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

- 2 granthamiana
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49 Abstract

50 The Grantham's camellia (*Camellia granthamiana* Sealy) is a rare and endangered tea 51 species that is endemic to southern China, and was first discovered in Hong Kong in 1955. 52 Despite its high conservation value, genomic resources of C. granthamiana remain limited. 53 Here, we present a chromosome-scale draft genome of the tetraploid C. granthamiana (2n =54 4x = 60) using a combination of PacBio long read sequencing and Omni-C data. The 55 assembled genome size is ~2.4 Gb with most sequences anchored to 15 pseudochromosomes 56 that resemble a monoploid genome. The genome is of high contiguity, with a scaffold N50 of 57 139.7 Mb, and high completeness with a 97.8% BUSCO score. Gene model prediction 58 resulted in a total 76,992 protein-coding genes with a BUSCO score of 85.9%. 1.65 Gb of 59 repeat content was annotated, which accounts for 68.48% of the genome. The Grantham's 60 camellia genome assembly provides a valuable resource for future investigations on its 61 biology, ecology, phylogenomic relationships with other *Camellia* species, as well as set up a 62 foundation for further conservation measures.

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64 Introduction

65 Camellia is a large genus in the family Theaceae with more than 230 described 66 species (POWO, 2021). While some camellias are well-known for their ornamental and 67 economical values as tea and woody-oil producing plants that derived into tens of thousands 68 of cultivars (Wang et al., 2021), more than 60 Camellia species were regarded as globally 69 threatened due to natural habitat fragmentation or loss and small population size (Beech et al., 70 2017). The Grantham's camellia (*Camellia granthamiana*) (Figure 1A) is a rare species once 71 discovered in Hong Kong and named after Sir Alexander Grantham and is narrowly 72 distributed in Hong Kong and Guangdong, China (Beech et al., 2017). It is listed as 73 vulnerable in the IUCN Red List and recorded as endangered in the China Plant Red Data 74 Book (Fu & Chin, 1992). In Hong Kong, the Grantham's camellia is a protected species by 75 law and has been actively being propagated and reintroduced to the wild by the Agriculture, 76 Fisheries and Conservation Department (Hu, 2003).

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78 Context

In view of the high conservation value of Grantham's camellia, several molecular studies have been previously conducted. They include sequencing the chloroplast genomes of C. granthamiana (Jiang et al., 2019; Li et al., 2018), using pan-transcriptomes to reconstruct the phylogeny of over a hundred of *Camellia* species (Wu et al., 2022), and population genetics study (Chen et al., 2023). However, nuclear genomic resources of *C. granthamiana* remain lacked. While most *Camellia* species possess a karyotype of 2n = 30, *C.* granthamiana is one of the exceptions with a karyotype of 2n = 4x = 60 (Huang et al., 2013;

86 Kondo et al., 1977).

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

- 92
- 93 Methods

94 Sample collection and high molecular weight DNA extraction

95 Fresh leaf tissues were sampled in transplanted individual on the campus of the 96 Chinese University of Hong Kong. High molecular weight (HMW) genomic DNA was 97 isolated from 1g leaf tissues using a CTAB pretreatment followed by NucleoBond HMW 98 DNA kit (Macherey Nagel Item No. 740160.20). Briefly, the tissues were ground with liquid 99 nitrogen and digested in 5 mL CTAB buffer (Doyle & Doyle, 1987) with an addition of 1% 100 polyvinylpyrrolidone (PVP) for 1 h. The lysate was treated with RNAse A, followed by an 101 addition of 1.6 mL of 3M potassium acetate and two round of chloroform:IAA (24:1) washes. 102 The supernatant was transferred to a new 50 mL tube using a wide-bore tip. H1 buffer from 103 the NucleoBond HMW DNA kit was added to the supernatant for a total volume of 6 mL 104 mixture, from which the DNA was isolated by following the manufacturer's protocol. After 105 the DNA was eluted with 60 µL elution buffer (PacBio Ref. No. 101-633-500), quality check was carried out with NanoDrop[™] One/OneC Microvolume UV-Vis Spectrophotometer, 106 Qubit[®] Fluorometer, and overnight pulse-field gel electrophoresis. 107

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109 Pacbio library preparation and sequencing

110 The qualified DNA was sheared with a g-tube (Covaris Part No. 520079) for 6 passes 111 of centrifugation at 1,990 x g for 2 min and was subsequently purified with SMRTbell[®] 112 cleanup beads (PacBio Ref. No. 102158-300). 2 µL sheared DNA was taken for fragment size 113 examination through overnight pulse-field gel electrophoresis. Two SMRTbell libraries were constructed with the SMRTbell® prep kit 3.0 (PacBio Ref. No. 102-141-700), following the 114 manufacturer's protocol. The final library was prepared with the Sequel[®] II binding kit 3.2 115 (PacBio Ref. No. 102-194-100) and was loaded with the diffusion loading mode with the 116 117 on-plate concentration set at 90 pM on the Pacific Biosciences SEQUEL IIe System, running 118 for 30-hour movies to output HiFi reads. In total, three SMRT cells were used for the 119 sequencing. Details of the resulting sequencing data are summarized in Supplementary 120 Information 1.

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123

122 Omni-C library preparation and sequencing

Nuclei was isolated from 3 g fresh leaf tissues ground with liquid nitrogen using the

modified 124 al. PacBio protocol from Workman et (2018)125 (https://www.pacb.com/wp-content/uploads/Procedure-checklist-Isolating-nuclei-from-plant-t 126 issue-using-TissueRuptor-disruption.pdf). The nuclei pellet was snap-frozen with liquid 127 nitrogen and stored at -80 °C. Upon Omni-C library construction, the nuclei pellet was 128 resuspended in 4 mL 1X PBS buffer and processed with the Dovetail® Omni-C® Library 129 Preparation Kit (Dovetail Cat. No. 21005) by following the manufacturer's procedures. The concentration and fragment size of the resulting library was assessed by Qubit[®] Fluorometer 130 131 and TapeStation D5000 HS ScreenTape, respectively. The qualified library was sent to 132 Novogene and sequenced on an Illumina HiSeq-PE150 platform. Details of the resulting 133 sequencing data are summarized in Supplementary Information 1.

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135 Genome assembly and gene model prediction

De novo genome assembly was first proceeded with Hifiasm (Cheng et al., 2021) and then was processed with searching against the NT database with BLAST to remove possible contaminations using BlobTools (v1.1.1) (Laetsch & Blaxter, 2017). Subsequently, haplotypic duplications were removed according to the depth of HiFi reads using "purge_dups" (Guan et al., 2020). Proximity ligation data from Omni-C were used to scaffold the assembly with YaHS (Zhou et al., 2022).

142 Gene models were trained, predicted and updated by funannotate (Palmer & Stajich, 143 2020) with the following parameters "--repeats2evm --protein_evidence uniprot_sprot.fasta 144 --genemark_mode ET --optimize_augustus --organism other --max_intronlen 350000". 145 Seven RNA sequencing data were downloaded from NCBI (SRA Accessions: SRR16685015, 146 SRR16685016, SRR16685017, SRR19086193, SRR19266768, SRR24821546, and 147 SRR24821547) and aligned to the repeat soft-masked genome using Hisat2 to run the 148 genome-guided Trinity (Grabherr et al., 2011), from which 289,554 transcripts were derived. 149 The Trinity transcript alignments were converted to GFF3 format and used as input to run the 150 PASA alignment to generate PASA models trained by TransDecoder, which were screened 151 using Kallisto TPM data. The PASA gene models were used to train Augustus in the 152 funannotate-predict step. The predicted gene models were combined from various prediction 153 sources, including GeneMark, high-quality Augustus predictions (HiQ), pasa, Augustus, 154 GlimmerHM and snap, and were integrated to produce the annotation files with Evidence 155 Modeler. UTRs were further captured in the funannotate-update step using PASA.

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157 *Repeat annotation*

The annotation of transposable elements (TEs) were performed by the Earl Grey TE annotation pipeline (version 1.2, https://github.com/TobyBaril/EarlGrey) (Baril et al., 2022).

- 160
- 161 *Macrosynteny analysis*

162 The longest gene transcripts from the predicted gene models of *Camellia* 163 granthamiana and *Camellia sinensis* (accession number: GWHASIV00000000; Zhang et al., 164 2021) were used to retrieve orthologous gene pair with reciprocal BLASTp (e-value 1e-5) 165 using diamond (v2.0.13) (Buchfink et al., 2021). The BLAST output was passed to MCScanX 166 (Wang et al., 2012) to infer macrosynteny of the pseudochromosomes between *C*. 167 granthamiana and *C. sinensis* with default parameters.

168

169 **Results and discussion**

170 Genome assembly of Camellia granthamiana

171 A total of 54.4 Gb HiFi reads was yielded from PacBio sequencing with an average 172 length of 10,731 bp (Table 1; Supplementary Information 1). Together with 233.8 Gb 173 Omni-C data, the genome of Camellia granthamiana was assembled with a final size of 174 2,412.5 Mb, from which 79.87% of the sequences were anchored into 15 175 pseudochromosomes (Figure 1B-1D; Supplementary Information 1). The scaffold N50 was 176 139.7 Mb and the BUSCO score was 97.8% (Figure 1B; Table 1). Gene model prediction 177 yielded a total of 76,992 protein-coding genes with a mean length of 301 bp and BUSCO 178 score of 85.9%.

179 Repeat content analysis annotated 1.65 Gb of transposable elements (TEs), 180 comprising 68.48% of the C. granthamiana genome. Among the classified TEs, LTR 181 retransposons accounted for the largest proportion (20.99%), followed by DNA transposons 182 (5.30%), LINE (1.60%) and Rolling-circle transposons (1.21%) (Figure 1D; Table 2). The 183 large proportion of repeat content in the C. granthamiana genome is comparable to other tea 184 species, such as the Tieguanyin cultivar of *Camellia sinensis* (78.2%) (Zhang et al., 2021), 185 wild oil-Camellia Camellia oleifera (76.1%) (Lin et al., 2022), and Camellia chekiangoleosa 186 (79.09%) (Shen et al., 2022).

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188 Macrosynteny between Camellia granthamiana and Camellia sinensis

189 Macrosynteny analysis revealed a 1-to-1 pair relationship between the 15 190 pseudochromsomes of *C. granthamiana* and that of *C. sinensis* (Figure 2). This indicates that 191 the assembled 15 pseudochromosomes resemble a monoploid genome of the tetraploid *C.* 192 granthamiana.

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194 Conclusion and future perspective

195 This study presents the first *de novo* genome assembly of the rare and endangered *C*. 196 *granthamiana*. This valuable genome resource is of great potential for the use in future 197 studies on the conservation biology of the Grantham's camellia, its relationship with other 198 *Camellia* species from a phylogenomic perspective and further investigations on the 199 biosynthesis of secondary metabolites of tea species.

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201 Data validation and quality control

For 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 Omni-C library was checked by Qubit[®]
 Fluorometer and TapeStation D5000 HS ScreenTape.

- During genome assembly, BlobTools (v1.1.1) (Laetsch & Blaxter, 2017) was employed to remove possible contaminations (Supplementary Information 2). The resulting genome assembly was run with Benchmarking Universal Single-Copy Orthologs (BUSCO, v5.5.0) (Manni et al., 2021) with the Viridiplantae dataset (Viridiplantae Odb10) to assess the
- 210 completeness of the genome assembly and gene annotation.
- 211

212 Disclaimer

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

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216 Data availability

The final genome assembly in this study was submitted to NCBI under accession number JAXFYN000000000. The raw reads generated were deposited in the NCBI database under under the SRA accessions SRR26895683 and SRR26909376. The genome annotation files were uploaded to Figshare (https://figshare.com/s/4b13376ad27ae0647fd1).

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222 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 macrosynteny analysis.

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229 Competing interest

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The authors declare that they do not have competing interests.

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- 236
- 237 **References**

238 1. Baril T, Imrie RM, Hayward A. Earl Grey: a fully automated user-friendly transposable 239 2022. element annotation and analysis pipeline. bioRxiv. 240 https://doi.org/10.1101/2022.06.30.498289 241 2. Beech E, Barstow M, Rivers M. The red list of Theaceae. Botanic Gardens Conservation 242 International: 2017. 243 3. Buchfink B, Reuter K, Drost HG. Sensitive protein alignments at tree-of-life scale using 244 DIAMOND. Nature methods. 2021;18(4):366-8. 245 4. Chen S, Li W, Li W, Liu Z, Shi X, Zou Y, Liao W, Fan Q. Population genetics of Camellia 246 granthamiana, an endangered plant species with extremely small populations in China. 247 Frontiers in Genetics. 2023;14. 5. Cheng H, Concepcion GT, Feng X, Zhang H, Li H. Haplotype-resolved de novo assembly 248 249 using phased assembly graphs with hifiasm. Nature methods. 2021;18(2):170-5. 250 6. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf 251 tissue. Phytochemical bulletin. 1987. 252 7. Fu L, Chin CM. China plant red data book. Science press; 1992. 253 8. Guan D, McCarthy SA, Wood J, Howe K, Wang Y, Durbin R. Identifying and removing 254 haplotypic duplication in primary genome assemblies. **Bioinformatics**. 255 2020;36(9):2896-8. 256 9. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, 257 Raychowdhury R, Zeng Q, Chen Z. Full-length transcriptome assembly from RNA-Seq 258 data without a reference genome. Nature biotechnology. 2011;29(7):644-52. 259 10. Hu Q. Rare and Precious Plants of Hong Kong. Agriculture Fisheries and Conservation 260 Department, the Government of the Hong Kong Special Administrative Region; 2003. 261 11. Huang H, Tong Y, Zhang QJ, Gao LZ. Genome size variation among and within Camellia 262 species by using flow cytometric analysis. PLoS One. 2013;8(5):e64981. 263 12. Jiang Z, Jiao P, Qi Z, Qu J, Guan S. The complete chloroplast genome sequence of 264 Camellia granthamiana. Mitochondrial DNA Part B. 2019;4(2):4113-5. 265 13. Kondo K. Chromosome numbers in the genus Camellia. Biotropica. 1977:86-94. 266 14. Kong W, Wang Y, Zhang S, Yu J, Zhang X. Recent Advances in assembly of plant 267 complex genomes. Genomics, Proteomics & Bioinformatics. 2023;21(3):427-439. 268 15. Laetsch DR, Blaxter ML. BlobTools: Interrogation of genome assemblies. 269 F1000Research. 2017;6(1287):1287. 270 16. Li W, Shi X, Guo W, Banerjee AK, Zhang Q, Huang Y. Characterization of the complete 271 chloroplast genome of *Camellia granthamiana* (Theaceae), a vulnerable species endemic 272 to China. Mitochondrial DNA Part B. 2018;3(2):1139-40. 273 17. Lin P, Wang K, Wang Y, Hu Z, Yan C, Huang H, Ma X, Cao Y, Long W, Liu W, Li X. The 274 genome of oil-Camellia and population genomics analysis provide insights into seed oil 275 domestication. Genome Biology. 2022;23:1-21.

18. Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Molecular biology and evolution.
2021;38(10):4647-54.

- 280 19. Palmer JM, Stajich J. Funannotate v1. 8.1: Eukaryotic genome annotation. Zenodo
 281 https://doi.org/10.5281/zenodo.2020;4054262.
- 282 20. POWO. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew.
 283 Published on the Internet. 2024. <u>http://www.plantsoftheworldonline.org/</u>. Accessed 2 Jan
 284 2024.
- 285 21. Rivers MC, & Wheeler L. 2015. *Camellia granthamiana*. The IUCN Red List of
 286 Threatened Species 2015: e.T62053240A62053244.
 287 https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T62053240A62053244.en. Accessed
 288 on 13 December 2023.
- 289 22. Shen TF, Huang B, Xu M, Zhou PY, Ni ZX, Gong C, Wen Q, Cao FL, Xu LA. The
 290 reference genome of Camellia chekiangoleosa provides insights into Camellia evolution
 291 and tea oil biosynthesis. Horticulture Research. 2022;9:uhab083.
- 23. Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H,
 Kissinger JC. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny
 and collinearity. Nucleic acids research. 2012;40(7):e49.
- 24. Wang Y, Zhuang H, Shen Y, Wang Y, Wang Z. The dataset of *Camellia* cultivars names in
 the world. Biodiversity Data Journal. 2021;9.
- 297 25. Workman R, Timp W, Fedak R, Kilburn D, Hao S, Liu K. High molecular weight DNA
 298 extraction from recalcitrant plant species for third generation sequencing. *Protocol*299 *Exchange* (published online 18 June 2018.
 300 https://protocolexchange.researchsquare.com/article/nprot-6785/v1).
- 26. Wu Q, Tong W, Zhao H, Ge R, Li R, Huang J, Li F, Wang Y, Mallano AI, Deng W, Wang
 W. Comparative transcriptomic analysis unveils the deep phylogeny and secondary
 metabolite evolution of 116 Camellia plants. The Plant Journal. 2022;111(2):406-21.
- 27. Zhang X, Chen S, Shi L, Gong D, Zhang S, Zhao Q, Zhan D, Vasseur L, Wang Y, Yu J,
 Liao Z. Haplotype-resolved genome assembly provides insights into evolutionary history
 of the tea plant Camellia sinensis. Nature Genetics. 2021;53(8):1250-9.
- 307 28. Zhou C, McCarthy SA, Durbin R. YaHS: yet another Hi-C scaffolding tool.
 308 Bioinformatics. 2023;39(1):btac808.
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- **Table 1.** Genome statistics and sequencing information.
- **Table 2.** Summary of classified transposable elements in the genome.
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- 313 Figure 1. Genomic information of Camellia granthamiana. A) Picture of Camellia

- 314 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
- 316 landscape plot (bottom) of repetitive elements in the genome.
- 317
- Figure 2. Macrosynteny dot plot between *Camellia granthamiana* and *Camellia sinensis*.
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- 320 Supplementary Information 1. Summary of genomic sequencing data.
- 321 Supplementary Information 2. Genome assembly QC and contaminant/cobiont detection.



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

)		Camellia granthamiana
	Genome size (bp)	2,412,502,632
	Number of scaffolds	1,681
	N_count	0.05%
	N50	139,717,271
	N50n	8
	BUSCO (Genome)	97.80%
	Gene models	74,088
	Protein-coding genes	76,992
	BUSCO (Proteome)	85.90%







