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The draft genome assembly of the critically endangered *Nyssa yunnanensis*, a plant species with extremely small populations endemic to Yunnan Province, China

Weixue Mu[1], Jinpu Wei[1], Ting Yang[1], Yannan Fan[1], Le Cheng[2], Jinlong Yang[2], Ranchang Mu[3], Jie Liu[3], Jianming Zhao[3], Xun Xu[1,6], Xin Liu[1], Radoje Drmanac[1,5*], Huan Liu[1,4*]

1. BGI-Shenzhen, Shenzhen 518083, China
2. BGI-Yunnan, BGI-Shenzhen, Kunming, 650106, China
3. Forestry Bureau of Ruili, Yunnan Dehong, Ruili 678600, China
4. Department of Biology, University of Copenhagen, DK-2100 Copenhagen, Denmark.
5. Complete Genomics Inc., 2904 Orchard Pkwy, San Jose, CA 95134 USA.
6. Guangdong Provincial Key Laboratory of Genome Read and Write, Shenzhen 518083, China

ORCIDs:

Weixue Mu: 0000-0002-2675-261X; Jinpu Wei: 0000-0002-1463-0236; Ting Yang: 0000-0002-2872-4954; Yannan Fan: 0000-0003-3308-6878; Jinlong Yang: 0000-0003-1323-7755; Xun Xu: 0000-0002-5338-5173; Xin Liu: 0000-0003-3256-2940; Huan Liu: 0000-0003-3909-0931

Abstract

Nyssa yunnanensis is a deciduous tree in family Nayssaceae within the order Cornales. As only 8 individuals in 2 sites recorded in Yunnan province of China, the species was listed as the China's national grade-I protection species in 1999, and also as one of 120 PSESP(Plant Species with Extremely Small Populations) in Implementation Plan of Rescuing and Conserving China's Plant Species with extremely Small Populations(PSESP) (2011-2-15). *N. yunnanensis* was also been evaluated as Critically Endangered in IUCN red list and Threatened Species List of China's Higher Plants. Hence understanding the genomic characteristics of this highly endangered Tertiary relict tree species is essential, especially for developing conservation strategies. Here

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we sequenced and annotated the genome of *N. yunnanensis* using 10X genomics linked-reads sequencing data. The *de novo* assembled genome is 1474Mb in length with a scaffold N50 length of 985.59kb. We identified 823.51Mb of non-redundant sequence as repetitive elements and annotated 39,803 protein-coding genes in the assembly. Our result provided the genomic characteristics of *N. yunnanensis*, which will provide valuable resources for future genomic and evolutionary studies, especially for conservation biology studies of this extremely threatened tree species.

Data Description

Nyssa yunnanensis belonging to the family Nyssaceae is an extremely threatened range-restricted tree species evaluated as Critically Endangered (CR) in the IUCN Red List of Threatened Species[1] and also a national key protected species under gradeI protection in China[2]. *N. yunnanensis* also was listed as one of 120 PSESP(Plant Species with Extremely Small Populations) in *Implementation Plan of Rescuing and Conserving China's Plant Species with extremely Small Populations(PSESP)*（2011-2-15）and as a critically endangered in the Threatened Species List of China's Higher Plants[3, 4]. It is a canopy tree reaching 30m in height and functionally dioecious, consisting two types of individuals. One bearing staminate flowers while the other bearing morphologically perfect flowers but inaperturate and inviable pollen grains. *N. yunnanensis* does not appear to exhibit parthenogenesis[5]. A survey on population status and ecological characteristics of *N. yunnanensis* had been carried out suggesting that this species is at risk having only two natural populations and eight individuals in Yunnan, China due to both ecological and human factors [6]. Since 2009, an integrated strategy for PSESP conservation of this species was initiated and applied over a 7-year period resulted in secure protection of natural populations, development of propagation technologies and production of vigorous seedlings, as well as establishment of three new populations and four ex situ germplasm collections of *N. yunnanensis* [7]. The phylogenetic study of the six species of Nyssa genus recognized in the Flora of China (*N. yunnanensis, N. javanica, N. sinensis, N. shangszeensis, N. shweliensis and N.*

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wenshanensis) based on morphological and molecular data suggesting that only *N. sinensis, N. yunnanensis and N. javanica* to be recognized and reticulate evolution is possible[8].

Recent advances in whole genome sequencing technology have provided valuable genomic resources to help us better understand the origin and evolutionary history as well as to enhanced approaches for conservation of endangered species [9]. The recently published genome of *Nyssa sinensiss* along with the genome of *Camptotheca acuminata* are the only two genome assemblies have been sequenced within the Nyssaceae family. The *N. sinensiss* genome having a total length of 1,001.42 Mb and an N50 scaffold size of 3.62 Mb[10], and the *C. acuminate* genome having a total length of 403.17 Mb and an N50 scaffold size of 1753Kb[11] respectively. *Acer yangbiense* is another plant species with extremely small populations endemic to Yunnan Province, and had been sequenced in 2019. The *A. yangbiense* genome has a total length of 666Mb with 13 chromosomes and a scaffold N50 size of 45Mb[12].

Although *N. yunnanensis* is not the first sequenced species in the Nyssaceae family, detailed understanding of this endangered species' genomic makeup along with other information such as population structure and reproductive biology is urgently required to help improve the current PSESP conservation strategy for its continued survival.

Methods

Sample collection

The wild individual of *Nyssa yunnanensis* (NCBI: txid161873) sequenced was 70cm in height (Fig. 1), and sampled growing in Ruili, Yunan, China. (97°56′20.99″ N, 24°03′02.72″ E, altitude 843M). Fresh young leaf samples were collected for DNA extraction. Voucher specimens and images were collected and stored in the CNGB herbarium (Fig. 2). The extracted DNA is stored in the BGI-sample center.

DNA extraction

Leaf samples of *N. yunnanensis* were used for DNA extraction using the cetyl-

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triethylammonium bromide (CTAB) method[13]. Quality control was done using a Sage Science Pippin pulse electrophoresis system and high-molecular-weight (HMW) gDNA with a length of around 50kb was obtained.

Library preparation and sequencing

The HMW DNA was loaded onto a Chromium Controller chip with 10X Chromium reagents and gel beads, and rest of the library preparation procedures were carried out according to the manufacturer's protocol [14]. Subsequently the sequencing was performed on a BGISEQ-500 platform according to the manufacturer's instructions [15] using the whole-genome shotgun sequencing strategy and a total of 1.64 Gb of raw data (150 bp, paired-end) was eventually generated which covered about 100× of the 1.64Gb estimated genome size.

Genome size estimation

The raw data of the *N. yunnanensis* was trimmed with Trimmomatic-0.38[16] with the parameters "ILLUMINACLIP: 2:35:4 HEADCROP:5 LEADING:3 TRAILING:3 SLIDINGWINDOW:5:15 MINLEN:50". The 1.64 Gb *N. yunnanensis* genome size was then estimated by k-mer frequency analysis software gce-1.0.0 (GCE, RRID:SCR_017332) [17] with the clean data. In addition, the genome size estimation performed automatically by the supernova-2.0.0 assembler software (Supernova assembler, RRID:SCR_016756)[18] resulted in an estimated genome size of 1.43 Gb.

***De novo* genome assembly**

De novo assembly was carried out using supernova-2.0.0 software[18] with the "--maxreads 691040000" parameter. Linked read data without trimming were used as the software recommended. Then the gaps within the scaffolds were filled by GapCloser version 1.12 (GapCloser, RRID:SCR_015026)[19] with the parameters "-l 150 -t 32" using barcode trimmed pair-end reads.

Repeat annotation

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Repetitive elements were identified using both homology-based and *de novo* predictions in the *N. yunnanensis* genome assembly. For homology-based prediction, RepeatMasker v3.3.0 (RepeatMasker , RRID:SCR_012954)[20] and RepeatProteinMasker v3.3.0[20] were applied, aligning the *N. yunnanensis* genome sequences against the Repbase v16.10[21] and to identify the known repetitive elements. For *de novo* prediction, RepeatModeler v1.0.5 (RepeatModeler, RRID:SCR_015027)[22] was first executed to build a de novo repeat library and using the *N. yunnanensis* genome assembly. Then RepeatMasker v3.3.0[20] was employed to align the *N. yunnanensis* genome sequences against the *de novo* repeat library to identify the repetitive elements. LTR_FINDER v1.05 (LTR_Finder, RRID:SCR_015247) [23] was used for *ab initio* LTR retrotransposon finding and Tandem Repeats Finder v4.07b[24] was used for Tandem repeats identification respectively.

Gene prediction

The *N. yunnanensis* genome with repetitive regions masked were further used to carry out gene prediction. MAKER-P v2.31 (MAKER, RRID:SCR_005309) [25] was utilized to predict protein-coding genes based on both homology and *de novo* prediction evidence. Protein sequences of *Camptotheca acuminate* and *Arabidopsis thaliana* were used as the homology evidence. Genemark-ES v4.21 (GeneMark, RRID:SCR_011930) [26] was self-trained with the default criteria. The first round of MAKER-P analysis was run using the default parameters on the basis of the above evidence, with the "protein2genome" parameter set to "1" to obtain protein-supported gene models. SNAP[27] was then used to train with these gene models. The default parameters were used to run the second and final rounds of MAKER-P to generate the final gene models.

Functional annotation

The predicted gene models were further functionally annotated by aligning their protein sequences against the Kyoto Encyclopedia of Genes and Genomes (KEGG)[28], Clusters of Orthologous Groups (COG)[29], SwissProt[30], TrEMBL, and National

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Center for Biotechnology Information (NCBI) non-redundant (NR) protein databases with BLASTP (BLASTP, RRID:SCR_001010) (E-value ≤ 1e-05). InterProScan v5.21 (InterProScan, RRID:SCR_005829) [31] was used for protein motifs and domains searching by comparing the sequences against domain databases including the PFAM, PANTHER, PRINTS, PROSITE, ProDom, and SMART databases. tRNAscan-SE v1.23[32] was used for tRNA genes identification and the rRNA sequences of *Arabidopsis thaliana* and *Oryza sativa* were BLAST against the *N. yunnanensis* assembly using BLASTN (BLASTN, RRID:SCR_001598) (E-value ≤ 1e-05) for rRNA genes identification respectively. miRNAs and snRNAs were predicted by searching the sequences against the Rfam database[33] using INFERNAL (Infernal, RRID:SCR_011809) [34] software.

Assembly and annotation of the *N. yunnanensis* genome

We assembled the nuclear genome of the highly endangered tree species *N. yunnanensis* with BGISEQ-500 data from a 10X genomics linked-reads library. The final genome assembly was 1.474Gb in length which is close to the estimated genome size of 1.64Gb, with a scaffold N50 of 985.59Kb and a contig N50 of 32.33Kb, respectively. The *N. yunnanensis* genome size we assembled is also close to the estimated genome size of 1.228Gb from the digitization of Ruili Botanical Garden project [35]. 9.76% of the genome regions were presented as Ns (Table 1). The GC content of the *N. yunnanensis* assembly excluding gaps was 42.18%, and a total of 54.24% of the assembly was composed of repetitive elements (Table 2). We ultimately obtained 39,803 protein-coding genes and the functional annotation successfully annotated 96.57% of the *N. yunnanensis* gene loci (Table 3).

Data validation and quality control

The completeness of *N. yunnanensis* assembly was estimated with two strategies. Firstly, we performed the completeness assessment using BUSCO ((BUSCO; v3.0.2, RRID:SCR_015008))[36] with Embryophyta odb10. The result showed that up to 1244 (90.5%) of the expected 1375 conserved plant orthologs were detected as complete in

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the *N. yunnanensis* assembly and 81.9% of them were identified as complete and single-copy genes (Table 4). Secondly, the RNA of the *N. yunnanensis* was extracted and sequenced generating 8.85Gb raw data, which was further filtered using SOAPfilter v2.2 (SOAP, RRID:SCR_000689) with following parameters "-q 33 -i 200 -g 1 -M 2 -Q 20". Then all the clean reads were aligned to the genome assembly using BWA-MEM (BWA, version 0.7.16, RRID:SCR_010910)[37] with default parameters. In total, 98.95% of the reads could be mapped back to the genome assembly and 83.74% of them were properly paired. These results demonstrated the high completeness of the *N. yunnanensis* assembled genome.

Re-use potential

Here, we report a draft genome assembly of the PSESP plant species *N. yunnanensis*. The completeness assessment carried out by reads mapping and BUSCO assessment indicated the high completeness of this draft assembly. Thus, it is useful for future phylogenetics and comparative genomics analyses, such as resolving the controversial phylogenetic relationships within the Nyssa genus, as well as providing valuable genomic data for the 10KP (10,000 Plants) Genome Sequencing Project. In particular, due to the extremely small population structure of *N. yunnanensis,* the genomic resources released in this study will be supportive for further researches on the conservation biology studies of this highly endangered species, as well as other PSESP species.

Abbreviations

BUSCO: Benchmarking Universal Single-Copy Orthologs; HMW: high-molecular-weight; PSESP: Plant species with extremely small populations

Availability of supporting data

The raw sequencing reads are deposited in NCBI under the BioProject accession PRJNA438407, with SRA accession number SRX8345787 and SRX8373586. The raw

reads are also deposited in the CNGB Nucleotide Sequence Archive (CNSA) with accession number CNP0001048. Genome assembly, protein-coding gene and repeat annotations are deposited in the *GigaScience* GigaDB [38].

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Competing interests

The authors declare that they have no competing interests.

Author contributions

H.L, R.D, X.L and X.X conceived and supervised the study; J.W, L.C, J.Y, R.M, J.L and J.Z prepared the samples; W.M, Y.F and T.Y analyzed the results; W.M wrote the manuscript with the inputs from all authors. All authors read and approved the final manuscript.

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Figures

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Figure 1. Photograph of *Nyssa yunnanensis* from Ruili, Yunnan Province, China.

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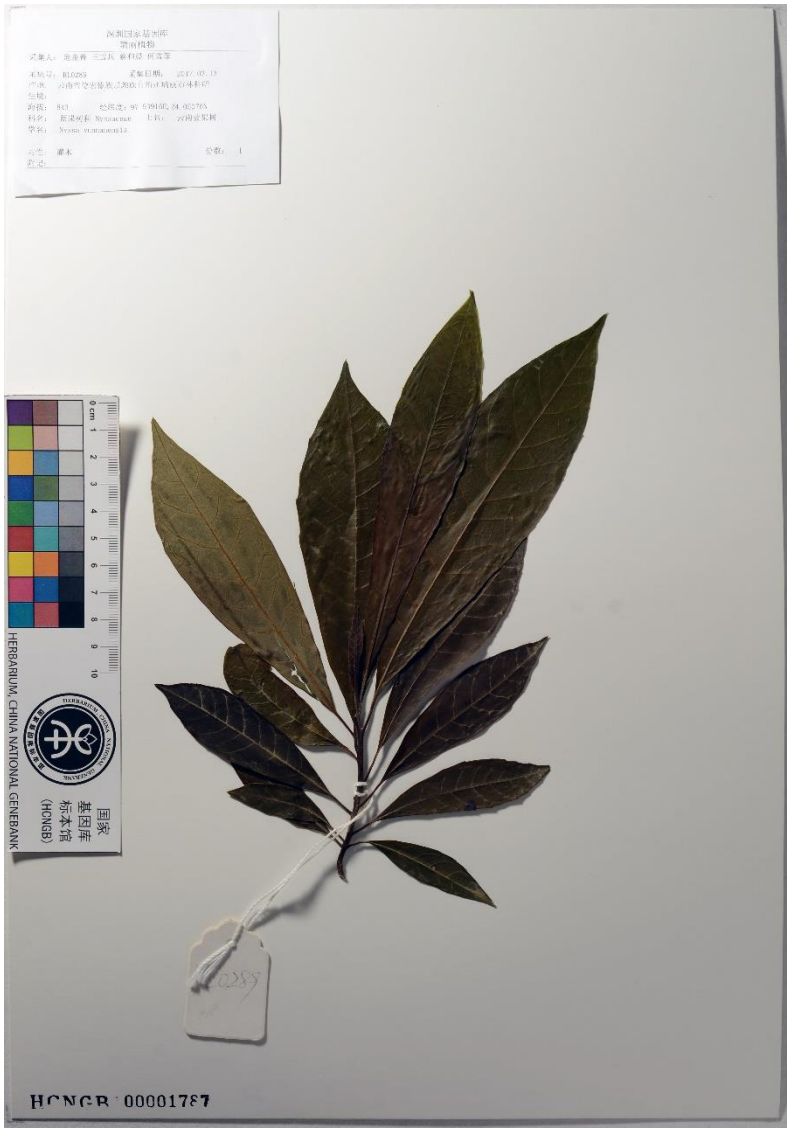

Figure 2. Photograph of the voucher specimens of *Nyssa yunnanensis*, stored in the CNGB herbarium (voucher RL0289)

Tables

Table 1: Statistics of the *N. yunnanensis* genome assembly.

Parameters	Scaffold		Contig	
	Length (bp)	Number	Length (bp)	Number
Maximal length (bp)	17,928,324		636,521	
N90	3,636	38,756	2,907	66,981
N80	8,907	13,172	6,794	37,587
N70	27,765	2,691	12,646	22,959
N60	313,394	525	21,403	14,902
N50	985,593	280	32,349	9,848

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N40	1,699,409	166	45,744	6,367
N30	2,587,311	96	62,631	3,874
N20	3,773,602	49	86,839	2,056
N10	6,183,248	17	127,405	771
Total length (bp)	1,474,110,738		1,330,612,708	
number>=100bp		288,486		320,761
number>=2000bp		57680		79,893
Percentage of N content		9.73%		

Table 2. Statistics of repetitive sequences identified in the *N. yunnanensis* genome.

Category	Total repeat length (bp)	% of assembly
DNA	133,291,367	9.04%
LINE	47,813,283	3.24%
SINE	1,053,993	0.07%
LTR	690,959,337	46.87%
Tandem repeats	6,108	0.0004%
Unknown	135	0.000009%
Combined	799,507,629	54.24%

Note: DNA: DNA transposon; LINE: long interspersed nuclear element; SINE: short interspersed nuclear elements; LTR: long terminal repeat.

Table 3. Summary of protein-coding genes annotated in the *N. yunnanensis* genome.

Characteristics of protein-coding genes	
Total number of protein-coding genes	39,803
Mean gene size (bp)	2576.05
Mean CDS length (bp)	957.64
Mean exon number per gene	4.16
Mean exon length (bp)	230.26
Mean intron length (bp)	512.32
Functional annotation by searching public databases	
% of proteins with hits in Swiss-Prot database	76.01
% of proteins with hits in NCBI nr database	96.30
% of proteins with hits in KEGG database	72.15
% of proteins with hits in TrEMBL database	95.90
% of proteins with hits in Interpro database	70.99
% of proteins with functional annotation (combined)	96.57

Table 4: BUSCO assessment of *N. yunnanensis* genome.

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BUSCO benchmark	Number of genes	Percentage
Complete BUSCOs	1244	90.5
Complete and single-copy BUSCOs	1126	81.9
Complete and duplicated BUSCOs	118	8.6
Fragmented BUSCOs	63	4.6
Missing BUSCOs	68	4.9
Total	1375	/