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A high-quality genome assembly of the endangered golden snub-nosed monkey (Rhinopithecus roxellana)

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Abstract:	Background: The golden snub-nosed monkey (Rhinopithecus roxellana), is an endangered colobine monkey species endemic to China. This species has several distinctive traits, and it is an ideal model for analysing evolutionary development of the social structure due to its unique social organization. Although there has been reported a genome assembly of the subspecies R. roxellana hubeiensis, the assembly is incomplete and fragmented due to employing short reads sequencing technology. This drawback may lose information, such as structural variation and repeat sequences which are important for understanding this endangered species. Therefore, to have a better understanding of evolutionary history and genetic-specific signatures, a high-quality reference genome of the taxon is need. Findings: To obtain a high-quality chromosome assembly of R. roxellana qinlingensis, we combined a total of five techniques including Pacific Bioscience's single-molecule real-time sequencing, Illumina's paired-end sequencing, BioNano optical maps, 10X Genomics link-reads and high-throughput chromosome conformation capture. The results indicate the assembled genome is about 3.04 Gb with a contig N50 of 5.72 Mbp and a scaffold N50 of 144.56 Mbp, which have made a 10-fold improvement compared to past published. It is shown that a total of 22497 protein coding genes were predicted, of which 22053 were functionally annotated. Moreover, gene family analysis shows that 993 and 2745 gene families are expanded and contracted in the R. roxellana genome, respectively. Conclusion: We present the updated high-quality genome assembly of R. roxellana with superior continuity and accuracy. The assembled genome can be used as reference for future genetic studies of the species. Also, the updated genome assembly may contribute to our comprehensive understanding of the species, which is particularly helpful in the conservation of this endangered species. Furthermore, such genome with superior continuity and accuracy can provide a new standard reference for Colobine primates.
Corresponding Author:	Xiao-Guang Qi CHINA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Xiao-Guang Qi
First Author Secondary Information:	
Order of Authors:	Xiao-Guang Qi Lu Wang Jinwei Wu Xiaomei Liu

	Dandan Di
	Yuhong Liang
	Yifei Feng
	Baoguo Li
Order of Authors Secondary Information:	
Additional Information:	
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A high-quality genome assembly of the endangered golden snub-nosed monkey (*Rhinopithecus roxellana*)

Lu Wang[1,†], Jinwei Wu[1,†], Xiaomei Liu[1], Dandan Di[1], Yuhong Liang[1], Yifei Feng[1], Baoguo Li[1,2], Xiao-Guang Qi[1,*]

[1] Shaanxi Key Laboratory for Animal Conservation, College of Life Sciences, Northwest University, Xi'an, 710069, China.

[2] Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, 650223, China.

[*]**Correspondence address.** Xiao-Guang Qi, E-mail: qixg@nwu.edu.cn

[†]These authors contributed equally to this work.

ABSTRACT

Background: The golden snub-nosed monkey (*Rhinopithecus roxellana*), is an endangered colobine monkey species endemic to China. This species has several distinctive traits, and it is an ideal model for analysing evolutionary development of the social structure due to its unique social organization. Although there has been reported a genome assembly of the subspecies *R. roxellana hubeiensis*, the assembly is incomplete and fragmented due to employing short reads sequencing technology. This drawback may lose information, such as structural variation and repeat sequences which are important for understanding this endangered species. Therefore, to have a better understanding of evolutionary history and genetic-specific signatures, a high-quality reference genome of the taxon is need.

Findings: To obtain a high-quality chromosome assembly of *R. roxellana qinlingensis*, we combined a total of five techniques including Pacific Bioscience's single-molecule real-time sequencing, Illumina's paired-end sequencing, BioNano optical maps, 10X Genomics link-reads and high-throughput chromosome conformation capture. The results indicate the assembled genome is about 3.04 Gb with a contig N50 of 5.72 Mbp and a scaffold N50 of 144.56 Mbp, which have made a 10-fold improvement compared to past published. It is shown that a total of 22497 protein coding genes were predicted, of which 22053 were functionally

annotated. Moreover, gene family analysis shows that 993 and 2745 gene families are expanded and contracted in the *R. roxellana* genome, respectively.

Conclusion: We present the updated high-quality genome assembly of *R. roxellana* with superior continuity and accuracy. The assembled genome can be used as reference for future genetic studies of the species. Also, the updated genome assembly may contribute to our comprehensive understanding of the species, which is particularly helpful in the conservation of this endangered species. Furthermore, such genome with superior continuity and accuracy can provide a new standard reference for Colobine primates.

Keywords: high-quality; *Rhinopithecus roxellana*; genome assembly; annotation; BioNano optical maps

Data Description

Background information

Snub-nosed monkeys (*Rhinopithecus*) consist of five endangered species narrowly restricted to China and Vietnam [1]. Among those, the golden snub-nosed monkey (*Rhinopithecus roxellana*) is also referred to as the Sichuan snub-nosed monkey, with the northernmost distribution of all Asian colobine species, found only in three isolated regions (Sichuan and Gansu, Shaanxi and Hubei provinces) in central and northwest China [2, 3]. This species is characterized by several distinctive traits, such as golden fur, blue facial colour, odd-shaped nose, more folivorous, most striking unique social system with multilevel societies, a rare and complex system that is found only in a few mammal species, including human beings [4]. Therefore, *R. roxellana* is an ideal model for the studies analysing evolutionary development of the social structure in primates and understanding the behaviour patterns of human society in social-anthropology.

As a research hotspot, studies on *R. roxellana* have investigated various aspects [4-6]. Recently, genomic analysis offered a powerful tool and has successfully been employed to underlie the molecular evolution of several groups [7-9]. According to the morphological variation and distribution difference, *R. roxellana* can differentiate into three subspecies: *Rhinopithecus roxellana roxellana* from Minshan mountains of Sichuan and Gansu province,

R. r. Qinlingensis from Qinling mountains of Shaanxi province, *R. r. hubeiensis* from shennongjia mountains [3]. Up to now, the best genome assembly of *R. roxellana* was published in 2014 [10], which was derived from short reads sequencing on Illumina HiSeq 2000 platform. Based on this achievement, studies on its folivorous dietary adaptations and the evolutionary history of *R. roxellana* have been conducted [10-12]. Despite such progress, the information including structural variation and repeat sequences was largely absent or unreliable due to the incomplete and fragmented genome assembly [13, 14].

Owing to the advances in sequencing technology, it is possible to obtain high-quality genome assembly that can provide new insights into the understanding of the organisms. Indeed, transposable elements and lineage-specific genes have never been reported to be identified by using the new improved maize reference genome [15]. By combining new sequencing approaches, Seo et al. [13] discovered clinically relevant structural variants and genes never reported before in updated human genome. New sequencing technologies have also been widely used in Gorillas [16] and Sumatran orangutan [17] that have the closet genetic relationship with humans, and domestic goat [18]. More importantly, a lot of new findings have been reported based on these updated genome assembly. However, a high-quality genome assembly of *R. roxellana* has not been reported yet, lagging the progress of understanding this endangered species.

Here, we report a greatly improved assembly and annotation of the reference genome for *R. roxellana* from through combined five technologies: Pacific Bioscience's single-molecule real-time sequencing (SMRT), Illumina's HiSeq paired-end sequencing (HiSeq), BioNano optical maps (BioNano), 10X Genomics link-reads (10X Genomics) and high-throughput chromosome conformation capture (Hi-C). Also, this is the first Colobine genome sequenced and assembled with both long reads and short reads. The updated genome assembly may allow us to comprehensively understand *R. roxellana*, offering new opportunities in analysing evolutionary history and genetic-specific signatures for this species, which may provide new insights in the conservation of this endangered primate. In addition, this genome with superior continuity and accuracy will provide a new standard reference for Colobine primates.

Sample collection and sequencing

An adult dead male *R. roxellana qinlingensis* used for sequencing was from Louguantai Breeding Centre, Xi'an, Shaanxi province, China, originally from Qinling Mountains. Total genomic DNA was extracted from heart tissue. To acquire a high-quality genome assembly, we applied a combined five sequencing methods. Initially, PacBio's SMRT sequencing was conducted on the SEQUEL platform according to manufactures, after removing adaptors in polymerase reads, resulting a total of 304.84 Gb clean long reads (95.86X coverage). Different

from PacBio sequencing, paired-end sequencing was performed using an Illumina NovaSeq 6000 platform with an insert size of 350 bp. Short reads derived from this step were filtered by SOAPfilter v. 2.2 [19] (a package from SOAPdenovo2) with the following criteria: filtering those reads with adapters, contaminations, N bases more than 10% and low quality, which generated 423.32 Gb sequencing clean reads (133.12X coverage). In addition, a high-quality optical genome map was constructed with Irys platform (BioNano Genomics), from which we acquired 463.75 Gb clean reads (145.83X coverage). Besides, 10X genomic link-reads sequencing was carried out on Illumina Hiseq Xten platform, and 348.41 Gb clean reads (109.56X coverage) were generated in total. Finally, a Hi-C library was prepared and sequenced with an Illumina NovaSeq 6000 platform for chromosome-scale scaffolding of genome assembly. Adapter sequences and low quality reads were discarded by using Cutadapter v1.0 [20], yielding a total of 310.92 Gb clean data (97.77X coverage). Statistics of the sequencing data was detailed in **Table 1**.

***De novo* assembly of the *R. roxellana* genome**

Estimation of genome size is helpful to our understanding of *R. roxellana*. Generally, we estimated the genome size of *R. roxellana* with the formula of $G = (K_{total} - K_{error})/D$, in which G represents genome size, while K_{total}, K_{error} and D indicates the total number of k-mers, number of k-mers which caused by sequencing errors and k-mer depth respectively. Finally, 109,210,004,556 k-mers were generated, and the peak k-mer depth was 34. Thus, the genome

size of *R. roxellana* was estimated to be about 3.18 Gb. The distribution of k-mer frequency was shown in **Supplementary Fig. S1**.

The *de novo* assembly of newly sequenced *R. roxellana* genome was performed in four progressive steps. Firstly, the assembly was conducted with the FALCON assembler (default parameters) [21] with the long reads obtained from the PacBio platform, which mainly includes three steps: 1) detection of overlap and reads correction; 2) detection of overlap between corrected reads; and 3) construction of string graph. Following FALCON step, the string graph assembly was further polished by Quier with long reads [22] and then corrected by Pilon with Illumina short reads [23]. Based on this initial genome assembly, sspace-longreads [19] with default settings was implemented for getting a longer scaffold genome by using PacBio long reads. Despite attempts have been made, scaffolding gaps were still found, those gaps were further closed with the help of PBjelly software under default settings, which generated a phased genome assembly with scaffold N50 of 8.20 Mbp (**Supplementary Table S1**).

Secondly, a hybrid assembly with scaffold N50 of 9.22 Mbp was constructed on the basis of Bionano optical map data using Bionano Solve3.1 (www.bionanogenomics.com) with default parameters (**Supplementary Table S2**). Thirdly, 10X genomic linked reads were employed to connect scaffolds from the second step by fragScaff software [24], which has updated the scaffold N50 of genome assembly to 24.09 Mbp (**Supplementary Table S3**).

Subsequently , those short-reads derived from Illumina were applied to correcting errors due to Burrows-Wheeler Aligner (BWA) [25] and pilon-1.18 [23].

Finally, to build chromosome-level assembly scaffolds, we mapped the Hi-C reads to the assembled scaffolds with BWA [25]. Then Hi-C data was subsequently applied to cluster, order, and orient scaffolds by Lachesis software [26]. The chromosome-level scaffolds for *R. roxellana* allowed us to estimate the interaction frequency between chromosome loci, the interaction heatmap shown in **Fig. 2**.

These processes together yielded a updated genome assembly of *R. roxellana* with its genome size of 3.04 Gb, contig N50 of 5.72 Mbp and scaffold N50 of 144.56 Mbp (**Table 2**). In comparison, the newly acquired *R. roxellana* reference genome has 100-fold higher contiguity than its previous (contig N50: 5.72 Mb versus 25.5 kb and scaffold N50: 144.56 Mb versus 1.55 Mb) [10]. We suppose that the remarkable improvement in contiguity can be attributed to the longer read length, deeper sequencing depth, properly assembled gaps, and increased sophisticated assembly algorithm.

To assess the genome assembly accuracy, we aligned the Illumina short reads to the assembly by BWA program [25]. With a ratio number of 99.17%, mapped read covered approximately 99.27% of the assembly (**Supplementary Table S4**). In addition, we estimated the assembly completeness by conducting Benchmarking Universal Single-copy Orthologs (BUSCO) analysis with BUSCO V3.0 [27]. Among the 4,104 mammalian BUSCOs, 94% was

detected in the genome assembly (**Supplementary Table S5**). The assembly completeness was also checked by core eukaryotic gene-mapping approach (CEGMA) [28]. The results showed that 93.95% (233 of 248) conserved genes were found in our genome assembly (**Supplementary Table S6**). Together, these analyses indicated a high accuracy and completeness of our genome assembly.

Identification of repeat elements

Repeat sequences occupy a large proportion of the genome sequences. Thus, it is necessary for us to identified those repeat elements. In our study, we combined homolog based and *de novo* based approach to predict and classify repeat elements. As for the homolog approach, we searched transposable elements from the RepBase database [29] with RepeatMasker v4.0.6 (http://www.repeatmasker.org/) and RepeatProteinMask (implemented in RepeatMasker). The *de novo* method was employed with RepeatModeler V1.0.11 [30], RepeatMasker v4.0.6 and Tandem Repeat Finder (TRF) (Version 4.07b) [31]. We merged the findings from both methods. Results showed that 45.43% of the genome was predicted as repeat elements (**Supplementary Table S7)**. A closer investigation indicated that the largest category of repeat elements in the species is the short (SINEs) and long (LINEs) interspersed nuclear elements. The detailed categories of repeat elements are summarized in **Supplementary Table S8**.

Non-coding RNA prediction

Non-coding RNA consists of several RNAs, as such ribosomal RNA (rRNA), transfer RNA (tRNA), microRNAs (miRNA) and small nuclear RNA (snRNA). This RNA group mainly plays a regulation role in biological processes. In our study, we detected rRNA from a Human rRNA database with BLASTN command, and the E-value was set as 1E-10. Similarly, miRNAs and snRNAs were searched against the Rfam database [32] with INFERNAL 1.1rc4 [33]. The tRNAs were predicted by tRNAscan-SE 1.3.1 software [34]. The numbers of rRNA, miRNA, snRNA and tRNA were 608, 17,813, 3,656 and 460, respectively in the genome of the species (**Supplementary Table S9**).

Gene prediction and functional annotation

We combined prediction methods based on *de novo*, homolog prediction and transcriptome data to estimate genes. As for *ab initio* based prediction, a total of five programs, namely Augustus v. 3.2.2 [35], GlimmeHMM v. 3.0.1 [36], GENSCAN [37], GENEID [38] and SNAP V2013-11-29 [39] were employed to predict protein-coding genes. Subsequently, we used the homolog-based prediction approach. Protein sequences from five homolog species (*Homo sapiens, Gorilla gorilla, Macaca mulatta, Rhinopithecus bieti, Rhinopithecus roxellana hubeiensis*) were downloaded from Ensemble Release 75

(http://www.ensembl.org/info/data/ftp/index.html), and used to perform TBLASTN blast against the repeat-masked genome sequences [40]. The related homologous genome sequences were then annotated to the matching proteins by GeneWise 2.4.1 [41]. Finally, we estimated genes based on transcriptome data. During this process, high-quality RNAs from heart and skin tissue were sequenced by an Illumina Novaseq 6000 platform. RNA-seq reads were assembled with trinityrnaseq-2.1.1 [42]. The assembled transcript sequences were aligned to the *R. roxellana* genome by Assemble Spliced Alignment (PASA) [43] with default parameters. In addition, we estimated the expression levels of transcripts by Tophat 2.0.13 [44] and Cufflinks [45].

The genes predicted from those three approaches were merged with EVidenceModeler [46]. Furthermore, untranslated regions and alternative splicing of those predicted gene sets were further checked by PASA with the help of transcriptome data [43]. Finally, a total of 22497 genes were predicted for the assembly genome of *R. roxellana* (**Table 3**), and each of them consisted of 7.71 exons on average. The detailed results generated during the gene prediction process were shown in **Table 3.** And, the gene prediction evidence based on different methods were shown in **Fig. 3**. In addition, we made a comparison between the *R. roxellana qinlingensis* and other mammals, suggesting a comparable pattern of the genome assembly for *R. roxellana qinlingensis* **(Supplementary Fig. S2)**.

To have a better understanding the biological functions of those predicted genes, they were annotated with several databases including NCBI nonredundant protein database (NR), SwissProt [47], Kyoto Encyclopedia of Genes and Genomes (KEGG) [48], InterPro [49], Pfam [50] and GO database [51]. In total, 22053 genes (98.42%) were functionally annotated (**Supplementary Table S10**).

Phylogenetic relationship analysis and gene family estimation

Coding regions and protein sequences of 11 representative mammals were downloaded from Ensemble (Ensemble Release 75). The longest transcript was chosen if genes possess many transcript isoforms. Treefam [52] approach was adopted to estimate gene families. Following all-to-all blast, a total of 17,560 gene families were identified. We reconstructed the phylogenetic relationship between *R. roxellana* and other mammals based on four-fold degenerate sites extracted from the 5,418 single-copy gene families. Phyml (version 3.2) [53] was employed to construct a maximum-likelihood tree under the GTR + gamma model that was inferred from JMODELTEST (version 2.1.10) [54]. Furthermore, we estimated the divergence time with MCMCTREE in PAML [55]. MCMCTREE was performed on the basis of bayesian method and the fossil calibration times from timetree were used as input. The reconstructed phylogeny confirmed the close relationship between *R. rollexana* and *M. mulatta*.

Moreover, we estimated that *R. rollexana* and *M. mulatta* diverged approximately 13.4 million years ago (Mya) (**Fig. 4**).

To have a better understanding the evolutionary history of *R. roxellana*, we estimated the expansion and contraction of gene family in *R. roxellana* by using CAFE 3.0 [56]. A gene family with *p*-value less than 0.05 was considered for further analysis. As a result, 993 and 2,745 gene families were expanded and contracted in *R. roxellana* genome, respectively (**Fig. 4**). Its genome showed substantial expansion of gene families which are mainly related to hemoglobin complex, energy metabolisms and oxygen transport (**Supplementary Table S11**).

Conclusion

In this study, we generated a high-quality genome assembly of the golden snub-nosed monkey (*R. roxellana*) by using five advanced technologies. This will be helpful to investigate the origin and evolutionary history of snub-nosed monkey. In addition, the genome may lay a foundation to survey the mechanisms about the formation of distinct characters and understand the unique multilevel societies in *R. roxellana*. Also, such genome may provide new insights for amending the conservation strategies and management of this endangered species. Furthermore, this genome with superior continuity and accuracy can provide a new standard reference for Colobine primates.

Declarations

Availability of supporting data

The raw data discussed in this publication have been deposited in NCBI's short read archive under the accession number PRJNA524949. Supporting data are available in the GigaDB database.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

Gb: gigabase; kb: kilobase; Mb: megabase; PE: paired-end; PacBio: Pacfic Biosciences; SMRT: single molecule real-time sequencing; Hi-C: high-throughput chromosome conformation capture; BUSCO: Benchmarking Universal Single-copy Orthologs; GEGMA: core eukaryotic gene-mapping approach; GO: gene ontology; TFS: transposable element; TRF: Tandem Repeat Finder; SINEs: Short interspersed nuclear elements; LINEs: long

interspersed nuclear elements; PASA: genome by Assemble Spliced Alignment; NR: NCBI nonredundant protein database; KEGG: Kyoto Encyclopedia of Genes and Genomes. Mya: million years ago.

Author contributions

X.G.Q. conceived and designed the project, L.W., J.W. contributed to the work on genomic sequencing and performing data analyses. B.G.L. helped with sample collection. L.W., J.W. and X.G.Q. wrote the manuscript. All authors provided input for the paper and approved the final version.

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Figures and tables

Figure legends:

Fig. 1. The photo of *R. roxellana* taken in the Qinling mountains.

Fig. 2. Hi-C heatmap between chromosome loci throughout the genome. Hi- C interactome within and among chromosomes of *R. roxellana* (Chr1–Chr22).

Fig. 3. The gene prediction evidence based on different methods. (a). Number of the genes estimated by the prediction approaches based on de novo (blue color), homolog prediction (pink color) and RNA_seq data (green color). The rna_0.5, denove_0.5 and homolog_0.5 indicates those genes predicted with an overlap are larger than 50% in each method; **(**b) Number of the genes shown in combination with the prediction approaches detailed in fig 2a and the expression level standard (rpkm). The rna_0.5, denovo_0.5, homolog_0.5 indicates that those genes predicted with an overlap are larger than 50% in each method, while rpkm>1 indicates those genes with an expression level larger than 1.

Fig. 4. The phylogenetic relationships of *R. roxellana* and other mammals and Gene family analysis in *R. roxellana* genome. Phylogenetic relationship was inferred from 5418 single-copy gene families. All nodes received 100% support values. The estimated divergence times are indicated near the nodes. The images in the figure are credited as “Illustrations

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MYA: million years ago. The numbers on each branch correspond to the numbers of gene families that have expanded (red) and contracted (green) in mammalian genome. MRCA: most recent common ancestor.

Table 1. Reads generated from five different sequencing methods

Pair-end libraries	Insert size (bp)	Total clean data (Gb)	Read length (bp)	Sequence coverage (X)
Illumina reads	350	423.32	150	133.12
Pacbio reads	20 k	304.84	\	95.86
10X Genomics	500 -700	348.41	150	109.56
Bionano	\	463.75	\	145.83
Hi-C	350	310.92	\	97.77
Total	\	1,851.24	\	582.15

Note: The sequence coverage was calculated with an estimated genome size of 3.18 Gb. The sign of backslash indicates that the insert size was absent.

Table 2. The final genome assembly statistics of *R. roxellana*

Sample ID	length		number	
	Contig[a] (bp)	Scaffold (bp)	Contig[b]	Scaffold
Total	3,038,184,325	3,038,467,325	6,099	3,269
Max	30,757,641	206,558,726	\	\
Number >= 2000	\	\	5,708	2,879
N50	5,723,610	144,559,847	151	9
N60	4,241,389	141,075,955	211	11
N70	3,173,235	135,203,321	292	14
N80	2,063,823	118,350,466	408	16
N90	896,517	83,045,532	622	19

Note: [a] Contig after scaffolding. The sign of backslash indicates that the length/number was absent.

Table 3. Summary of predicted protein-coding genes and their characteristics

Gene set		Number	Average transcript length (bp)	Average CDS length (bp)	Average intron length (bp)	Average exon length (bp)	Average exons per gene
De novo	Augustus	32,928	23,441	1,052	196	5,112	5.38
	GlimmerHMM	618,957	4,204	404	166	2,654	2.43
	SNAP	97,298	49,851	755	144	1,1597	5.23
	Geneid	36,863	35,242	1,035	188	7,615	5.49
	Genscan	50,419	40,635	1,137	167	6,800	6.81
Homolog	Ggo	25,281	19,893	1,055	184	3,971	5.74
	Hsa	38,444	14,763	826	182	3,942	4.54
	Mmu	21,959	29,709	1,470	187	4,123	7.85
	Rbi	25,320	25,685	1,387	196	3,991	7.09
	Rro	24,121	28,439	1,420	185	4,043	7.68
RNASeq	PASA	66,620	28,449	1,219	164	4,247	7.41
	Cufflinks	73,199	31,497	2,737	409	5,052	6.69
EVM		30,102	22,298	1,098	182	4,199	6.05
Pasa-update*		29,403	27,638	1,180	181	4,782	6.53
Final set*		22,497	34,153	1,369	178	4885	7.71

Note: Pasa-update* indicates only the UTRs（Untranslated regions）were considered during the filter process, and other regions were not included. Final set* indicates the results were acquired following the Pasa-update process, with the criteria of the longest isoform was chosen if there were multiple splicing isoforms, and the redundant single exons were also discarded.

Supplementary files:

Supplementary Fig. S1. Genome size estimation using k-mer method

Supplementary Fig. S2. The comparison of each element in the genome of homologous species

Supplementary Table S1. The results of assembly with PacBio long reads and gap filling

Supplementary Table S2. The results of assembly with Bionano optical map data

Supplementary Table S3. The results of assembly with 10X Genomics link reads

Supplementary Table S4. The mapping rate of reads and coverage of assembled genome with BWA

Supplementary Table S5. Assessment results by using BUSCO annotation

Supplementary Table S6. The completeness test results of assembled genome with CEGMA software

Supplementary Table S7. Results of repeats elements predictions from the genome assembly

Supplementary Table S8. The results of TEs elements predicted from the genome assembly

Supplementary Table S9. Summary of predicted RNAs and their characteristics

Supplementary Table S10. The functional annotation of the genes predicted from *R. roxellana* genome

Supplementary Table S11. The GO annotation results of expansion gene families in *R. roxellana* genome

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Fig 1

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Fig 2

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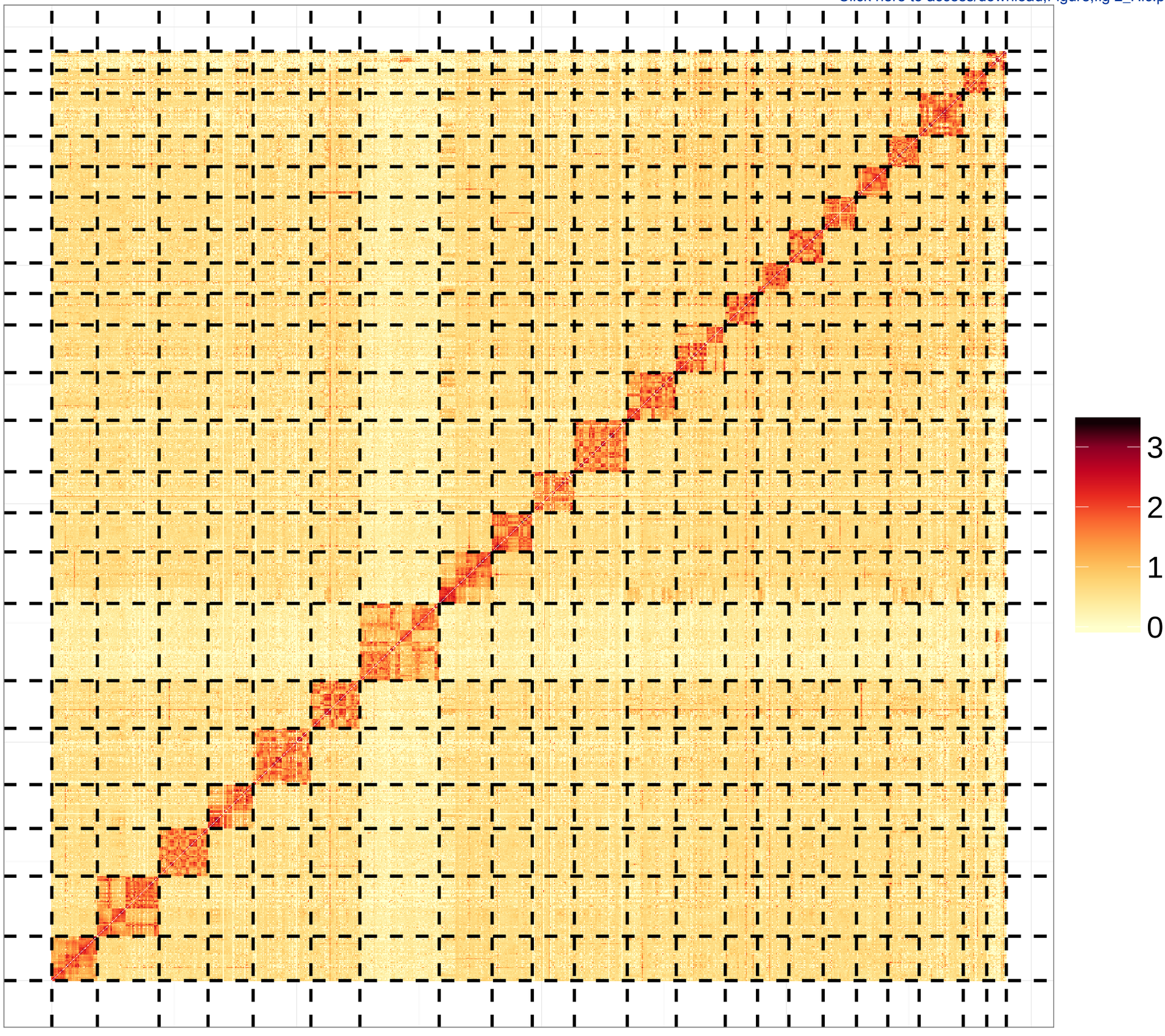

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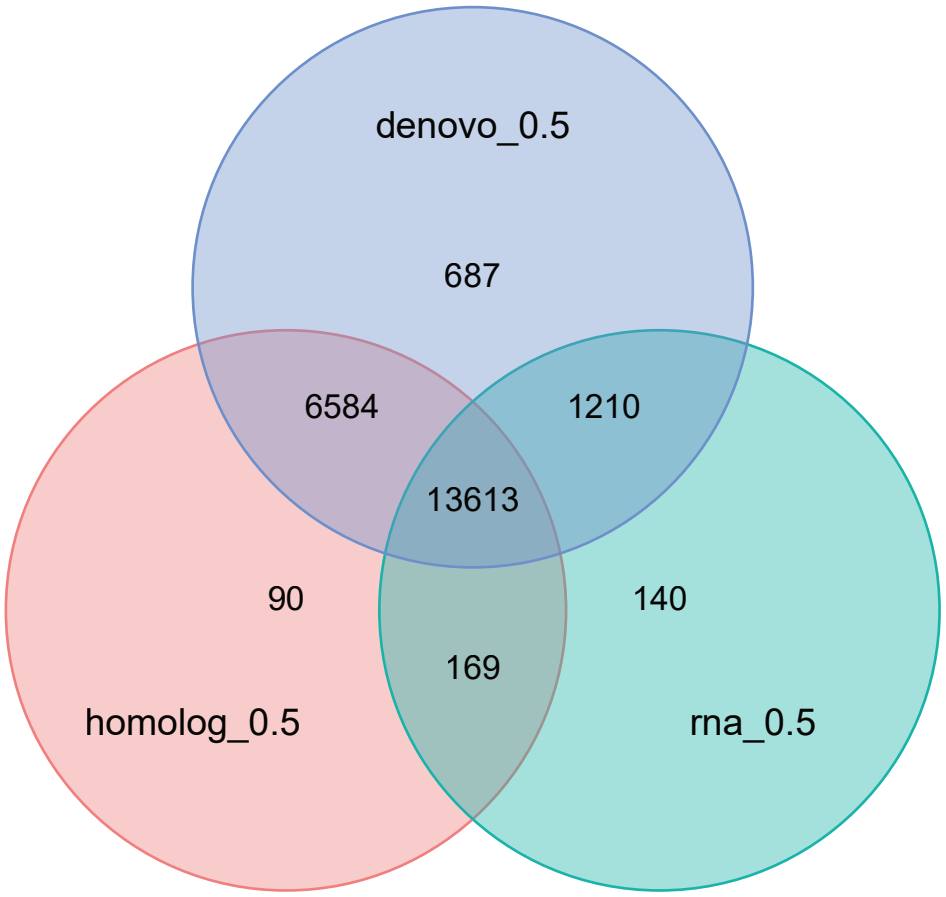

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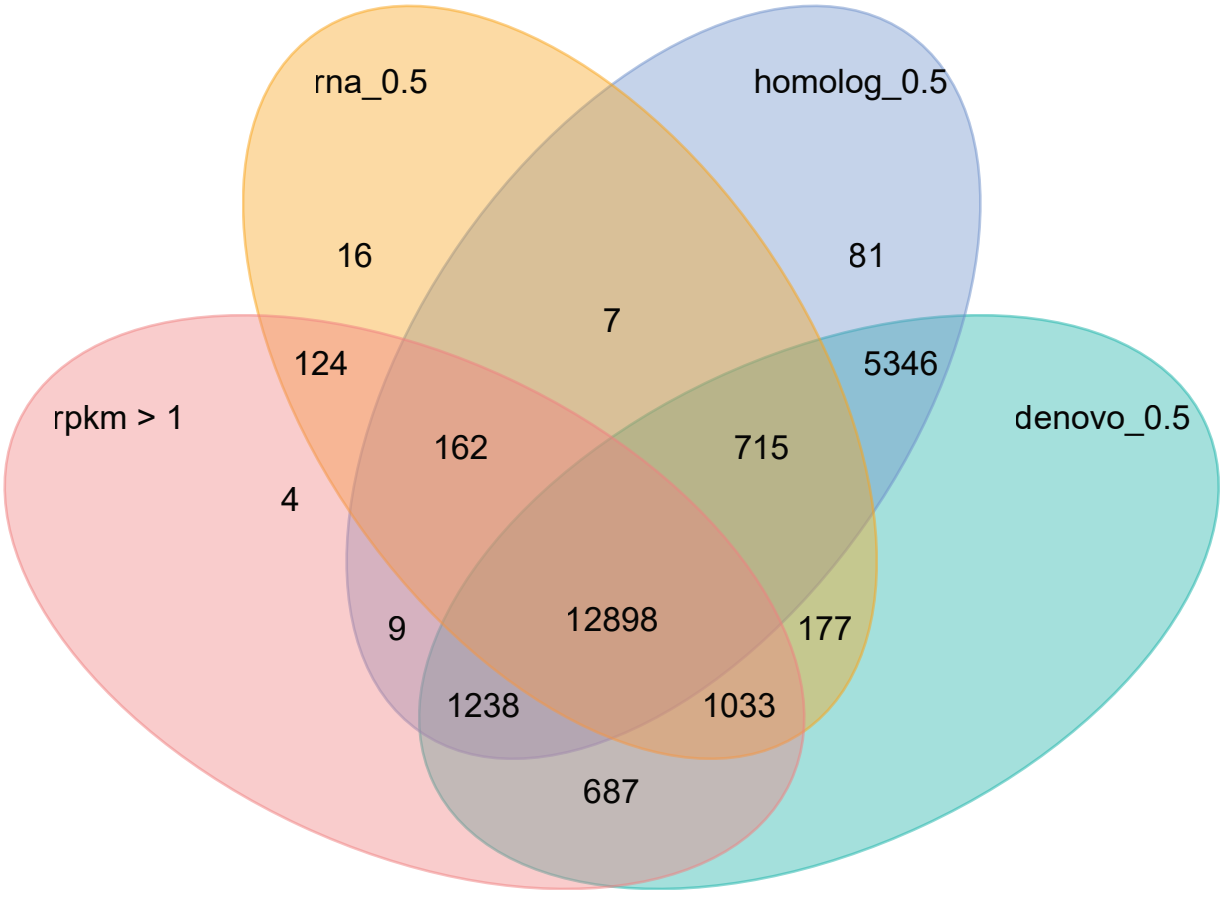

Fig 4

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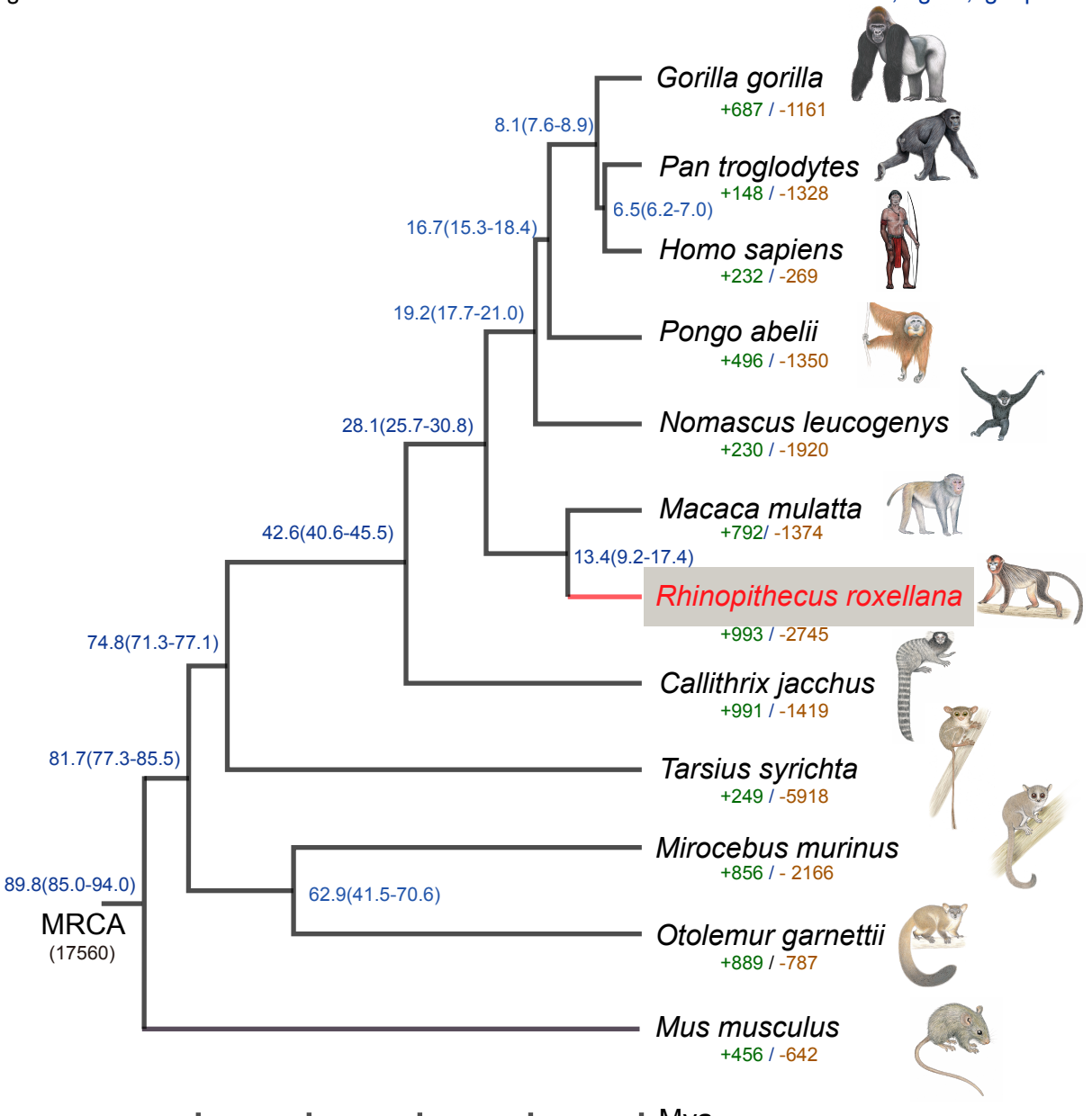

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Supplementary Material

SI_V1.docx

March 12, 2019

Dear *GigaScience* editor,

Thanks a lot for having reviewed our manuscript titled **"A high-quality genome assembly of the endangered golden snub-nosed monkey *Rhinopithecus roxellana*"** for consideration of publication in *GigaScience*. Now we have revised the manuscript according to the reviewer's comments. Most explanations regarding the revisions of our manuscript are as follows.

1 The raw data discussed in this publication have been deposited in NCBI's short read archive under the accession number PRJNA524949. Supporting data are available in the GigaDB database.

2 We have attached a photo of *Rhinopithecus roxellana* taken in the Qinling mountains in the attached file "Figures" (fig 1).

3 We have improved the language by a native speaker.

4 We have updated the abstract, introduction and conclusion.

4 We have improved the introduction to highlight the reason why we sequence the species (On Page 6, Line 84-89).

5 We demonstrated our genome improvement by comparing with previously reported (On Page 9, Line 143-149).

As a result, we believe that our work will be of general interest to a broad scientific audience including evolutionary biologists, phylogeneticists, geneticists, zoologists, ecologists, and popular media. Thank you for your time and consideration.

Yours sincerely,

Xiao-Guang Qi
Shaanxi Key Laboratory for Animal Conservation
College of Life Sciences
Northwest University
Email: qixg@nwu.edu.cn