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DATA RELEASE

Genome assembly of the rare and endangered Grantham's camellia, *Camellia granthamiana*

Hong Kong Biodiversity Genomics Consortium*,†

ABSTRACT

Grantham's camellia (*Camellia granthamiana* Sealy) is a rare and endangered tea species discovered in Hong Kong in 1955 and endemic to southern China. Despite its high conservation value, the genomic resources of *C. granthamiana* are limited. Here, we present a chromosome-scale draft genome of the tetraploid *C. granthamiana* ($2n = 4x = 60$), combining PacBio long-read sequencing and Omni-C data. The assembled genome size is ~2.4 Gb, with most sequences anchored to 15 pseudochromosomes resembling a monoploid genome. The genome has high contiguity, with a scaffold N50 of 139.7 Mb, and high completeness (97.8% BUSCO score). Our gene model prediction resulted in 68,032 protein-coding genes (BUSCO score of 90.9%). We annotated 1.65 Gb of repeat content (68.48% of the genome). Our Grantham's camellia genome assembly is a valuable resource for investigating Grantham's camellia's biology, ecology, and phylogenomic relationships with other *Camellia* species, and provides a foundation for further conservation measures.

Subjects Genetics and Genomics, Botany, Plant Genetics

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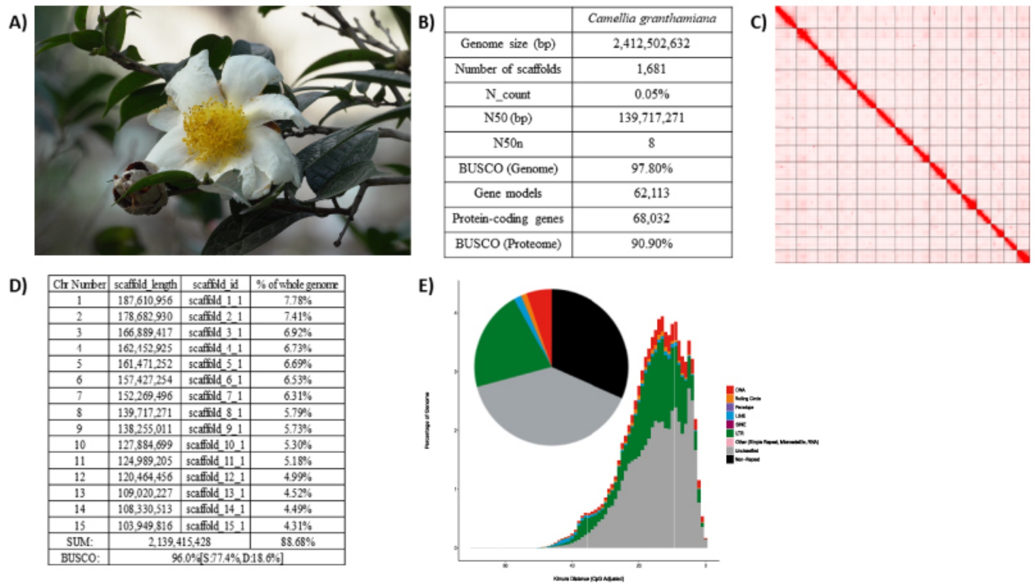
INTRODUCTION

Camellia is a large genus in the family Theaceae with more than 230 described species [1]. Camellias are well-known for their ornamental and economic values as tea and woody-oil producing plants, with tens of thousands of cultivars derived from them [2]; however, more than 60 *Camellia* species are regarded as globally threatened due to their natural habitat fragmentation or loss, and to their small population size [3]. The Grantham's camellia (*Camellia granthamiana*) (Figure 1A) is a rare species first discovered in Hong Kong and named after the former Governor Sir Alexander Grantham, and is narrowly distributed in Hong Kong and Guangdong, China [3]. It is listed as vulnerable in the Red List of the International Union for Conservation of Nature and recorded as endangered in the China Plant Red Data Book [4]. In Hong Kong, Grantham's camellia is a protected species by law and has been actively propagated and reintroduced to the wild by the Agriculture, Fisheries and Conservation Department [5].

CONTEXT

In view of the high conservation value of Grantham's camellia, several molecular studies have been done. They included sequencing the chloroplast genomes of *C. granthamiana* [6, 7], using pan-transcriptomes to reconstruct the phylogeny of over a hundred *Camellia* species [8], and population genetics studies [9]. However, the nuclear genomic resources of *C. granthamiana* are still missing. While most *Camellia* species possess a karyotype of $2n = 30$, *C. granthamiana* is an exception with a karyotype of $2n = 4x = 60$ [10, 11].



	Camellia granthamiana
Genome size (bp)	2,412,502,632
Number of scaffolds	1,681
N_count	0.05%
N50 (bp)	139,717,271
N50n	8
BUSCO (Genome)	97.80%
Gene models	62,113
Protein-coding genes	68,032
BUSCO (Proteome)	90.90%

Chr Number	scaffold_length	scaffold_id	% of whole genome
1	187,610,956	scaffold_1_1	7.78%
2	178,682,930	scaffold_2_1	7.41%
3	166,889,417	scaffold_3_1	6.92%
4	162,452,925	scaffold_4_1	6.73%
5	161,471,252	scaffold_5_1	6.69%
6	157,427,254	scaffold_6_1	6.53%
7	152,269,496	scaffold_7_1	6.31%
8	139,717,271	scaffold_8_1	5.79%
9	138,255,011	scaffold_9_1	5.73%
10	127,884,699	scaffold_10_1	5.30%
11	124,989,205	scaffold_11_1	5.18%
12	120,464,456	scaffold_12_1	4.99%
13	109,020,227	scaffold_13_1	4.52%
14	108,330,513	scaffold_14_1	4.49%
15	103,949,816	scaffold_15_1	4.31%
SUM:	2,139,415,428		88.68%
BUSCO:	96.0%[S:77.4%,D:18.6%]		

Figure 1. Genomic information of *Camellia granthamiana*. (A) Picture of *Camellia granthamiana*; (B) Summary of genome statistics; (C) Omni-C contact map of the genome assembly; (D) Information of 15 pseudochromosomes; (E) Pie chart (Top) and repeat landscape plot (bottom) of repetitive elements in the genome.

In Hong Kong, *C. granthamiana* was chosen as one of the species listed for sequencing in the Hong Kong Biodiversity Genomics Consortium (also known as EarthBioGenome Project Hong Kong), which is formed by investigators from eight publicly funded universities. Here, we report the genome assembly of *C. granthamiana*, which can serve as a solid foundation for further investigations of this rare and endangered species.

METHODS

Sample collection and high molecular weight DNA extraction

Fresh leaf tissues were sampled in transplanted individuals on the campus of the Chinese University of Hong Kong. High molecular weight (HMW) genomic DNA was isolated from 1 g leaf tissues using pretreatment with cetyltrimethylammonium bromide (CTAB) followed by the NucleoBond HMW DNA kit (Macherey Nagel Item No. 740160.20). Briefly, tissues were ground with liquid nitrogen and digested in 5 mL CTAB buffer [12] with the addition of 1% polyvinylpyrrolidone for 1 h. The lysate was treated with RNAse A, followed by the addition of 1.6 mL of 3 M potassium acetate and two rounds of chloroform:IAA (24:1) washes. The supernatant was transferred to a new 50 mL tube using a wide-bore tip. H1 buffer from the NucleoBond HMW DNA kit was added to the supernatant for a total volume of 6 mL, from which the DNA was isolated following the manufacturer's protocol. After the DNA was eluted with 60 μL elution buffer (PacBio Ref. No. 101-633-500), a quality check was carried out with NanoDrop™ One/OneC Microvolume UV–Vis Spectrophotometer, Qubit® Fluorometer, and overnight pulse-field gel electrophoresis.

Pacbio library preparation and sequencing

The qualified DNA was sheared with a g-tube (Covaris Part No. 520079) with six passes of centrifugation at 1,990 × *g* for 2 min. Next, it was purified with SMRTbell® cleanup beads

Table 1. Genome and transcriptome sequencing information.

Library	Reads	Bases	Accession number
PacBio HiFi	5,071,365	54,421,045,547	SRR26895683
Omnic	1,558,845,532	233,826,829,800	SRR26909376
mRNA CamG_YL_H	41,179,150	5,987,388,039	SAMN40925022

(PacBio Ref. No. 102158-300). A total of 2 μL sheared DNA was taken for fragment size examination through overnight pulse-field gel electrophoresis. Then, two SMRTbell libraries were constructed with the SMRTbell® prep kit 3.0 (PacBio Ref. No. 102-141-700) following the manufacturer's protocol. The final library was prepared with the Sequel® II binding kit 3.2 (PacBio Ref. No. 102-194-100) and was loaded, using the diffusion loading mode, with the on-plate concentration set at 90 pM on the Pacific Biosciences SEQUEL IIe System, running for 30-hour movies to output HiFi reads. In total, three SMRT cells were used for the sequencing. Details of the resulting sequencing data are summarized in Table 1.

Omni-C library preparation and sequencing

Nuclei were isolated from 3 g fresh leaf tissues ground with liquid nitrogen using the PacBio protocol modified from Workman *et al.* [13]. The nuclei pellet was snap-frozen with liquid nitrogen and stored at −80 °C. Upon Omni-C library construction, the nuclei pellet was resuspended in 4 mL 1× PBS buffer and processed with the Dovetail® Omni-C® Library Preparation Kit (Dovetail Cat. No. 21005) following the manufacturer's procedures. The concentration and fragment size of the resulting library were assessed by Qubit® Fluorometer and TapeStation D5000 HS ScreenTape, respectively. The qualified library was sent to Novogene and sequenced on an Illumina HiSeq-PE150 platform. Details of the resulting sequencing data are summarized in Table 1.

Total RNA isolation and transcriptome sequencing

Approximately 0.5 g of young leaf tissue was ground into powder after being frozen in liquid nitrogen. Total RNA was then isolated using a CTAB pretreatment method [14], followed by the mirVana miRNA Isolation Kit (Ambion, cat no. AM1560). The quality of the RNA sample was assessed using NanoDrop® One/OneC Microvolume UV–Vis Spectrophotometer and 1% agarose gel electrophoresis. Next, the sample was sent to Novogene Co. Ltd (Hong Kong, China) for transcriptome sequencing. Details of the sequencing data are listed in Table 1.

Genome assembly and gene model prediction

De novo genome assembly was first generated with Hifiasm (RRID:SCR_021069) [15] and then was processed by searching it against the NT database with BLASTn (RRID:SCR_004870) to remove possible contaminations using Blobtools (v1.1.1; RRID:SCR_017618) [16]. Subsequently, haplotypic duplications were removed according to the depth of HiFi reads using purge_dups (RRID:SCR_021173) [17]. Proximity ligation data from Omni-C were used to scaffold the assembly with YaHS (RRID:SCR_022965) [18].

To remove low-quality and contaminated reads, RNA sequencing data were first processed using Trimmomatic (v0.39; RRID:SCR_011848) [19], with parameters "TruSeq3-PE.fa:2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25" [19], and kraken2 (v2. 0.8 with kraken2 database k2_standard_20210517; RRID:SCR_005484) [20].

Then, RNA sequencing data were aligned to the repeat soft-masked genome using Hisat2 (RRID:SCR_015530) [21] to generate the bam file. A total of 6,219,463 Tracheophyta reference protein sequences were downloaded from NCBI as protein hits, along with the RNA bam file, to perform genome annotation using Braker (v3.0.8; RRID:SCR_018964) [22] with default parameters.

Repeat annotation

The annotation of transposable elements (TEs) was performed by the Earl Grey TE annotation pipeline (version 1.2) [23].

Macrosynteny analysis

The longest gene transcripts from the predicted gene models of *C. granthamiana* and *Camellia sinensis* (accession number: GWHASIV00000000) [24] were used to retrieve orthologous gene pairs with reciprocal BLASTp (e-value 1e-5; RRID:SCR_001010) using diamond (v2.0.13; RRID:SCR_016071) [25]. The BLAST output was passed to MCScanX (RRID:SCR_022067) [26] to infer the macrosynteny of the pseudochromosomes between *C. granthamiana* and *C. sinensis* with default parameters.

DATA VALIDATION AND QUALITY CONTROL

For the HMW DNA and Pacbio library samples, NanoDrop® One/OneC Microvolume UV–Vis Spectrophotometer, Qubit® Fluorometer, and overnight pulse-field gel electrophoresis were used for quality control. The quality of the Omni-C library was checked by Qubit® Fluorometer and TapeStation D5000 HS ScreenTape. Hi-C contact maps used to validate the pseudochromosomes were generated using the Juicer tools (version 1.22.01; RRID:SCR_017226) [27], following the Omni-C manual (Figure 1C) [28].

During genome assembly, BlobTools (v1.1.1) [16] was used to remove possible contaminations (Figure 3). The resulting genome assembly was run with BUSCO v5.5.0 [29], using the Viridiplantae dataset (Viridiplantae Odb10) to assess the completeness of the genome assembly and gene annotation.

Omni-C reads and PacBio HiFi reads were used to measure the assembly completeness and the consensus quality (QV) using Merqury (v1.3; RRID:SCR_022964) [30] with kmer 21, resulting in a 95.7267% kmer completeness for the Omni-C data and 52.3372 QV values for the HiFi reads, corresponding to 99.999% accuracy.

RESULTS AND DISCUSSION

Genome assembly of *C. granthamiana*

A total of 54.4 Gb HiFi reads was yielded from PacBio sequencing with an average length of 10,731 bp (Tables 1, 2). Together with 233.8 Gb Omni-C data, the genome of *C. granthamiana* was assembled to a final size of 2,412.5 Mb with 6,572 gaps and 37.64% GC content, from which 88.68% of the sequences were anchored into 15 pseudochromosomes (Figure 1B–D). The scaffold N50 was 139.7 Mb and the BUSCO score (RRID:SCR_015008) was 97.8% (Figure 1B; Table 2). Our gene model prediction yielded a total of 68,032 protein-coding genes with a mean length of 298 amino acids and a BUSCO score of 90.9%, which is comparable to other *Camellia* species (Tables 3, 4).

Repeat content analysis annotated 1.65 Gb of transposable elements (TEs), comprising 68.48% of the *C. granthamiana* genome. Among the classified TEs, long terminal repeats

Table 2. Genome statistics and sequencing information.

	Camellia granthamiana
Total length (bp)	2,412,502,632
number	1,681
Mean length (bp)	1,435,159
Longest	187,610,956
Shortest	1,000
N_count	0.054%
Gaps	6,572
N50	139,717,271
N50n	8
N70	124,989,205
N70n	11
N90	1,975,528
N90n	27
BUSCOs (Genome)	C:97.9%[S:79.3%,D:18.6%],F:0.5%,M:1.6%,n:425
HiFi Reads	5,071,365
HiFi Bases	54,421,045,547
HiFi Q30%	3
HiFi Q20%	5
HiFi GC%	38
HiFi Nppm	0
HiFi Ave_len	10,731
HiFi Min_len	100
HiFi Max_len	50,499
Gene models	74,088
No. of protein-coding genes	76,992
Total length of protein-coding genes (AA)	23,158,643
Mean length of protein-coding genes (AA)	301
BUSCOs (Proteome)	C:85.9%[S:66.1%,D:19.8%],F:8.5%,M:5.6%,n:425

Table 3. Camellia genome statistics.

Species	Assembly accession	BUSCOs (Genome)	genome_size (bp)	N50
Camellia sinensis var. sinensis	GCA_004153795.2	C:87.8%[S:77.9%,D:9.9%],F:4.9%,M:7.3%,n:425	2,863,254,423	1,320,966
Camellia sinensis	GCA_013676235.1	C:97.9%[S:88.7%,D:9.2%],F:0.9%,M:1.2%,n:425	3,113,463,150	204,241,410
Camellia sinensis	GCF_004153795.1	C:94.4%[S:83.3%,D:11.1%],F:3.5%,M:2.1%,n:425	3,105,370,065	1,388,941
Camellia lanceoleosa	GCA_025200525.1	C:98.8%[S:80.9%,D:17.9%],F:0.7%,M:0.5%,n:425	2,999,357,698	186,426,707
Camellia granthamiana	**GCA_036172215.1**	**C:97.9%[S:79.3%,D:18.6%],F:0.5%,M:1.6%,n:425**	**2,412,502,632**	**139,717,271**
Camellia sinensis var. sinensis	GCA_017311205.1	C:97.4%[S:85.9%,D:11.5%],F:0.7%,M:1.9%,n:425	3,062,881,361	213,467,978
Camellia sinensis var. sinensis	GCA_020536495.1	C:97.1%[S:84.9%,D:12.2%],F:1.4%,M:1.5%,n:425	3,062,744,301	213,458,217
Camellia sinensis	GCA_020536515.1	C:97.2%[S:85.4%,D:11.8%],F:0.9%,M:1.9%,n:425	3,062,857,199	213,466,203
Camellia sinensis var. lasiocalyx	GCA_020536555.1	C:97.4%[S:85.4%,D:12.0%],F:0.9%,M:1.7%,n:425	3,062,765,809	213,459,538
Camellia sinensis var. assamica	GCA_020536565.1	C:97.4%[S:85.9%,D:11.5%],F:0.7%,M:1.9%,n:425	3,062,795,309	213,462,283
Camellia sinensis	GCA_020536595.1	C:97.4%[S:85.6%,D:11.8%],F:0.7%,M:1.9%,n:425	3,062,747,348	213,457,662
Camellia sinensis var. assamica	GCA_020536795.1	C:97.4%[S:85.9%,D:11.5%],F:0.9%,M:1.7%,n:425	3,062,621,441	213,448,988
Camellia sinensis var. assamica	GCA_020536855.1	C:97.1%[S:84.9%,D:12.2%],F:1.2%,M:1.7%,n:425	3,062,795,300	213,461,895
Camellia sinensis var. assamica	GCA_020536865.1	C:97.4%[S:86.1%,D:11.3%],F:0.9%,M:1.7%,n:425	3,062,765,203	213,459,320
Camellia oleifera	GCA_022316695.1	C:96.0%[S:69.6%,D:26.4%],F:0.7%,M:3.3%,n:425	2,889,508,820	185,364,083
Camellia japonica	GCA_030407325.1	C:97.9%[S:78.6%,D:19.3%],F:0.2%,M:1.9%,n:425	2,803,480,011	175,506,177
Camellia sinensis	GCA_032173705.1	C:88.4%[S:79.5%,D:8.9%],F:7.5%,M:4.1%,n:425	2,679,620,955	146,057,547

retrotransposons accounted for the largest proportion (20.99%), followed by DNA transposons (5.30%), long interspersed nuclear elements (1.60%), and rolling-circle transposons (1.21%) (Figure 1D; Table 5). The large proportion of repeat content in the *C. granthamiana* genome is comparable to other tea species, such as the Tieguanyin cultivar

Table 4. Camellia genome annotations statistics.

Species	*Camellia sinensis* var. *sinensis*	*Camellia sinensis*	*Camellia sinensis*	*Camellia lanceoleosa*	*Camellia granthamiana*
Assembly Accession	GCA_004153795.2	GCA_013676235.1	GCF_004153795.1	GCA_025200525.1	**GCA_036172215.1**
genome_size(bp)	2,863,254,423	3,113,463,150	3,105,370,065	2,999,357,698	**2,412,502,632**
BUSCO (Prot)	C:71.6% [S:64.5%,D:7.1%], F:12.7%, M:15.7%, n:425	C:83.1% [S:80.7%,D:2.4%], F:8.5%, M:8.4%, n:425	C:96.5% [S:44.5%,D:52.0%], F:1.9%, M:1.6%, n:425	C:95.0% [S:81.6%,D:13.4%], F:3.8%, M:1.2%, n:425	**C:90.9% [S:68.5%,D:22.4%], F:3.1%, M:6.0%, n:425**
Number_of_Proteins	30,173	32,356	76,698	54,167	**68,032**
Sum_of_Amino_Acids (aa)	13,483,688	12,149,973	31,189,428	18,299,499	**20,299,147**
Mean_of_Proteins (aa)	447	376	407	338	**298**
Sum_of_Exons (bp)	49,451,194	36,546,930	176,395,894	55,437,744	**60,897,420**
Mean_of_Exons (bp)	285	213	294	218	**267**
Sum_of_Introns (bp)	170,601,000	187,621,926	1,233,047,059	335,774,258	**196,881,812**
Mean_of_Introns (bp)	1,191	1,307	2,521	1,674	**1,225**
Numer_of_gene_loci	30,173	32,356	62,338	54,167	**62,113**
Sum_of_gene_region_(bp)	220,052,194	255,832,614	355,988,266	390,833,969	**218,557,015**
%_of_gene_loci_in_genome	7.69%	8.22%	11.46%	13.03%	9.06%
Average_gene_region(bp)	7,293	7,907	5,711	7,215	**3,519**

Table 5. Summary of the classified TEs in the genome.

Classification	Total length (bp)	Count	Proportion (%)	No. of distinct classifications
DNA	127,797,240	123,181	5.30	7,315
LINE	38,512,116	34,251	1.60	5,403
LTR	506,291,722	183,247	20.99	8,722
Other (Simple Repeat, Microsatellite, RNA)	1,176,745	2,020	0.05	598
Penelope	138,228	226	0.01	131
Rolling Circle	29,291,650	25,351	1.21	3,413
SINE	421,211	1,239	0.02	285
Unclassified	948,396,501	917,104	39.31	9,029
SUM:	**1,652,025,413**	**1,286,619**	**68.48**	**34,896**

of *C. sinensis* (78.2%) [24], wild oil-Camellia *Camellia oleifera* (76.1%) [31], and *Camellia chekiangoleosa* (79.09%) [32].

Macrosynteny between *C. granthamiana* and *C. sinensis*

Our macrosynteny analysis revealed a 1-to-1 pair relationship between the 15 pseudochromsomes of *C. granthamiana* and *C. sinensis* (Figure 2). This indicates that the assembled 15 pseudochromosomes resemble a monoploid genome of the tetraploid *C. granthamiana*.

CONCLUSION AND FUTURE PERSPECTIVES

This study presents the first *de novo* genome assembly of the rare and endangered *C. granthamiana*. This valuable genome resource has excellent potential for use in future studies on the conservation biology of Grantham's camellia, its relationship with other Camellia species from a phylogenomic perspective, and further investigations on the biosynthesis of secondary metabolites in tea species.

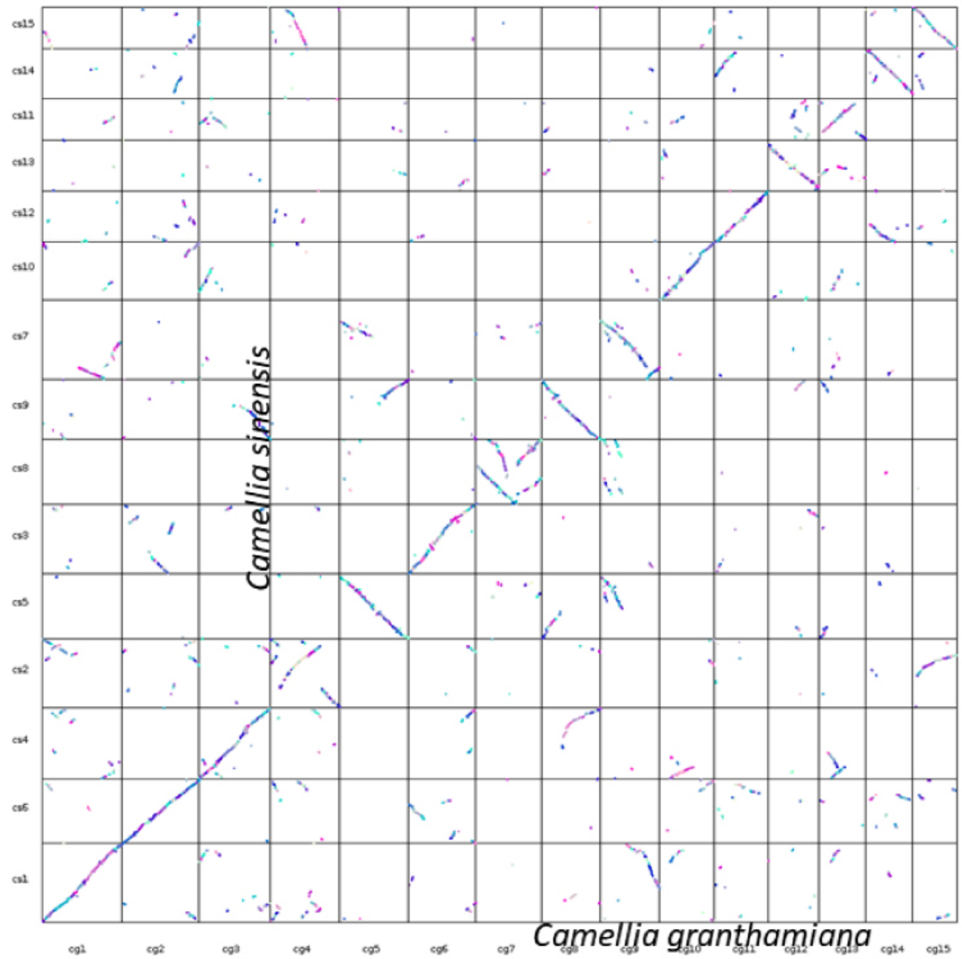


Figure 2. Macrosynteny dot plot between *Camellia granthamiana* and *Camellia sinensis*.

DISCLAIMER

The genomic data generated in this study was not fully haplotype-resolved for a tetraploid genome, and the genome heterozygosity was not assessed.

DATA AVAILABILITY

The final genome assembly of this study was submitted to NCBI under the accession number JAXFYN000000000. The generated raw reads were deposited in the NCBI database under the SRA accessions SRR26895683, SRR26909376, and SAMN40925022. The genome annotations and other supporting data files are available in Figshare [33].

ABBREVIATIONS

CTAB, cetyl trimethylammonium bromide; HMW, high molecular weight; QV, consensus quality; TE, transposable elements.

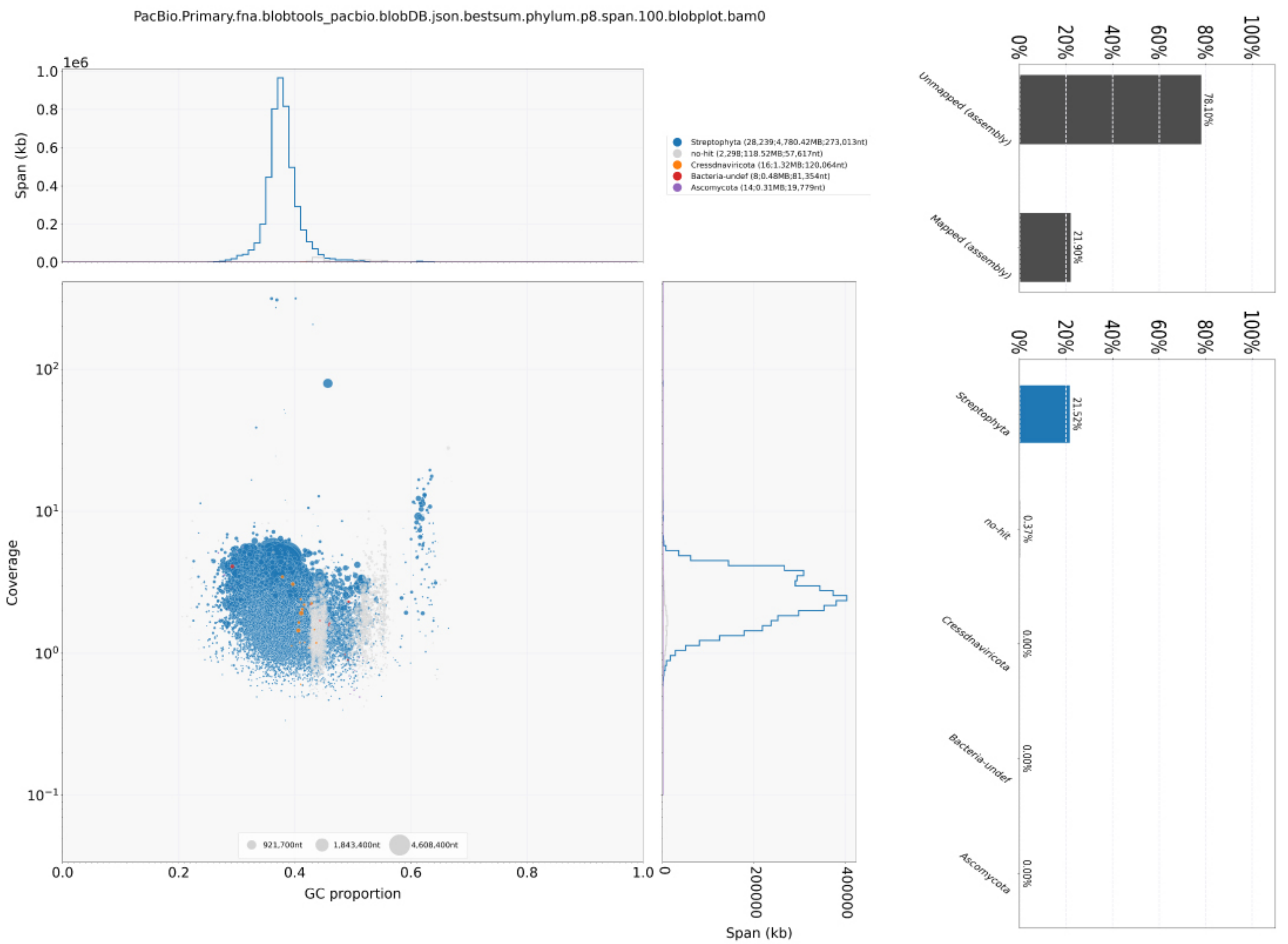


Figure 3. Genome assembly quality control and contaminant/cobiont detection.

DECLARATIONS

Ethics approval and consent to participate

The authors declare that ethical approval was not required for this type of research.

Competing interests

The authors declare that they do not have competing interests.

Authors' contribution

JHLH, TFC, LLC, SGC, CCC, JKHF, JDG, SCKL, YHS, CKCW, KYLY and YW conceived and supervised the study. DTWL collected the sample materials. STSL and WLS performed DNA extraction, library preparation and genome sequencing. HYY facilitated the logistics of samples. WN performed genome assembly, gene model prediction and genome quality check analyses. STSL carried out the macrosynteny analysis.

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REFERENCES

1 **POWO**. Plants of the world online. Facilitated by the Royal Botanic Gardens, Kew. 2023; https://powo.science.kew.org/.

2 **Wang Y**, **Zhuang H**, **Shen Y et al.** The dataset of Camellia cultivars names in the world. ***Biodivers. Data J.***, 2021; **9**: e61646. doi:10.3897/BDJ.9.e61646.

3 **Beech E**, **Barstow M**, **Rivers M et al.** The Red List of Theaceae. UK: Botanic Gardens Conservation International, 2017. ISBN-10: 1-905164-66-1.

4 **Fu L**, **Jin J**. China Plant Red Data Book-Rare and Endangered Plants, vol. 1, 1992; p. xviii + 741, ref. 394, 19940300703, ISBN: 7-03-000484-1.

5 **Hu Q**, **Xia N**, **Wu D et al.** Rare and Precious Plants of Hong Kong. Agriculture. Hong Kong: Fisheries and Conservation Department, 2003; pp. 220–222, https://www.herbarium.gov.hk/en/publications/books/book2/text/camellia-granthamiana/index.html.

6 **Jiang Z**, **Jiao P**, **Qi Z et al.** The complete chloroplast genome sequence of *Camellia granthamiana*. ***Mitochondrial DNA Part B***, 2019; **4**(2): 4113–4115. doi:10.1080/23802359.2019.1692703.

7 **Li W**, **Shi X**, **Guo W et al.** Characterization of the complete chloroplast genome of *Camellia granthamiana* (Theaceae), a Vulnerable species endemic to China. ***Mitochondrial DNA Part B***, 2018; **3**(2): 1139–1140. doi:10.1080/23802359.2018.1521310.

8 **Wu Q**, **Tong W**, **Zhao H et al.** Comparative transcriptomic analysis unveils the deep phylogeny and secondary metabolite evolution of 116 Camellia plants. ***Plant J.***, 2022; **111**(2): 406–421. doi:10.1111/tpj.15799.

9 **Chen S**, **Li W**, **Li W et al.** Population genetics of *Camellia granthamiana*, an endangered plant species with extremely small populations in China. ***Front. Genet.***, 2023; **14**: 1252148. doi:10.3389/fgene.2023.1252148.

10 **Huang H**, **Tong Y**, **Zhang Q-J et al.** Genome size variation among and within Camellia species by using flow cytometric analysis. ***PLoS One***, 2013; **8**(5): e64981. doi:10.1371/journal.pone.0064981.

11 **Kondo K**. Chromosome numbers in the genus Camellia. ***Biotropica***, 1977; **9**(2): 86–94. https://doi.org/10.2307/2387663.

12 **Doyle JJ**, **Doyle JL**. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. ***Phytochem. Bull.***, 1987; **19**: 11–15.

13 **Workman R**, **Timp W**, **Fedak R et al.** High molecular weight DNA extraction from recalcitrant plant species for third generation sequencing. protocols.io. 2019; https://dx.doi.org/10.17504/protocols.io.4vbgw2n.

14 **Jordon-Thaden IE**, **Chanderbali AS**, **Gitzendanner MA et al.** Modified CTAB and TRIzol protocols improve RNA extraction from chemically complex Embryophyta. ***Appl. Plant Sci.***, 2015; **3**(5): apps.1400105. doi:10.3732/apps.1400105.

15 **Cheng H**, **Concepcion GT**, **Feng X et al.** Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. ***Nat. Methods***, 2021; **18**(2): 170–175. doi:10.1038/s41592-020-01056-5.

16 **Laetsch DR**, **Blaxter ML**. BlobTools: Interrogation of genome assemblies. ***F1000Research***, 2017; **6**: 1287. doi:10.12688/f1000research.12232.1.

17 **Guan D**, **Guan D**, **McCarthy SA et al.** Identifying and removing haplotypic duplication in primary genome assemblies. ***Bioinformatics***, 2020; **36**(9): 2896–2898. doi:10.1093/bioinformatics/btaa025.

18 **Zhou C**, **McCarthy SA**, **Durbin R**. YaHS: yet another Hi-C scaffolding tool. ***Bioinformatics***, 2023; **39**: btac808. doi:10.1093/bioinformatics/btac808.

19 **Bolger AM**, **Lohse M**, **Usadel B**. Trimmomatic: a flexible trimmer for Illumina sequence data. ***Bioinformatics***, 2014; **30**: 2114–2120. doi:10.1093/bioinformatics/btu170.

20 **Wood DE**, **Lu J**, **Langmead B**. Improved metagenomic analysis with Kraken 2. ***Genome Biol.***, 2019; **20**: 257. doi:10.1186/s13059-019-1891-0.

21 **Kim D**, **Paggi JM**, **Park C et al.** Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. ***Nat. Biotechnol.***, 2019; **37**: 907–915. doi:10.1038/s41587-019-0201-4.

22 **Hoff KJ**, **Lomsadze A**, **Borodovsky M**, **Stanke M**. Whole-genome annotation with BRAKER. ***Methods Mol. Biol.***, 2019; **1962**: 65–95. doi:10.1007/978-1-4939-9173-0_5.

23 **Baril T**, **Galbraith J**, **Hayward A**. Earl Grey: a fully automated user-friendly transposable element annotation and analysis pipeline. ***Mol. Biol. Evol.***, 2024; **41**(4): msae068. doi:10.1093/molbev/msae068.

24 **Zhang X**, **Chen S**, **Shi L et al.** Haplotype-resolved genome assembly provides insights into evolutionary history of the tea plant *Camellia Sinensis*. ***Nat. Genet.***, 2021; **53**: 1250–1259.

25 **Buchfink B**, **Xie C**, **Huson DH**. Fast and sensitive protein alignment using DIAMOND. ***Nat. Methods***, 2015; **12**: 59–60.

26 **Wang Y**, **Tang H**, **Debarry JD et al.** MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. ***Nucleic Acids Res.***, 2012; **40**: e49. doi:doi.org/10.1093/NAR/GKR1293.

27 **Durand NC**, **Shamim MS**, **Machol I et al.** Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. ***Cell Syst.***, 2016; **3**: 95–98. doi:10.1016/j.cels.2016.07.002.

28 Omni-C manual. https://omni-c.readthedocs.io/en/latest/contact_map.html.

29 **Manni M**, **Berkeley MR**, **Seppey M et al.** BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. ***Mol. Biol. Evol.***, 2021; **38**: 4647–4654. doi:10.1093/molbev/msab199.

30 **Rhie A**, **Walenz BP**, **Koren S et al.** Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies. ***Genome Biol.***, 2021; **21**(1): 245. doi:10.1186/s13059-020-02134-9.

31 **Lin P**, **Wang K**, **Wang Y et al.** The genome of oil-Camellia and population genomics analysis provide insights into seed oil domestication. ***Genome Biol.***, 2022; **23**(1): 14. doi:10.1186/s13059-021-02599-2.

32 **Shen T**, **Huang B**, **Xu M et al.** The reference genome of *Camellia chekiangoleosa* provides insights into Camellia evolution and tea oil biosynthesis. ***Hortic. Res.***, 2022; **9**: uhab083. doi:10.1093/hr/uhab083.

33 Genome assembly of the rare and endangered Grantham's camellia, *Camellia Granthamiana*. Figshare. [DataSet]. 2024; https://doi.org/10.6084/m9.figshare.24925233.

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