2013). Maximum likelihood analysis was run *via* IQTREE with 1000 bootstrap replicates for ML tree construction, and the best model TVM+F + I was selected by the Bayesian Information Criterion (BIC)

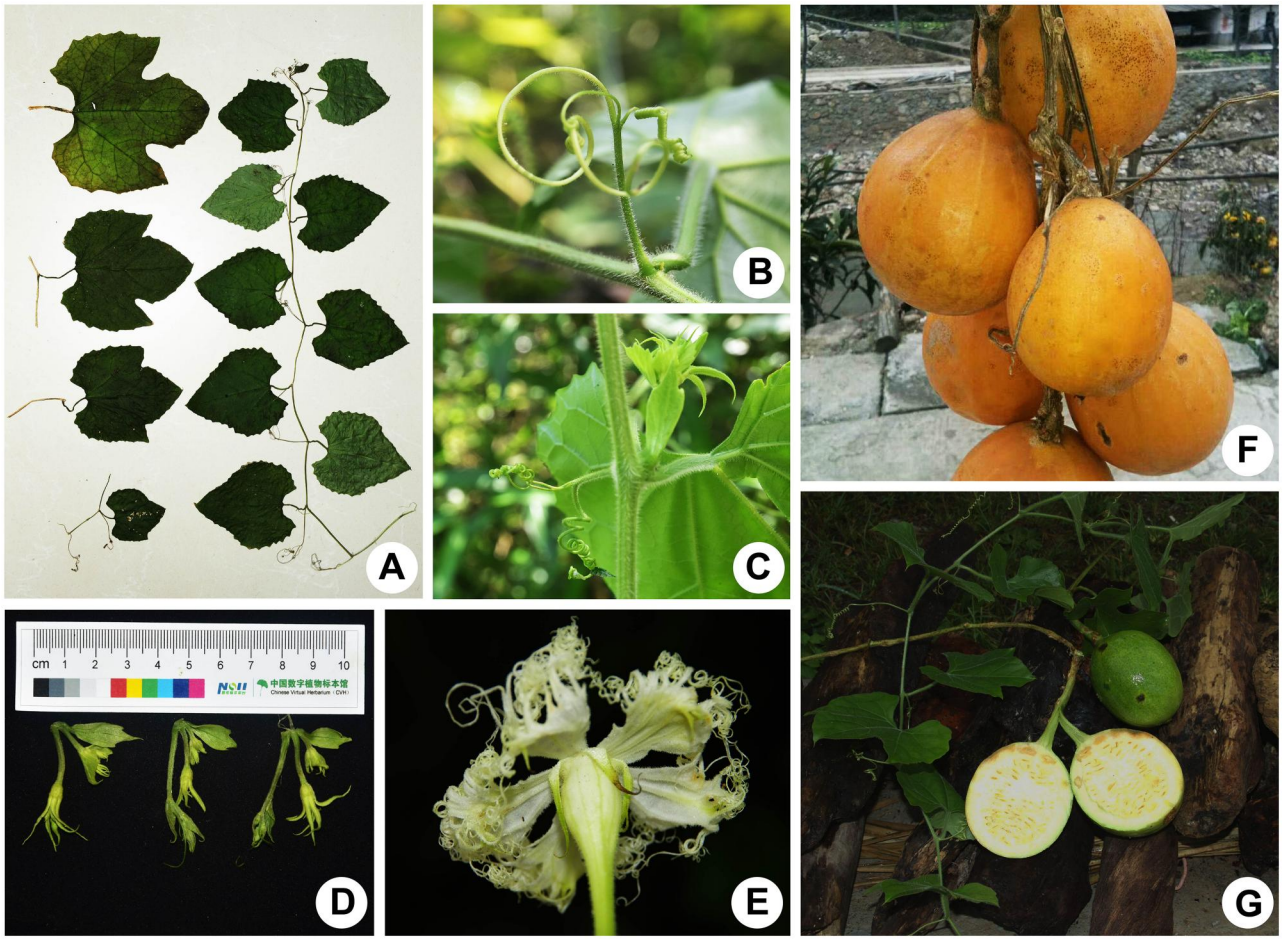

Figure 1. The photographs of *Trichosanthes sunhangii* taken in the Shennongjia forestry district (by Qun Liu), (A) variation in leaf shape; (B) tendril; (C,D) staminate racemes; (E) pistillate flower; (F,G) fruits. Morphology of *T. sunhangii* is unusual in that leaf blades of *T. sunhangii* are usually 3- or 5-lobed to 2/3 or not lobed, with the margin no longer lobed. Especially, the bracts of the staminate flowers of *T. sunhangii* are unlobed, oblanceolate, entire, sessile and attenuate at the base, and the apex of the bracts is tri-partite with lanceolate segments (Liu et al. 2021).

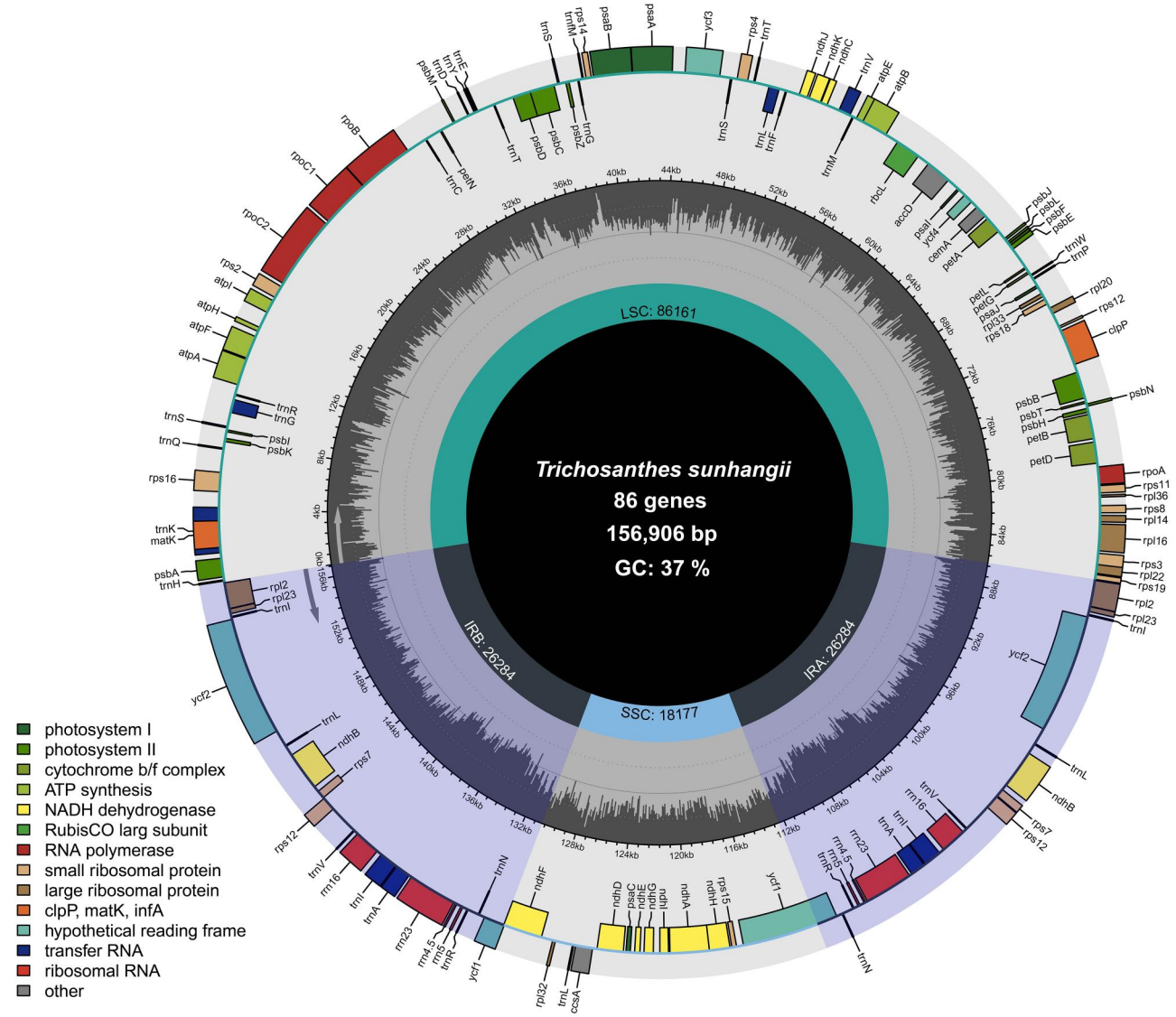

Figure 2. The circular plastid genomic map of *Trichosanthes sunhangii* was drawn using the Chloroplot to show the genes present in each region (LSC, SSC, and IRs). The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively, and each functional group of genes is distinctively color-marked. In the inner circle, the darker gray shades represent the GC content, and the lighter gray shades signify the AT content.

(Nguyen et al. 2015). Bayesian inference first chose the best nucleotide substitution model TVM+I+G based on Akaike Information Criterion (AIC) *via* JmodelTest 2 v.2.1.6 (Posada 2008). The Markov chain Monte Carlo analyses were run with four simultaneous chains of 10,000,000 generations and tree sampling every 1,000 generations. After the first 25% of trees were discarded as burn-in, the remaining trees were used to construct a majority-rule consensus BI tree with Bayesian posterior probabilities using MrBayes v.3.2 (Ronquist et al. 2012) on the CIPRES Portal (https://www.phylo.org/portal2).

Results

The newly generated complete circular plastid genome of *T. sunhangii*, with an average coverage of 1,334.59× (Figure S1), presented the typical quadripartite structure featuring a pair of 26,284 bp inverted repeat regions (IRs) separated by a large single-copy region of 86,161 bp (LSC) and a small single-copy region of 18,177 bp (SSC). The whole genome was annotated with 130 genes, including 85 CDS, 37 transfer RNA (tRNA) genes, and 8 ribosomal RNA (rRNA) genes (Figure 2). Among these genes, ten were identified as cis-splicing (*atp*F, *rpo*C1, *ycf*3, *clp*P, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, and *ndh*A) (Figure S2A), while the *rps*12 was a trans-splicing gene (Figure S2B). Three genes, *rps*19, *ycf*1, and *ndh*F were found to cross the borders between the IR regions and the SC regions. Additionally, the total GC content of the genome was 37.1%, with corresponding values for the IRs, LSC, and SSC being 42.8%, 34.9%, and 31.3%, respectively.

A sequence matrix of 79,438 bp was obtained by aligning 83 shared CDS, and phylogenetic trees with similar topologies were generated using Maximum likelihood analysis and Bayesian inference (Figure 3). Within the *Trichosanthes* lineage, the vast majority of nodes received strong support. Two species, *T. wallichiana* (Ser.) Wight and *T. tricuspidata* Lour., representing the sect. *Involucraria* of the subgen.

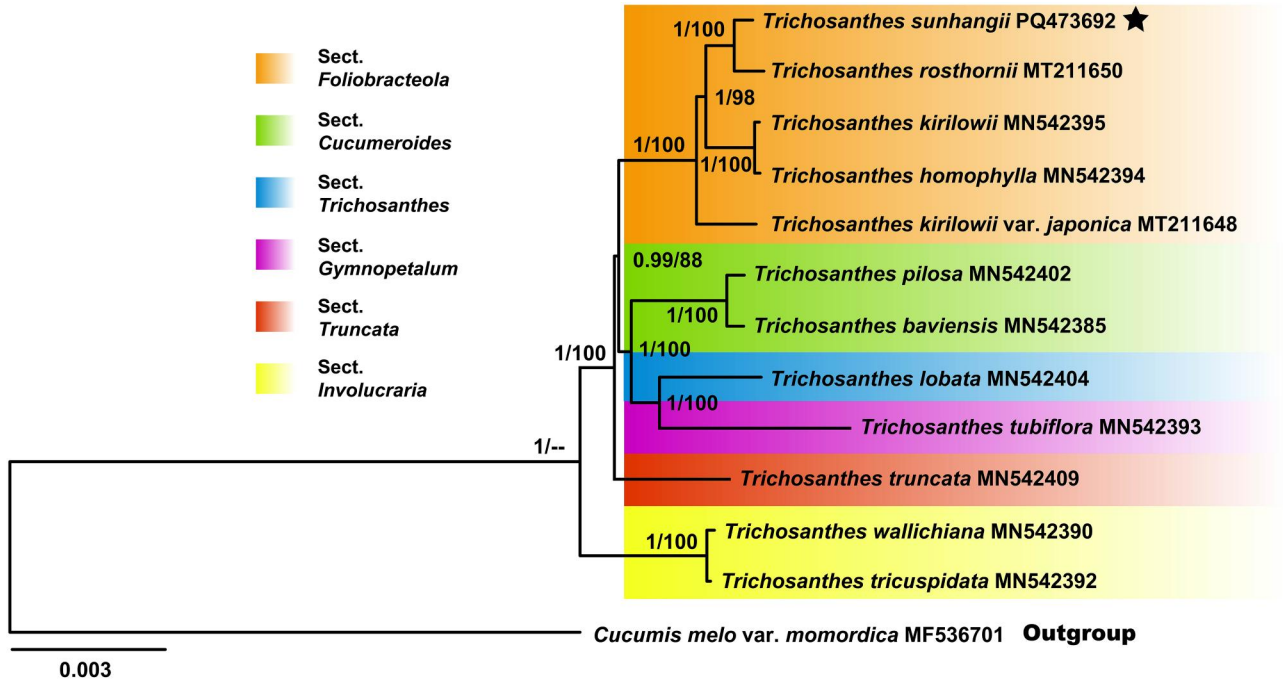

Figure 3. The phylogenetic tree derived from Bayesian inference based on 83 shared CDS, with each node showing Bayesian posterior probability (left, BP) and ML bootstrap support value (right, BS), and the position of *Trichosanthes sunhangii* is highlighted by the black star (Genbank: PQ473692). The sources of plastid genome data are as follows: *Cucumis melo* var. *momordica* (Genbank: MF536701) (Park et al. 2021), *Trichosanthes baviensis* (Genbank: MN542385) (Bellot et al. 2020), *Trichosanthes wallichiana* (Genbank: MN542390) (Bellot et al. 2020), *Trichosanthes tricuspidata* (Genbank: MN542392) (Bellot et al. 2020), *Trichosanthes tubiflora* (Genbank: MN542393) (Bellot et al. 2020), *Trichosanthes homophylla* (Genbank: MN542394) (Bellot et al. 2020), *Trichosanthes kirilowii* (Genbank: MN542395) (Bellot et al. 2020), *Trichosanthes pilosa* (Genbank: MN542402) (Bellot et al. 2020), *Trichosanthes lobata* (Genbank: MN542404) (Bellot et al. 2020), *Trichosanthes truncata* (Genbank: MN542409) (Bellot et al. 2020), *Trichosanthes kirilowii* var. *japonica* (Genbank: MT211648) (Park et al. 2021), *Trichosanthes rosthornii* (Genbank: MT211650) (Park et al. 2021).

Scotanthus, were sister to the remaining species (Subgen. *Trichosanthes*) (BP = 1, BS = 100). Within the subgen. *Trichosanthes*, five sections (*Foliobracteola*, *Cucumeroides*, *Trichosanthes*, *Gymnopetalum*, and *Truncata*) were recognized. *T. sunhangii* was resolved as a member of the sect. *Foliobracteola*, clustered with *T. rosthornii* (BP = 1, BS = 100) and then established a sister relationship with *T. kirilowii* and *T. homophylla* (BP = 1, BS = 98).

Discussion and conclusion

In the present study, we first reported the plastome characters of *T. sunhangii*, an endemic species in the Shennongjia forestry district of China. Its genome composition aligned closely with those of other *Trichosanthes* species that have been publically studied in terms of gene count and GC content (Bellot et al. 2020; Park et al. 2021), reflecting the relative conservation of the plastid genome within this genus. The phylogenetic analysis using shared CDS reshaped robust relationships among sections within *Trichosanthes*, in agreement with the findings of Park et al. (2021) study. Besides, our results re-emphasized the phylogenetic status of *T. sunhangii*, confirming its placement within the sect. *Foliobracteola* of the subgen. *Trichosanthes*. This species was more closely related to *T. rosthornii*, *T. kirilowii*, and *T. homophylla*, as supported by our previous analysis based on the combined sequences of plastid and nuclear genes (*mat*K, *rpl*20-*rps*12, and nrITS) (Liu et al. 2021). Morphologically, *T. sunhangii* possessed orange fruits, which was a key character of the sect. *Foliobracteola*. It closely resembles *T. kirilowii* and T. *rosthornii* with the leaf blades usually lobed, suborbicular, or broadly cordate-ovate but can be distinguished from the latter two species by the number of tendrils, the sparsely pubescent surface of the fruits, the bract shape of the staminate flowers, and other characters (Liu et al. 2021). The generation of the *T. sunhangii* plastid genome not only enriches the genomic databases of this genus but also contributes to a comprehensive understanding of the formation and evolution of this endemic species in the future.

Authors' contributions

Liu LF planned and designed the research. Liu Q collected the plant materials. Liu Q, Peng JY, and Di YN performed the experiments. Liu LF and Peng JY analyzed the data. Liu LF wrote the manuscript. Liu Q and Di YN reviewed the final manuscript. All authors agree to be accountable for all aspects of the work.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. The plant material used in the study complied with national and international standards and local laws and regulations. No endangered or protected species were involved in the study, and the collecting of the samples did not require specific permission from authorities.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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/ under the GenBank accession PQ473692. The associated BioProject, SRA, and BioSample numbers are PRJNA1173379, SRR31011141, and SAMN44303778, respectively.

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