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A high-quality chromosome-level genome assembly of herbaceous bamboo species *Lithachne pauciflora*

Ke-Cheng Qian^{1,2}, Jun-Feng Liu^{1,2}, Yang Yang¹, Cen Guo³✉ & Zhen-Hua Guo^{1,2}✉

Lithachne pauciflora is a herbaceous bamboo species characterized by its short height, low degree of lignification, and dimorphic florets, making it a special model for studying the key trait evolution in bamboo. Here, we assembled a high-quality, chromosome-level genome of *L. pauciflora* by integrating PacBio HiFi long reads, short reads, and Hi-C technology. The final genome assembly is 1.7 Gb with a scaffold N50 of 147.73 Mb. 99.3% of sequences were anchored onto 11 pseudo-chromosomes, with a BUSCO score of 98.6% and LAI value of 21. The total length of repeat elements is 1.43 Gb, accounting for 84.02% of the whole genome assembly. We annotated 32,696 protein-coding genes, of which 94.8% received functional annotations. This genome assembly of *L. pauciflora* provides valuable insights into chromosomal evolution and key trait evolution in herbaceous bamboo.

Background & Summary

Bamboo (Poaceae: Bambusoideae) comprises approximately 136 genera and 1,700 species in 19 subtribes¹, is one of the most diverse lineages within the grass family. According to their degree of lignification, bamboo can be classified into woody and herbaceous lineages. Woody bamboo, representing over 90% of the bamboo diversity, is well-known for tall and highly lignified culms, making it a major component within forest ecosystems. Certain woody bamboo species, such as moso bamboo (*Phyllostachys edulis*) are of great economic, ecological, and cultural value in Asia, Africa, and Latin America.

Previous studies have reported a total of 16 bamboo genomes representing different lineages and ploidal levels^{2–8}. These include three herbaceous bamboos with HH genome (*Olyra latifolia*, *Raddia guianensis*, and *Ra. distichophylla*), four temperate woody bamboos with CCDD genome (*Ampelocalamus luodianensis*, *Hsuehochloa calcarea*, *P. edulis*, *P. violascens* ‘Prevernalis’), three neotropical woody bamboos with BBCC genome (*Guadua angustifolia*, *Otatea glauca*, *Rhipidocladum racemiflorum*), and five paleotropical woody bamboos with AABBCC genome (*Bonia amplexicaulis*, *Dendrocalamus brandisii*, *D. latiflorus*, *D. sinicus*, *Melocanna baccifera*). Additionally, the report of the nonaploid woody bamboo genome (*Bambusa odashimae*) represents the deciphering of the highest chromosome count⁹. Most of these studies focused on woody bamboo, suggesting that the allopolyploid evolutionary history and subgenome dominance may collectively contribute to the key innovations of woody bamboo⁷.

Herbaceous bamboos play a crucial role in bridging the gap between woody bamboos and other grasses, however, they have received far less attention. These bamboos are mainly found in the Neotropical Americas, Africa (including Madagascar), and New Guinea. They are characterized by their short height, low degree of lignification, unbranched culms typically lacking culm sheaths, unisexual spikelets, and dimorphic florets¹⁰. *Lithachne pauciflora* is the most widely distributed species within *Lithachne* genus, which typically grows to a height of less than 30 centimeters and thrives in shaded understory environments (Fig. 1a). The leaf bases are

¹Germplasm Bank of Wild Species & Yunnan Key Laboratory of Crop Wild Relatives Omics, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, 650201, China. ²Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, 650201, China. ³Center for Integrative Conservation & Yunnan Key Laboratory for the Conservation of Tropical Rainforests and Asian Elephants, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Mengla, Yunnan, 666303, China. ✉e-mail: guocen@xtbg.ac.cn; guozhenhua@mail.kib.ac.cn



Fig. 1 An adult plant (a) and inflorescences (b) of *Lithachne pauciflora*.

	Reads number	Total length (Gb)	Coverage ($\times$)	N50 length of reads (bp)
Short reads	712,679,184	106.9	70	150
PacBio HiFi reads	5,055,427	94	62	18,657
Hi-C reads	1,921,585,906	278.5	183	150

Table 1. Sequencing data of *Lithachne pauciflora*.

	<i>L. pauciflora</i>
Estimated genome size by flow cytometry (Gb)	1.52
Estimated genome size by k-mer (bp)	1,520,249,662
Heterozygosity (%)	0.33
Number of contigs	460
N50 of contigs (bp)	140,574,293
Total length of contigs (bp)	1,707,736,184
Number of scaffolds	166
N50 of scaffolds (bp)	147,733,637
Total length of scaffolds (bp)	1,697,057,541
GC content (%)	45.75
Pseudo-chromosomes	11
Sequence in pseudomolecules (bp)	1,686,385,992
Mapping rate with Illumina reads (%)	98.65
Completeness BUSCOs (%)	98.60
Complete single-copy BUSCOs (%)	95.80
Complete duplicated BUSCOs (%)	2.80
LTR Assembly Index (LAI)	21.1

Table 2. Genome assembly statistics of *L. pauciflora*.

asymmetrical. The inflorescences are both terminal and axillary, with the terminal inflorescences generally bearing only male spikelets, while the inflorescences at the lower nodes contain a distal female spikelet and one or two male spikelets (Fig. 1b). Phylogenetic studies have shown that *Lithachne* is monophyletic and closely related to *Olyra*¹¹. Species within this genus are diploid, with a chromosome number reported as $2n = 2x = 22$ ¹². It is suggested that the ancestral herbaceous bamboo karyotype (AGK-H) was 11, with a potential nested chromosome fusion (NCF) event involving chr10-chr12 leading to a reduction of the ancestral number from 12 to 11⁷. However, since only chromosome-level genomes of two genera have been published, it remains to be settled whether this event occurs at the ancestor node of herbaceous bamboo or species-specific.

In this study, we generated a high-quality chromosome-level genome of *L. pauciflora* by integrating PacBio HiFi reads (94 Gb, 62 $\times$), short reads (106.9 Gb, 70 $\times$), and Hi-C data (278.5 Gb, 183 $\times$) (Table 1). The total length of primary assembled contigs is 1,707,736,184 bp, which is similar to the estimated genome size of flow cytometry and K-mer analyses. After scaffolding, the final genome assembly is 1,697,057,541 bp, with N50 of scaffolds of 147.73 Mb (Table 2). 99.3% of sequences were anchored onto 11 pseudo-chromosomes (Fig. 2a). Only

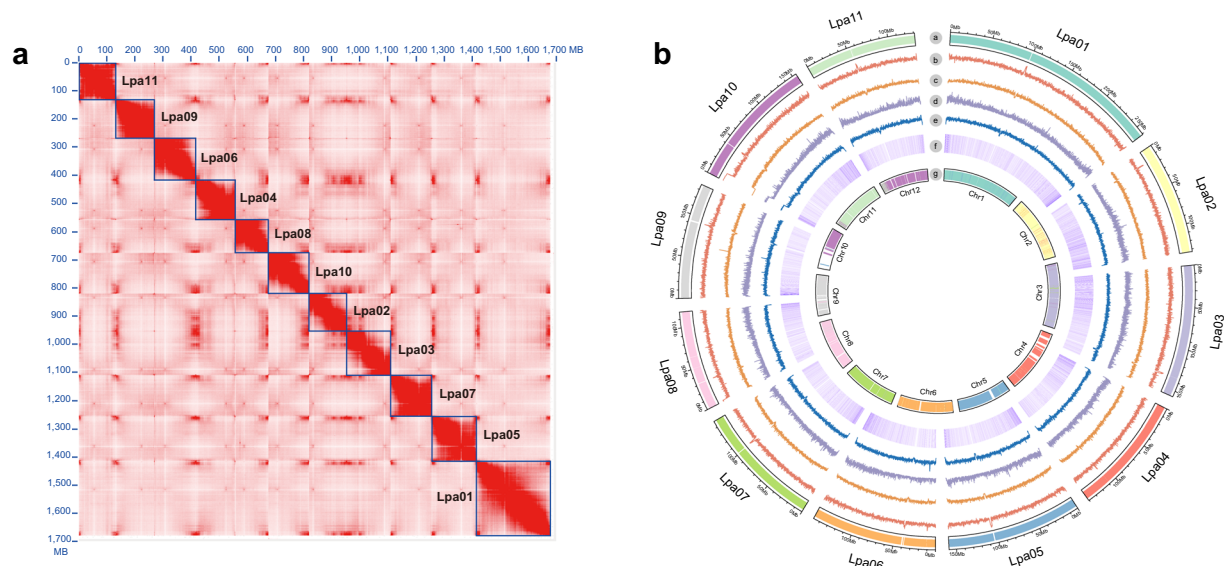


Fig. 2 Genome features of *L. pauciflora*. (a) Hi-C interaction heatmap of the *L. pauciflora* genome, with chromosomes labeled according to their collinearity with the rice genome. (b) Circos plot of the *L. pauciflora* genome. The tracks arranged from outermost to innermost are as follows: (a) 11 pseudo-chromosomes with filled regions indicating syntenic blocks with rice chromosomes, (b) GC contents, (c) LTR density, (d) TIR density, (e) repeat density, (f) gene density, and (g) syntenic blocks of rice genome compared to the *L. pauciflora* genome.

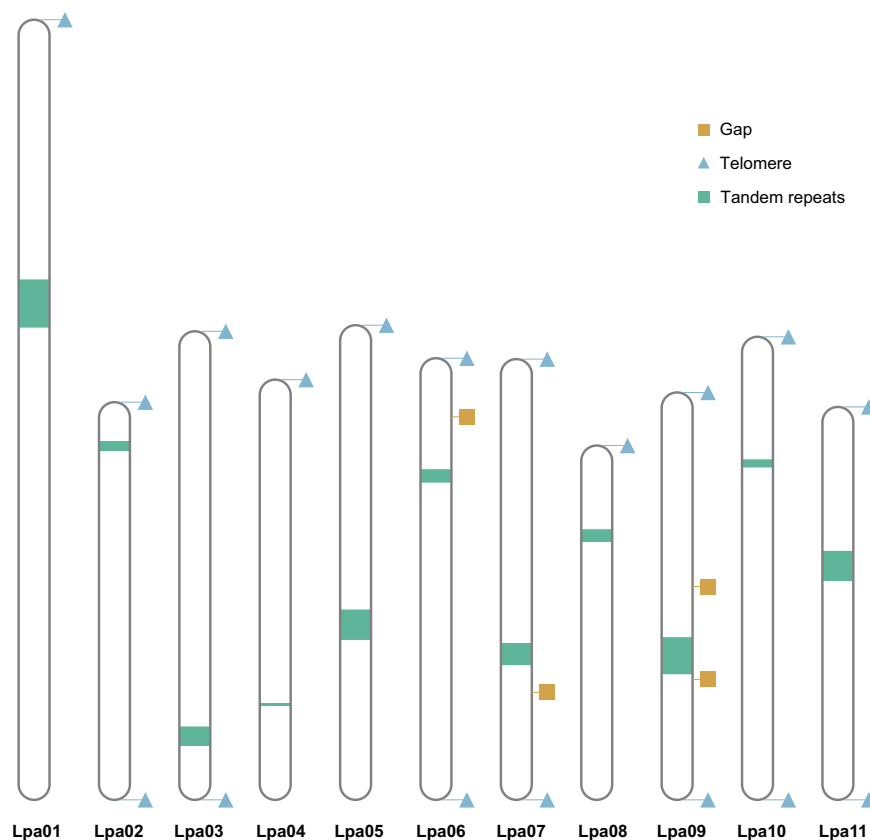


Fig. 3 Distribution of gaps, telomere regions, and tandem repeat regions across the chromosome sequences of *L. pauciflora*.

four gaps were detected in the assembled genome. Telomeres were successfully identified at both ends of seven chromosomes, while one end of four chromosomes (Lpa01, Lpa04, Lpa05, and Lpa08). Additionally, putative centromere regions were identified for all chromosomes (Fig. 3). A total of 1,409,694 repeat elements with a

	Class	Count	Masked (bp)	%masked
LTR	Copia	327,013	503,342,886	29.48
	Gypsy	340,420	570,661,796	33.42
	unknown	126,666	98,685,082	5.78
TIR	CACTA	74,817	40,133,269	2.35
	Mutator	72,152	40,972,746	2.40
	PIF_Harbinger	34,441	8,663,557	0.51
	Tcl_Mariner	129,154	37,015,396	2.17
	hAT	29,571	9,734,648	0.57
	polinton	35	23,310	0.002
nonLTR	LINE_element	3,101	2,102,101	0.12
	unknown	106	119,454	0.01
nonTIR	helitron	154,281	76,435,408	4.48
Unknown		117,937	46,953,222	2.75
Total		1,409,694	1,434,842,875	84.02

Table 3. Classification of repeat annotation in *L. pauciflora*.

	<i>L. pauciflora</i>
Number of protein-coding genes	32,696
Average gene length (bp)	3,763
Average CDS length (bp)	1,100
Average exon length (bp)	654
Average exon number per gene	4
Average intron length (bp)	502

Table 4. Genome annotation statistics of *L. pauciflora*.

length of 1,434,842,875 bp were identified, accounting for 84.02% of the whole genome assembly (Table 3). We annotated 32,696 protein-coding genes, with an average gene length of 3,763 bp (Table 4). The genome of *L. pauciflora* generated here provides valuable insights for studying the chromosomal evolution and key trait evolution in herbaceous bamboo.

Methods

Plant materials and sequencing. Fresh young leaves were collected from a single plant of *L. pauciflora* cultivated at the greenhouse of Kunming Institute of Botany, Chinese Academy of Sciences. High-quality genomic DNA was extracted following the standard operating procedure of the modified Cetyltrimethylammonium Bromide (CTAB) method¹³. Tissues of the vegetative leaf blade, female spikelet, and male spikelet, were sampled during the same time of day and frozen immediately in liquid nitrogen for RNA extraction and transcriptome sequencing.

The short-read library was prepared with a DNA-fragment insert size of 400 bp, and paired-end reads were generated using the DNBSEQ-T7 platform (BGI Inc., Shenzhen, China) with 70× sequencing depth (Table 1). The Pacbio Template Prep Kit 1.0 library preparation protocol was used for Long-read library preparation. High fidelity reads sequencing was performed using the PacBio Revio sequencing platform in the CCS mode, with a total of 94 Gb (62× sequencing depth) HiFi data with N50 sizes of 18.6 kb (Table 1). Hi-C libraries were prepared based on the standard library construction process of Illumina’s TruSeq DNA PCR-free prep kit reagents by the Personalbio Technology Company (Shanghai, China). Generally, fresh young leaves of *L. pauciflora* were first fixed in 2% formaldehyde solution, and then homogenized and centrifuged to isolate the nuclei. The cross-linked chromatin was then digested using the restriction enzyme *MboI*. Biotin-labeled adapters were subsequently used to fill in both sticky ends of the digested fragments. Ligation was performed using T4 DNA ligase, followed by further enrichment, purification, and trimming of the fragments to a size range of 300–700 bp. These Hi-C libraries were sequenced on the Illumina Novaseq platform to produce 150 bp paired-end reads with total data of 278.5 Gb (183× sequencing depth). Three different tissues were sampled for the transcriptome data sequencing. Each tissue was sequenced with three replicates, except for female and male spikelets that sequenced only twice due to material limitations. The DNBSEQ-T7 sequencing platform was used for 2 × 150 bp data sequencing. The sequencing library fragment size was 380 bp, and 6 Gb data was generated for each sample.

Genome size estimation. Both flow cytometry and k-mer frequency analyses were adopted to estimate the genome size of *L. pauciflora*. For the flow cytometry analysis, the genome size was determined using the BD FACScalibur flow cytometer following the standard procedure^{14,15} with the model plant tomato as reference. For the k-mer frequency analysis, we first used fastp v0.23.1¹⁶ to perform quality control on the short-read sequencing data. Next, we used Jellyfish v2.2.10¹⁷ to count k-mers on the filtered data and GenomeScope v1.0.0¹⁸ to evaluate the genome size and heterozygosity. The estimated genome size was about 1.5 Gb, with a heterozygosity of 0.33% (Table 1).

Chromosome-level genome assembly and quality evaluation. Fastp v0.23.1¹⁶ was used to evaluate the sequencing error rate and perform quality control on the Hi-C data. Subsequently, the clean HiFi reads were *de novo* assembled using the default parameters of the Hifiasm v0.19.9¹⁹ software to obtain the preliminary assembled genome contig sequence. The primary assembly contained 1,707.73 Mb in 460 contigs with a contig N50 of 140.57 Mb (Table 2). To generate a contact map, Juicer v2.20.00²⁰ was then used to align the Hi-C data to the preliminary assembled genome sequence. 3D-DNA v180114²¹ was used to sort and orient the contigs according to the strength of the Hi-C reads interaction relationship, and Hi-C-assisted chromosome assembly was performed. Juicebox v1.11.08²² was then used to manually check and adjust the preliminary chromosome assembly results, mainly for chromosome boundaries, incorrect insertions, and directions. 3D-DNA was then used to generate the adjusted chromosome sequence, and the final genome assembly consisted of 11 chromosome sequences (Fig. 2, Table 2). PMAT v2.0.1²³ was utilized to assemble the chloroplast and mitochondrial genomes, and the relevant contigs were excluded from the genome assembly.

We evaluate the quality of the chromosome-level genome sequence based on contiguity, correctness, and completeness. First, BWA v0.7.17²⁴ was used to map the short paired-end reads back to the genome sequences, and the reads mapping rate was 98.65% (Table 2). Subsequently, BUSCO v5.5.0 (Benchmarking Universal Single-Copy Orthologs)²⁵ was used to identify single-copy homologous genes in the OrthoDB database embryophyta_odb10, and the completeness of the assembled genome is evaluated as 98.6% with 95.8% complete single-copy BUSCOs (Table 2). Next, the LTR Assembly Index (LAI)²⁶ was used to assess the continuity of the genome assembly based on the repetitive elements prediction results by EDTA v2.2.0²⁷ and LTR_retriever²⁸ software. According to the standard of draft level ($0 \leq \text{LAI} < 10$), reference level ($10 \leq \text{LAI} < 20$), and gold level ($20 \leq \text{LAI}$), the LAI value 21 suggests high quality of the assembly of *L. pauciflora*.

Repetitive elements annotation and telomere prediction. EDTA v2.2.0²⁷ was adopted to annotate the transposable elements (TE) in the genome of *L. pauciflora* with default parameters. As a result, a total of 1,409,694 TE sequences with a total length of 1.43 Gb, accounting for 84.02% of the whole genome was identified. Of which, the long terminal repeats (LTRs, 68.68%) comprise the highest proportion, followed by terminal inverted repeats (TIRs, 8%) (Table 3). For telomere prediction, we utilized the TeloExplorer module of quarTeT v1.2.5²⁹, employing the canonical plant telomeric repeat sequence TTTAGGG as the search motif. We successfully predicted telomeric sequences for all 11 chromosomes. Among these, seven chromosomes (Lpa02, Lpa03, Lpa06, Lpa07, Lpa09, Lpa10, and Lpa11) exhibited telomeric repeats at both ends, while four chromosomes (Lpa01, Lpa04, Lpa05, and Lpa08) showed repeats at only one end (Fig. 3). To identify centromeres, we used the CentroMiner module of quarTeT²⁹, with inputs from the repetitive element annotation generated by EDTA v2.2.0²⁷ and our gene annotation file that includes information on alternative splicing. The results indicated that each chromosome contains a distinct region enriched with tandem repeats, likely representing the putative centromere.

Gene prediction and annotation. Protein-coding genes were annotated by integrating approaches including *ab initio* prediction, transcriptome-based, and homology-based strategy. Gene structure annotation was performed using a soft-masked genome sequence generated by EDTA v2.0.0²⁷. We utilized BRAKER3 v3.0.3³⁰ in ETP mode to integrate evidence from *ab initio* prediction, RNA-seq data, and protein homology. RNA-seq reads were aligned to the genome using HISAT2 v2.2.1³¹, and StringTie2 v2.2.1³² was employed to assemble transcript models. GeneMark-ETP³³ was trained and executed using both the RNA-seq alignments and protein evidence from the OrthoDB database, incorporating protein annotations from *Olyra latifolia* (GCA_036346145.1) and *Raddia guianensis* (GCA_036346415.1). Subsequently, AUGUSTUS v3.5.0³⁴ was trained and executed *ab initio* gene prediction guided by the same extrinsic evidence along with the output from GeneMark-ETP³³. TSEBRA³⁵ was then used to integrate the predictions from AUGUSTUS v3.5.0³⁴ and GeneMark-ETP³³, producing the final set of gene models. To enhance the quality of the annotations and recover untranslated regions (UTRs), Trinity v2.8.5³⁶ was used to perform *de novo* transcriptome assembly, and the resulting transcripts were further processed with PASA v2.5.3³⁷ to polish and refine the BRAKER3-derived gene models. Following the guidelines of Vuruputoor *et al.*³⁸, we excluded mono-exonic genes that lacked functional annotations based on evidence from domain or sequence similarity (see the section on Functional Prediction for details). This process led to our final gene annotation.

Overall, 32,696 protein-coding genes were annotated for the *L. pauciflora* genome (Table 4), which is slightly higher than the counts of 31,189 in *Ol. latifolia* and 27,496 in *Ra. guianensis*⁷. The gene length ranges from 98 bp to 347,248 bp, with an average gene length of 3,763 bp and a median of 2,288 bp. The average length of coding sequences (CDS) is 1,100 bp and a median of 882 bp. In addition, each gene has an average of 4 exons, with an average length of 654 bp.

Functional prediction. Three strategies were adopted for the functional prediction of protein-coding genes. GFAP v3.1³⁹ was used to align predicted genes with the GO, KEGG, and Pfam databases, with default values. DIAMOND v2.1.9⁴⁰ (-evalue 1e-5, -max-target-seqs. 5, Identity > 30%) and BLAST v2.15.0⁴¹ (-evalue 1e-5, -max_target_seqs. 5, -qcov_hsp_perc 30) were used to identify the best match by aligning the protein sequences to the SwissProt and NR databases, respectively. Finally, 31,000 genes were functionally annotated in at least one of the above databases, accounting for 94.8% of the predicted protein-coding genes (Fig. 4a; Supplementary Table S1).

Annotation of noncoding RNAs. The tRNAscan-SE v2.0.12⁴² was used to identify the transfer RNAs (tRNAs). RNAmmer v1.2⁴³ was adopted to annotate ribosomal RNAs (rRNAs). Other noncoding RNAs, such as microRNAs (miRNAs) and small nuclear RNAs (snRNAs), were identified using Infernal v1.15⁴⁴ by searching

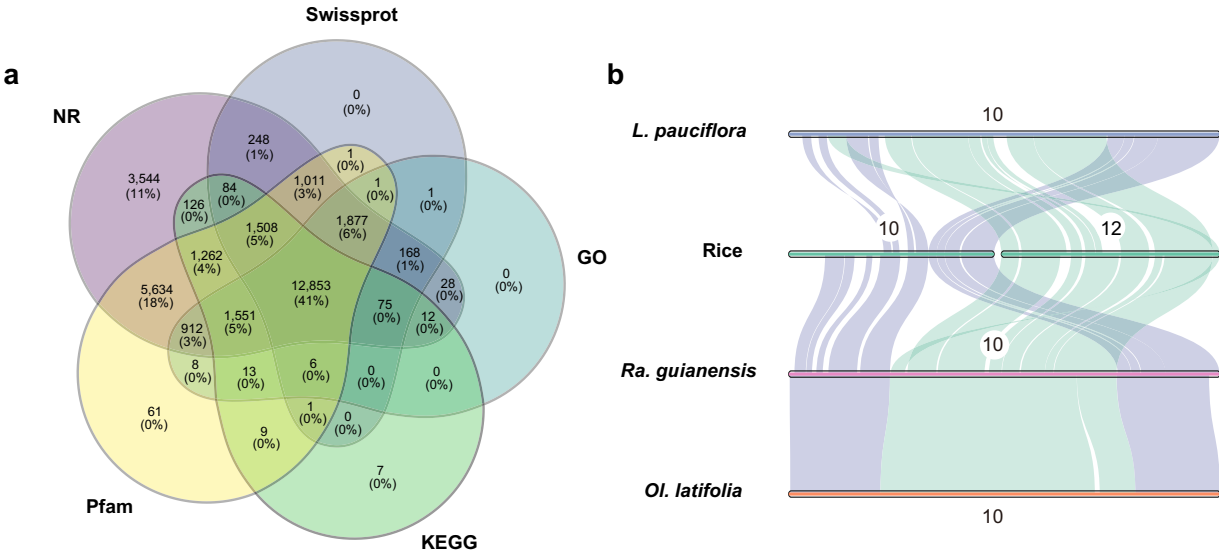


Fig. 4 Statistical Venn diagrams (a) of *L. pauciflora* genome and (b) synteny relationships of chromosomes 10 and 12 between herbaceous bamboo and the rice genome. The Venn diagrams illustrate the functional annotations of protein-coding genes from *L. pauciflora* against the five public databases. The diagrams illustrate the protein-coding genes categorized by different functional annotations, displaying the shared core genes (center), overlapping genes (overlapping regions), and specific genes (outer regions) for each functional annotation. The macrosynteny relationships of chromosomes 10 and 12 between herbaceous bamboo and rice genomes are presented, along with the nested chromosome fusion (NCF) shared by three herbaceous bamboos.

Type		Copy	Average length (bp)	Total length (bp)	% of genome
miRNA		801	189	151,593	0.009
tRNA		2,415	74	179,171	0.011
rRNA	rRNA	5,386	1,169	5,893,967	0.347
	18S	1,179	1,614	1,903,526	0.112
	28S	1,296	2,788	3,613,119	0.213
	5.8S	876	155	135,785	0.008
	5S	2,035	119	241,537	0.014
snRNA	snRNA	2,764	111	294,127	0.017
	CD-box	704	106	78,034	0.005
	HACA-box	8	112	895	0.000
	splicing	1,451	95	137,164	0.008
	scaRNA	601	130	78,034	0.005

Table 5. Statistics of non-coding RNA annotation results in *L. pauciflora* genome. MicroRNA (miRNA), Transfer RNAs (tRNAs), ribosomal RNA (rRNA), small nuclear RNA (snRNA), and small Cajal body-specific RNA (scaRNA).

against the Pfam database. In total, 2,415 tRNAs, 5,386 rRNAs, 801 miRNAs, and 2,764 snRNAs were predicted in *L. pauciflora* genome (Table 5; Supplementary Table S2).

Syntenic analysis. The syntenic analysis among the genomes of *L. pauciflora*, *Ol. latifolia*, and *Ra. guianensis*, with the rice genome serving as a reference, was performed using the jcv.compara.catalog ortholog pipeline from jcv v1.1.17⁴⁵. Syntenic blocks were identified with the following parameters: a C-score cutoff of 0.7, a search extent of 20 for flanking regions, and a minimum of 4 anchor pairs required to define a block. The clear overall 1:1 collinearity between the *L. pauciflora* genome and rice was observed (Fig. 2b), thus we named the chromosomes of *L. pauciflora* according to their collinearity with the rice genome. Previous research suggested that a nested chromosome fusion (NCF) involving chromosomes 12 and 10 of the rice genome occurred in the ancestral herbaceous bamboo karyotype, leading to a reduction in chromosome number from 12 to 11⁷. Our study further solidified this finding by revealing a shared NCF event observed in the *L. pauciflora* genome (Fig. 4b).

Data Records

The relevant data generated in this paper have been uploaded in the National Genomics Data Center (NGDC), Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under the BioProject accession number PRJCA036467. BGI short-reads, PacBio HiFi long-reads, Hi-C reads, and RNA-seq data have been deposited in the Genome Sequence Archive (GSA) in NGDC under the accession number CRA023401⁴⁶, CRA023429⁴⁷, CRA023437⁴⁸, and CRA023415⁴⁹. The genome assembly file has been deposited in the NCBI GenBank database under the accession number JBPVWQ000000000⁵⁰. Additionally, the genome assembly and annotation data have been deposited in Figshare⁵¹.

Technical Validation

Evaluation of the genome assembly and annotation. In this study, a total of $62 \times$ HiFi reads, $70 \times$ short reads, and $183 \times$ Hi-C data (Table 1) were generated to achieve a high-quality chromosome-level genome assembly of *L. pauciflora*. The high correctness, contiguity, and completeness of the assembly were supported by various lines of evidence. Firstly, 98.65% of the short reads were properly mapped back to the assembled genome sequences, suggesting the correctness of the *L. pauciflora* genome assembly (Table 2). The Hi-C heatmap displayed strong interaction signals across the 11 chromosomes (Fig. 2), revealing a well-executed chromosome scaffolding process with no obvious errors. Moreover, the N50 of the scaffolds in the *L. pauciflora* assembly reached 147.7 Mb, surpassing the chromosome-level assemblies of *Ol. latifolia* (57.04 Mb) and *Ra. guianensis* (56.58 Mb)⁷. Subsequently, BUSCO analysis revealed that the proportion of complete genes (including both single-copy and duplicated genes) was 98.6%, with only 0.9% of gene missing, highlighting the high level of completeness of the assembled genome. Additionally, the LTR Assembly Index (LAI)²⁶ was calculated to assess the continuity of the genome assembly. Based on the classifications of draft level ($0 \leq \text{LAI} < 10$), reference level ($10 \leq \text{LAI} < 20$), and gold level ($20 \leq \text{LAI}$), the LAI value of 21 suggests a high-quality assembly of *L. pauciflora*.

We identified only four gaps in the assembled genome, which are located on chromosomes Lpa06 (one gap), Lpa07 (one gap), and Lpa09 (two gaps) (Fig. 3). We successfully predicted telomeric sequences for all 11 chromosomes. Among these, seven chromosomes (Lpa02, Lpa03, Lpa06, Lpa07, Lpa09, Lpa10, and Lpa11) exhibited telomeric repeats at both ends, while four chromosomes (Lpa01, Lpa04, Lpa05, and Lpa08) showed telomeres at only one end. The results of centromere prediction indicated that each chromosome contains a distinct region enriched with tandem repeats, likely marking the location of the putative centromere. In summary, these evaluation demonstrates a high quality of our genome assembly.

The predicted number of coding genes, totaling 32,696, which is slightly higher than the counts of *Ol. latifolia* (31,189) and *Ra. guianensis* (27,496)⁷. The quality of the gene annotation was evaluated using BUSCO v5.5.0²⁵ with the embryophyta_odb10 database. The results indicate that our annotation is of high quality³⁸, with 95.4% complete BUSCOs, including 92.8% complete and single-copy BUSCOs, 2.6% complete and duplicated BUSCOs, 2.5% fragmented BUSCOs, and 2.1% missing BUSCOs. The proportion of single-exon genes was 0.36. To further assess the completeness and accuracy of the annotated gene sets, the predicted coding genes were functionally annotated through BLAST searches against multiple databases, including NR, SwissProt, KEGG, GO, and Pfam. The results indicated that 94.8% (31,000) of the predicted gene models were functionally annotated in at least one of these databases (Fig. 4; Supplementary Table S1).

Data availability

All sequencing and assembly data generated in this study have been deposited in public repositories. Raw sequencing data including BGI short-reads, PacBio HiFi long-reads, Hi-C reads, and RNA-seq data are available in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC) under the BioProject accession number PRJCA036467, with the following accession numbers: CRA023401⁴⁶, CRA023429⁴⁷, CRA023437⁴⁸, and CRA023415⁴⁹. The genome assembly has been deposited in the NCBI GenBank database under the accession number JBPVWQ000000000⁵⁰. Additionally, the genome assembly and annotation files are also deposited in Figshare (<https://doi.org/10.6084/m9.figshare.29608349.v2>)⁵¹.

Code availability

In this study, no custom code was developed. All data processing commands and pipelines were executed following the manuals and protocols provided by the published bioinformatics software. Details regarding the specific software versions and parameters utilized can be found in the Methods section.

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Author contributions

Zhen-Hua Guo and Cen Guo conceived and designed the research, Cen Guo, Ke-Cheng Qian, and Yang Yang prepared plant samples. Ke-Cheng Qian, Cen Guo, and Jun-Feng Liu performed data analysis. Ke-Cheng Qian, Cen Guo, and Zhen-Hua Guo drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to C.G. or Z.-H.G.

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