

The complete chloroplast genome assembly of *Amorphophallus kiusianus* makino 1913 (araceae) from Southern China

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ABSTRACT

The mountainous region of southern China has been characterized by its complicated environment and topography. *Amorphophallus kiusianus* Makino 1913 is a representative species of extreme habitat preference that resides mainly in this region. To help study the genetic differentiation mechanisms of *A. kiusianus* populations, we sequenced the first chloroplast genome of this species using next-generation sequencing. The chloroplast genome was 166,269 bp in length with an average GC content of 36% (GenBank accession number: PP072243). The lengths of the large single-copy region (LSC), small single-copy region (SSC), and two inverted repeats (IRs) were 90,701 bp, 14,802 bp, 31,383 bp, and 31,383 bp, respectively. One hundred and twenty-nine genes were annotated in the chloroplast genome, including 84 protein-coding genes, 8 rRNA genes, and 37 tRNA genes. The phylogenetic tree suggested a close relationship among *A. kiusianus*, *A. yunnanensis*, and *A. coetaneus*. The chloroplast genome reported in this study provides valuable genomic resources for the future phylogeographic research of *A. kiusianus*.

ARTICLE HISTORY

Received 18 January 2024
Accepted 9 April 2024

KEYWORDS

Amorphophallus kiusianus;
araceae; chloroplast
genome; phylogenetic
analysis

Introduction



Amorphophallus genus, a group of perennial plants in the Araceae family, is characterized by an underground stem and a solitary leaf (Figure 1) (Li et al. 2010). There are approximately 230 *Amorphophallus* species worldwide, mainly distributed in tropical and subtropical regions of West Africa and South Asia. In China, there are over 20 *Amorphophallus* species, making it one of the diversity centers of this genus (Srzednicki and Borompichaichartkul 2020). For the high content of glucomannan in the corm, several *Amorphophallus* species, such as *A. konjac* and *A. albus*, have been cultivated in China, Japan, and Southeast Asian countries for a long time (Srzednicki and Borompichaichartkul 2020). *Amorphophallus kiusianus* Makino 1913 is mainly distributed in mountainous areas of southern China, including Guangdong, Hunan, Jiangxi, Zhejiang, Fujian, and Taiwan provinces (Li et al. 2010). The natural habitat of this species is shaded environments with an altitude ranging from 300 m to 900 m, such as valleys, broad-leaved forests, plantations, and orchards.


For the complicated and diverse climate and topography, the mountainous region of southern China has been recognized as one of the world's plant diversity centers (Sun et al. 2016; Gao et al. 2015). Due to the strict preference for altitude, the natural populations of *A. kiusianus* showed discontinuous distribution with small populations across southern

China (Yin et al. 2022). However, the study on population genetic diversity and genetic differentiation of this species in isolated mountain systems has not been reported. The chloroplast genome is usually inherited uniparentally in flowering plants (Chen et al. 2018). For the low recombination rate and high transferability, chloroplast DNA sequences have been widely utilized in phylogenetic research, analyses of genetic diversity, and the development of DNA barcodes (Henriquez et al. 2020; Yin and Gao 2023b). In this study, we reported the first chloroplast genome, which will provide genomic resources for future phylogeographic studies of this species.

Materials and methods

One individual of *Amorphophallus kiusianus* was collected from Yichuan, Jiangxi province, China (115°23'31.6" E, 28°57'45.3" N). The specimen was deposited into the herbarium of the College of Biological Resource and Food Engineering, Qujing Normal University (JABF_6_Yinsi20220809, Yong Gao, gaoyong@mail.qjnu.edu.cn). Genomic DNA was isolated from 0.15 g of the fresh leaf material using a plant genomic DNA isolation kit (DP305; Tiangen, China). Novogene Bioinformatics Technology Co. Ltd (Tianjin, China) constructed the sequencing library and next-generation sequencing. Briefly, the DNA sample was first

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2024.2342934>.

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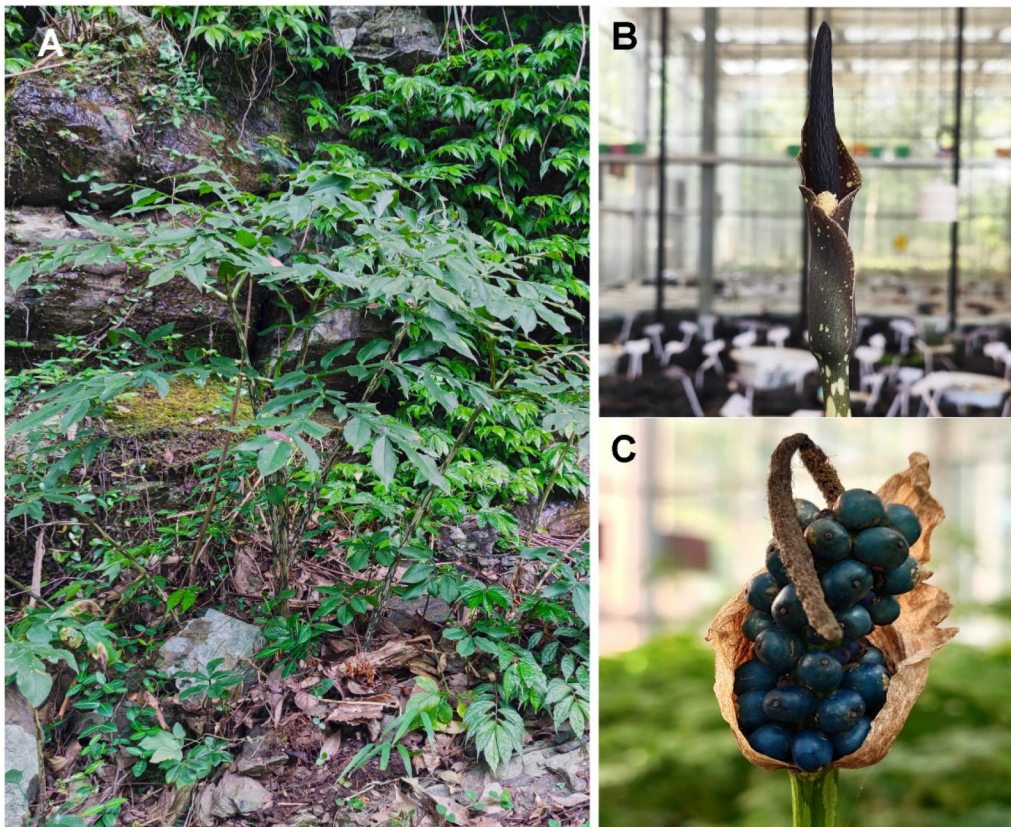


Figure 1. Morphological characteristics of the leaf (a), flower (B), and fruits (C) of *Amorphophallus kiusianus*. The photos were taken by the author Yong Gao. The petiole of *A. kiusianus* is glossy with dirty olive-green or grayish-green colors. Spathe is dark greenish outside with small, whitish spots. The berries are at first bright green, turning pinkish purple, and finally deep blue.

fragmented by sonication. DNA fragments with a size of approximately 350 bp were selected and ligated with adaptors, followed by PCR amplification. At last, the DNA library was sequenced on the Novaseq 6000 platform with PE (paired-end) 150 strategy (Illumina, CA, USA).

The raw sequencing data of *A. kiusianus* was filtered to remove reads containing unidentified nucleotides or over 50% of low-quality bases (Phred quality < 5). The chloroplast genome was assembled using GetOrganelle v1.7.8 (Jin et al. 2020). The assembling parameters were set as follows, the k-mers were set as 75, 95, 115, and 127, and the maximum extension rounds (R) were set to 40. The annotation of genome features was implemented with two online software, Cpgvas2 (<http://47.96.249.172:16019/analyzer/home>) and Geoseq (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) (Tillich et al. 2017; Shi et al. 2019). We manually adjusted the annotation result when necessary. The genome map of the chloroplast genome was drawn using the web-based software Cpgview (<http://www.1kmpg.cn/cpgview/>) (Liu et al. 2023). The quality of the assembly was assessed by mapping reads onto the genome using BWA v0.7.17 (Li and Durbin 2009). The cis-splicing and trans-splicing genes in the genome were analyzed by Cpgview (Liu et al. 2023). To infer the phylogenetic position of *A. kiusianus*, we downloaded chloroplast genome sequences of 18 Araceae species from the NCBI GenBank database. The genome sequences of these species were aligned using MAFFT v7.475 (Katoh and Standley 2013). ModelFinder was utilized to get the best nucleotide substitution model of the sequences

(Kalyanamoorthy et al. 2017). The maximum likelihood (ML) phylogeny among these species was constructed using IQ-TREE v1.6.12 with 1,000 bootstraps (Nguyen et al. 2015). The highly variable regions of the chloroplast genomes among *A. kiusianus* and the other six downloaded *Amorphophallus* species were analyzed using DnaSP v6.12 (Librado and Rozas 2009). The step size and window length were set to 125 bp and 500 bp, respectively.

Results

In total, 8.1 Gb of the raw sequencing data of *A. kiusianus* was produced using next-generation sequencing, and 5.38×10^7 clean reads were retained after filtering. The assembled chloroplast genome turned out to be 166,269 bp in length with an average GC content of 36%. We deposited the chloroplast genome sequence into the NCBI GenBank database with the accession number PP072243. A typical quadripartite structure was found in the genome, and the lengths of the large single-copy region (LSC), small single-copy region (SSC), and two inverted repeats (IRs) were 90,701 bp, 14,802 bp, 31,383 bp, and 31,383 bp, respectively (Figure 2). The average coverage depth of sequencing reads across the genome was 320 (Figure S1). One hundred and twenty-nine genes were annotated across the chloroplast genome, including 84 protein-coding genes, 8 rRNA genes, and 37 tRNA genes. Eleven genes (*atpF*, *ndhA*, *ndhB* (two copies), *petB*, *petD*, *rpl2* (two copies), *rpoC1*, *accD*, and *rps16*)

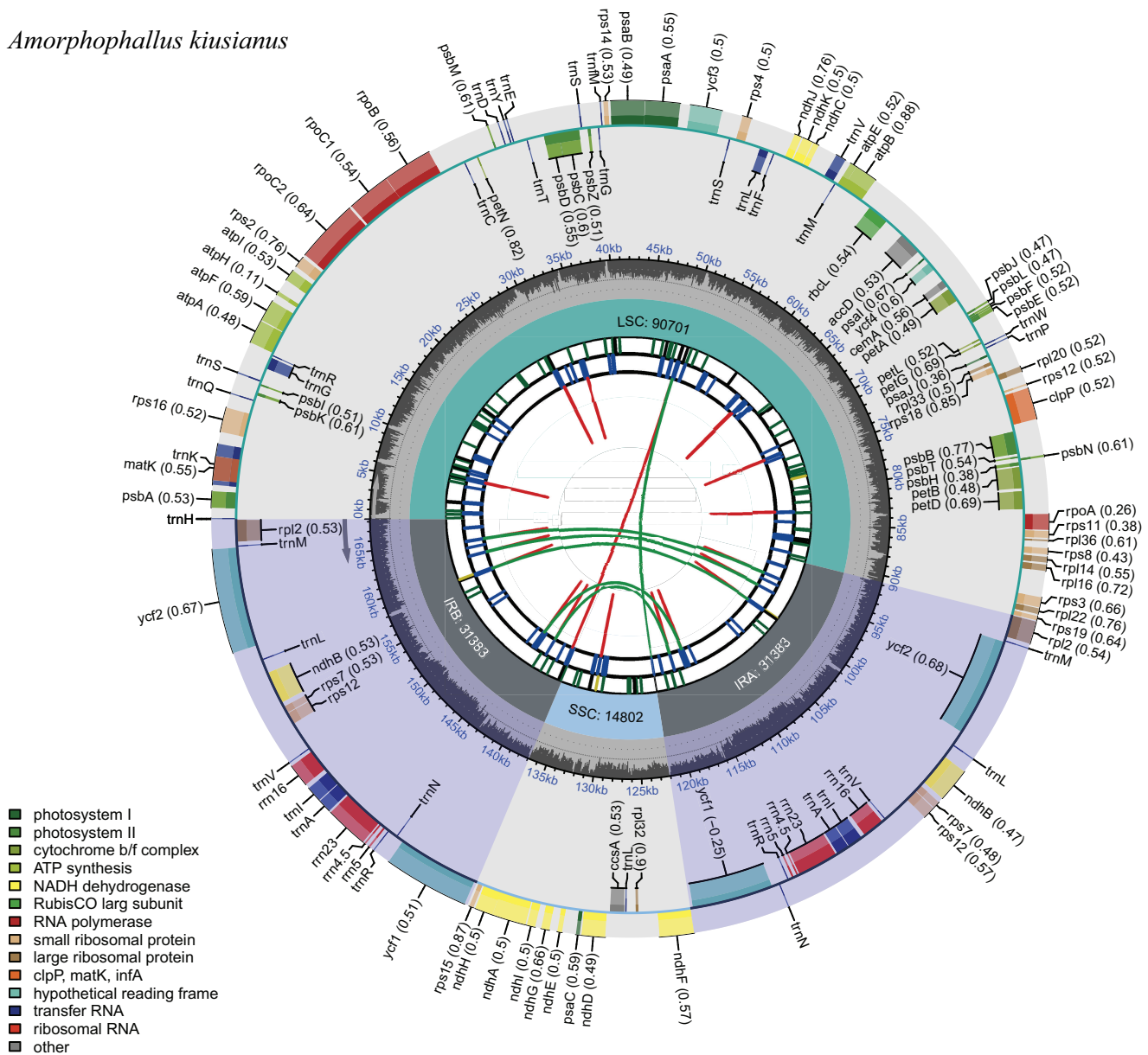
Amorphophallus kiusianus

Figure 2. The circular map of the chloroplast genome of *Amorphophallus kiusianus*. Genes belonging to different functional groups are plotted in the outer circle. The quadripartite structure, which consists of the LSC, the SSC, and two IR regions, is shown. The dark gray in the inner circle indicates the GC content of the chloroplast genome.

were detected to have one intron, and four genes (*clpP*, *rps12* (two copies), and *ycf3*) were found to have two introns (Figures S2 and S3). According to the Bayesian information criterion, ModelFinder suggested that the TVM + F + R3 model was the best nucleotide substitution model. The ML phylogenetic tree based on chloroplast genomes indicated that *Amorphophallus* species distributed in East Asia formed two clades, which was in accordant with the previous study with *LEAFY* and *trnL* sequences (Grob et al. 2004). However, the support values of each branch were much higher, which suggested that the complete chloroplast genome was more effective than several gene sequences in inferring the phylogenetic relationship among and within species (Pouchon et al. 2023).

Discussion and conclusion

In this study, we sequenced and assembled the first chloroplast genome of *A. kiusianus*. A typical quadripartite structure

and 129 genes had been found across the genome, which was consistent with the findings in chloroplast genomes of other *Amorphophallus* species (Li et al. 2024; Yin and Gao 2023a; Yin and Gao 2023b; Liu et al. 2019). Our phylogenetic tree based on chloroplast genomes indicated that *Amorphophallus* species distributed in East Asia formed two clades, which was in accordant with the previous study with *LEAFY* and *trnL* sequences (Grob et al. 2004). However, the support values of each branch were much higher, which suggested that the complete chloroplast genome was more effective than several gene sequences in inferring the phylogenetic relationship among and within species (Pouchon et al. 2023).

The mountainous region of Southern China is well-known for its complicated and fragmented geography and diverse environments (Qiu et al. 2011). As one species mainly distributed in this area, the natural populations of *A. kiusianus*

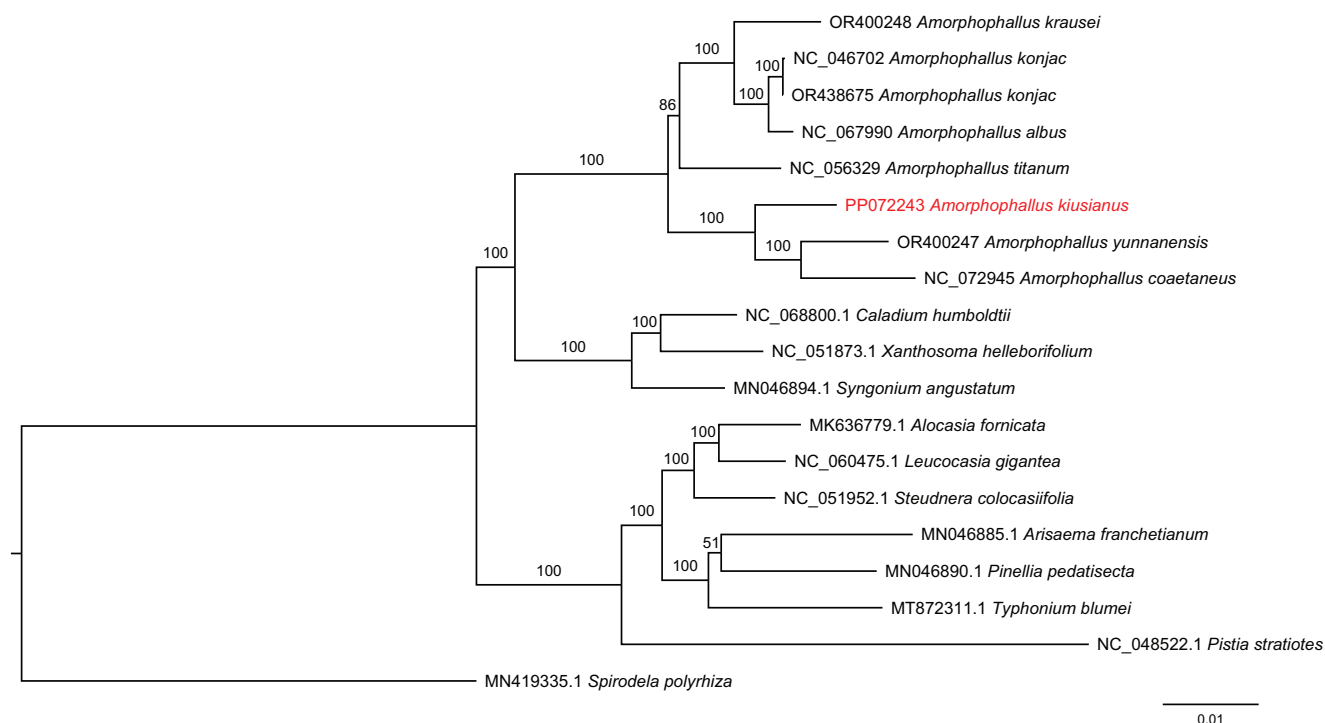


Figure 3. Maximum-likelihood phylogenetic tree of *Amorphophallus kiusianus* and 18 other related taxa in the family Araceae. *Spirodela polyrhiza* is used as an outgroup. The scale bar represents the number of substitutions at each locus. Accession numbers: *Amorphophallus kiusianus*, PP072243 (this study); *Amorphophallus krausei*, OR400248 (Yin and Gao 2023b); *Amorphophallus konjac*, NC_046702 (Hu et al. 2019); *Amorphophallus konjac*, OR438675 (Li et al. 2024); *Amorphophallus albus*, NC_067990 (Shan et al. 2023); *Amorphophallus titanum*, NC_056329 (Henriquez et al. 2021); *Amorphophallus yunnanensis*, OR400247 (Yin and Gao 2023a); *Amorphophallus coetaneus*, NC_072945 (Gao et al. 2023); *Caladium humboldtii*, NC068800 (reference not available); *Xanthosoma helleborifolium*, NC_051873 (reference not available); *Syngonium angustatum*, MN046894 (Henriquez et al. 2020); *Alocasia fornicata*, MN636779 (reference not available); *Leucocasia gigantea*, NC_060475 (reference not available); *Stuednera colocasiifolia*, NC_051952 (reference not available); *Arisaema franchetianum*, MN046885 (Henriquez et al. 2020); *Pinellia pedatisecta*, MN046890 (Henriquez et al. 2020); *Typhonium blumei*, MT872311 (Low et al. 2021); *Pistia stratiotes*, NC_048522 (Quan and Chen 2020); *Spirodela polyrhiza*, MN419335 (reference not available).

provide an excellent material for investigating processes driving genetic differentiation. Our study provides valuable genomic resources for the future phylogeographic research of this species.

Ethical approval

This study includes no human, animal, or endangered plant samples, and the sampling site was not in the natural reserve. No permissions are needed during the collection of samples.

Author contributions

Si Yin designed the study. Yong Gao collected the sample, and Si Yin extracted the genomic DNA. Yong Gao and Si Yin analyzed the data. Yong Gao and Si Yin drafted the paper. The authors have approved the final version of the manuscript.

Disclosure statement

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

Funding

This work was supported by the Special Basic Cooperative Research Programs of Yunnan Provincial Undergraduate Universities (202101BA070001-011).

Data availability statement

The chloroplast genome assembly of *A. kiusianus* is deposited into the NCBI GenBank database with accession number PP072243. The associated BioProject, BioSample, and SRA numbers are PRJNA1059772, SAMN39214234, and SRR27395334, respectively.

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