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The Genome Assembly and Annotation of the Oriental Ratsnake *Ptyas mucosa*

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Abstract: The Oriental ratsnake *Ptyas mucosa* is a common non-venomous snake of the colubrid family, with a wide geographic range spanning much of South and Southeast Asia. *P. mucosa* is widely cultivated dut to it used in traditional medicine, scientific research, and handicrafts. Therefore, genome resources could play an important role in the efficacy of traditional medicine and the analysis of the living environment of the species. We collected a snake sample in Hezhou, Guangxi, China, which was identified as *P. mucosa* by morphological identification. Here we present a highly continuous *P. mucosa* genome with a genome size of 1.74Gb. The scaffold N50 length is 9.57Mb and the maximal length of scaffold is 78.3Mb, the *P. mucosa* genome has a CG content of 37.9% and the integrity of the gene reached 86.6%.Assembled using long-reads, the total length of the repeat sequence in the genome reached 735 Mb, and its repeat content was as high as 42.19%. A total of 24,869 functional genes were annotated. This study will assist in the understanding of the *P. mucosa*, and also provide a basis for medicinal research.

Keywords: genetics and genomics; evolutionary biology; zoology

Introduction

Known as the Oriental ratsnake (Figure 1)[1], Indian ratsnake or Dhaman; *Ptyas mucosa* is a common non-venomous species of colubrid snakes. There are over 300 genera and 2000 species in the colubrid family, making it the largest snake family [2]. The ratsnake, is an excitable and fast-moving snake, but it is harmless to humans, preying upon small reptiles, birds, and mammals. Therefore, in some areas, farmers will obtain and move the Oriental ratsnake from other locations to catch mice and protect their crops. Adult snakes usually prefer to subdue their prey by sitting on it instead of constricting, using their weight to suppress the prey, which is a hunting mechanism of hunting uncommon in other snake species [3]. When they are threatened, they will inflate their necks, which can be used to imitate the king cobra or Indian cobra to scare off potential predators [4].

In southern China, the Indian rat snake is commonly eaten by humans, and its skin is used for making the membranes of a traditional musical instrument, the erhu [5]. In traditional Chinese medicine, its gallbladder is used make a medicinal wine to treat many diseases [6]. In the past, due to over hunting, its numbers were greatly reduced, but with the success of artificial breeding, their numbers have gradually recovered [6].

In this study, we present a highly continuous *P. mucosa* genome with a genome size of 1.74Gb by using single-tube long fragment reads (stLFR) sequencing data and combined with whole genome sequencing data for correction. Its repeat content reached 42.19%. These provide an important basis for follow-up studies elucidating the biology *of P. mucosa.* Taking this further high-quality reference genome and transcriptome data can provide effective help for subsequent targeted breeding.

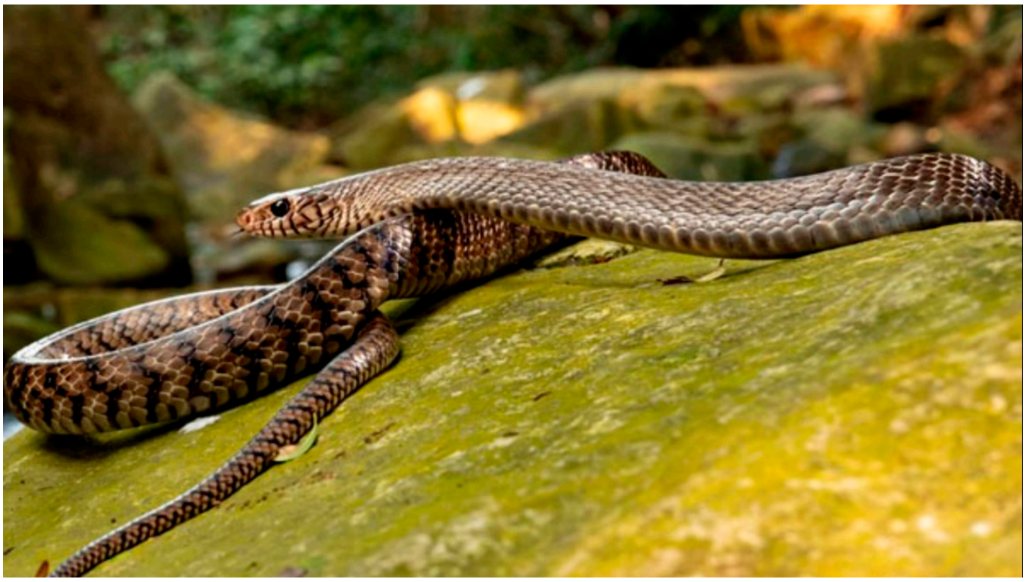


Figure 1. A *P.mucosa* individual Adam Francis.

Main content

Context

In this study we present a highly-continuous genome assembly of *P. mucosa*, finding the maximum genome size of *P. mucosa* is 1.74Gb. The scaffold N50 length is 9.57Mb and the maximal length of scaffold is 78.3Mb (Table 1). Furthermore, the *P. mucosa* genome has a CG content of 37.9% and using BUSCO (Figure 2) to measure its integrity reached 86.6%. Thus we can see from these genome assembly data that this reference is a highly contiguous genome. Here we report the draft reference genome sequence of *P. mucosa*. This data will provide a valuable resource in the study of nonpoisonous snakes.

Table 1. Summary of the features of the *P.mucosa* genome.

	contig	**Scaffold**
Maximal length (bp)	317010	78354666
N90 (bp)	4639	10835
N50 (bp)	23622	9579637
number>=100bp	189926	87170
number>=2kb	110746	35256
GC content (%)	40.3	37.9
Genome size (bp)	1743610025	

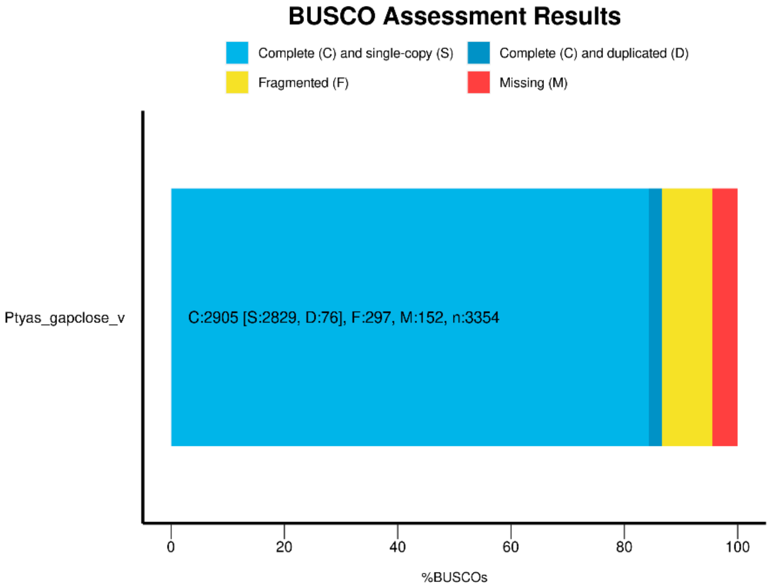
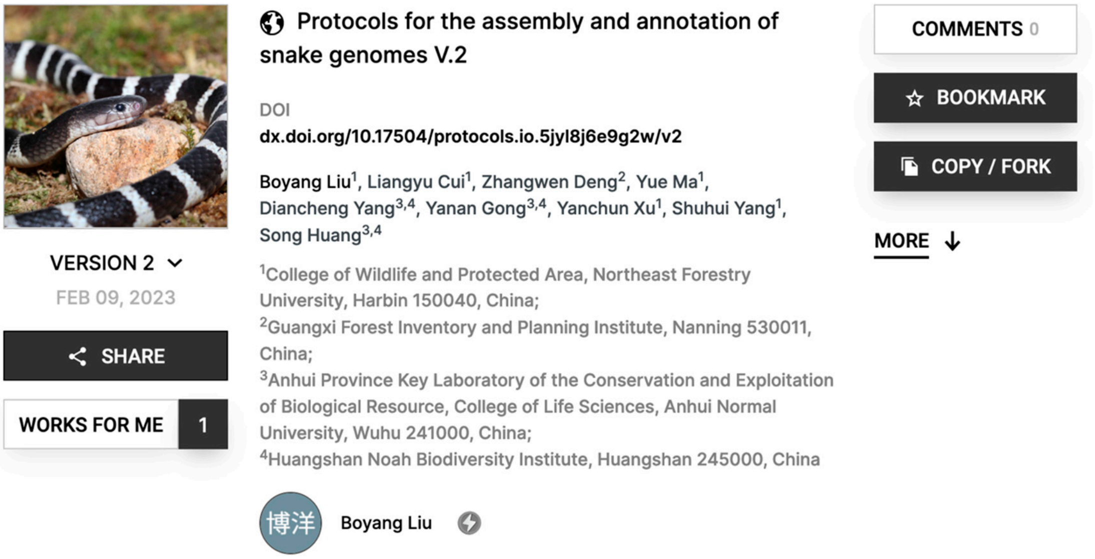


Figure 2. BUSCO Assessment result of the *P.mucosa* genome.

Methods

Detailed stepwise protocols are gathered in a protocols.io collection with some minor adaptations outlined below [7] (Figure 3).



Protocols for the assembly and annotation of snake genomes V.2

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Figure 3. A protocols.io collection of the standard protocols for sequencing snake genomes [7].

Sample collection and sequencing

In 2021 an adult *P. mucosa* (NCBI:txid31142) individual from Hezhou City in Guangxi province was collected for genome assembly and RNA sequencing. The individual died of natural causes and the samples were transferred to dry ice and quickly frozen, then kept at -80℃ until further use. We isolated 8 tissues and organs for RNA sequencing, including the heart, small intestine, large intestine, lung, liver, stomach, kidney and muscle. Furthermore, genomic DNA was extracted for whole-genome sequencing utilizing the AxyPrep genomic DNA kit (AxyPrep, USA).

The total RNA was isolated utilizing the TRlzol reagent (Invitrogen, USA) following the recommended guidelines. The assessment of RNA quality, purity, and quantity was performed using the Qubit 3.0 fluorometer (Life Technologies, USA) and the Agilent 2100 Bioanalyzer System (Agilent, USA). The cDNA libraries were generated through the reverse transcription of RNA fragments ranging from 200 to 400 bp. In addition, the liver sample was used for single-tube long fragment read (stLFR) sequencing and genome survey which it refers to the means of analyzing the second generation sequencing data through k-mer to obtain genome size, heterozygosity, repeat sequence proportion, GC-content and other genomic information.

Genome survey, assembly, annotation and assessment

The stLFR sequencing data were assembled with Supernova software (v2.1.1) [8]. NextPolish (v1.0.5) [9] program was then used to carry out a second round of correction and third round of polishing for this assembly by using the WGS data. To get a haploid representation of the genome, duplicates were purged with Purge_Dups pipeline [8] from the genome. The completeness of the genome was evaluated using sets of Benchmarking Universal Single-Copy Orthologs (BUSCO v5.2.2) with genome mode and lineage data from vertebrata_odb10 [10].

In order to detect the presence of known repeat elements in the genome of the many-banded P. mucosa, , the following approach was employed Repeat Finder (TRF) [11], LTR_FINDER (RRID:SCR_015247) [12] and RepeatModeler (v2.0.1,RRID:SCR_015027) (v1.0.8) [13]. RepeatMasker (v3.3.0, RRID:SCR_012954) [14] and RepeatProteinMask v3.3.0 [15] were used to search the genome sequences for known repeat elements. BRAKER2 pipeline[16] was used to perform gene prediction. Then the gene sets were aligned against several known databases, including SwissProt[17], TrEMBL[17], Kyoto encyclopedia of genes and genomes (KEGG)[18], GO and NR [19] database.

Results

In *P. mucosa*, the total length of the repeat sequence in the genome reached 735Mb, and its repeat content is as high as 42.16% (Tables 2 and 3). We analysed the content of various repetitive elements, and several different genome families were identified within the *P. mucosa* genome. We found that LINE repeat elements accounted for 35.51%, while LTR accounted for 9.15%, and DNA accounted for 4.66% (Figure 4). Long interspersed nuclear elements were the most numerous of these repeats. Research findings suggest that despite snake species sharing similar genome sizes, they demonstrate considerable variations in TE content, with limited diversity in the types of TEs. Species with a longer evolutionary history tend to exhibit greater diversity in TE content, as indicated by research findings.

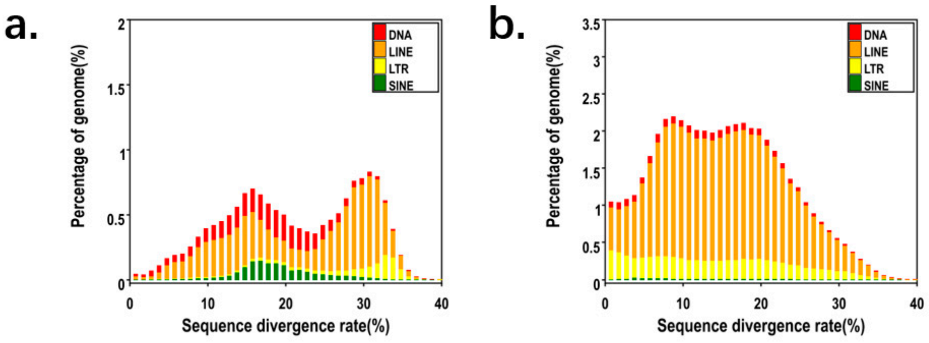


Figure 4. Distribution of transposable elements (TEs) in the *P. mucosa* genome. The TEs include DNA transposons (DNA) and RNA transposons (i.e. DNAs, LINEs, LTRs, and SINEs). (a) Known sequence divergence rate distribution (b). *De novo* sequence divergence rate distribution.

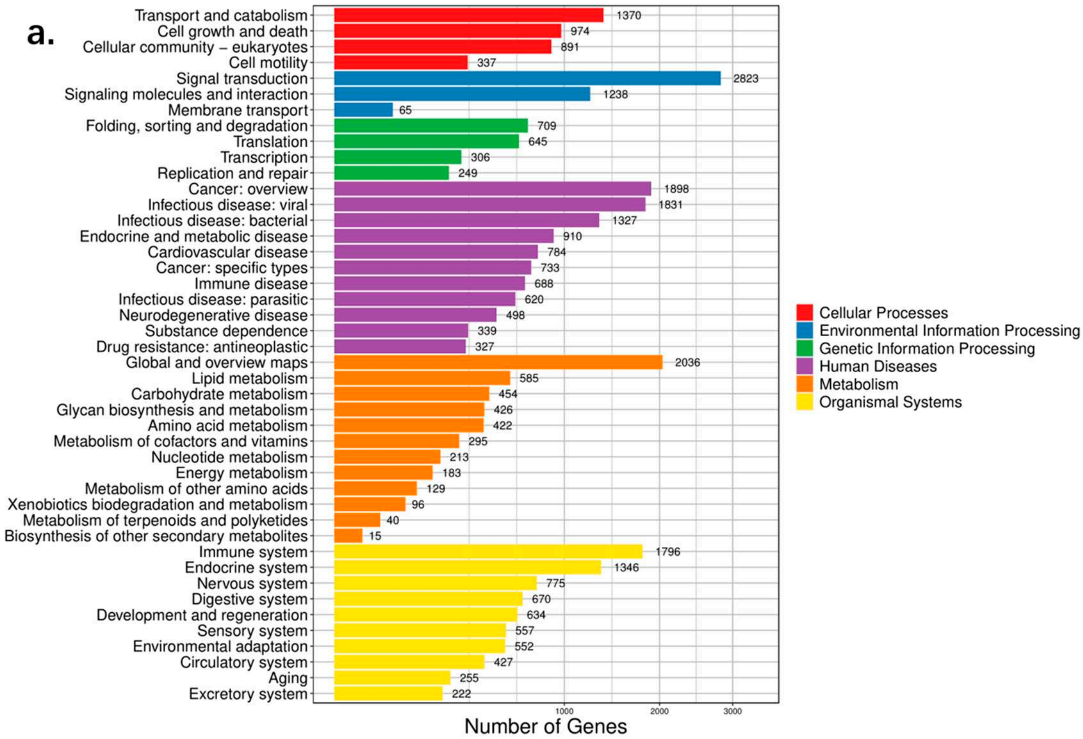
Table 2. Statistics for repetitive sequences identified in the *P. mucosa*.

Type	Length (bp)	% in genome
DNA	41761899	2.395689
LINE	581624764	33.365146
SINE	8061060	0.462426
LTR	149994747	8.604511
Other	0	0
Satellite	2433786	0.139615
Simple_repeat	10136004	0.581456
Unknown	5653213	0.324299
Total	735004828	42.163857

Table 3. Summary of transposable elements (TEs) in the *P. mucosa* genome.

	Repbase TEs		TE protiens		De novo		Combined TEs	
Type	Length (bp)	% in genome	Length (bp)	% in genome	Length (bp)	% in genome	Length (bp)	% in genome
DNA	39281826	2.35	6433176	0.38	37917702	2.26	71410039	4.27
LINE	186209051	11.14	150758176	9.02	449338074	26.89	511842308	30.63
SINE	20280301	1.21	0	0	2779035	0.16	22466386	1.34
LTR	34138399	2.04	53662430	3.21	224765038	13.45	234525215	14.03
Other	25447	0.002	0	0.	0	0	25447	0.002
Unknown	0	0	0	0	7924824	0.47	7924824	0.47
Total	266507708	15.95	210726751	12.61	667082033	39.92	705048693	42.19

A total of 24,869 functional genes were annotated using KEGG. This showed the highest number of annotated genes in pathways related to Human Diseases, Organismal Systems and Metabolism, and the highest number of Signal Transduction genes were in Environmental Information Processing. Moreover, GO gene enrichment for *P. mucosa* revealed that, among 25 biological process pathways, 247 genes were related to immune system processes, and 2 genes were related to detoxification (Figure 5)



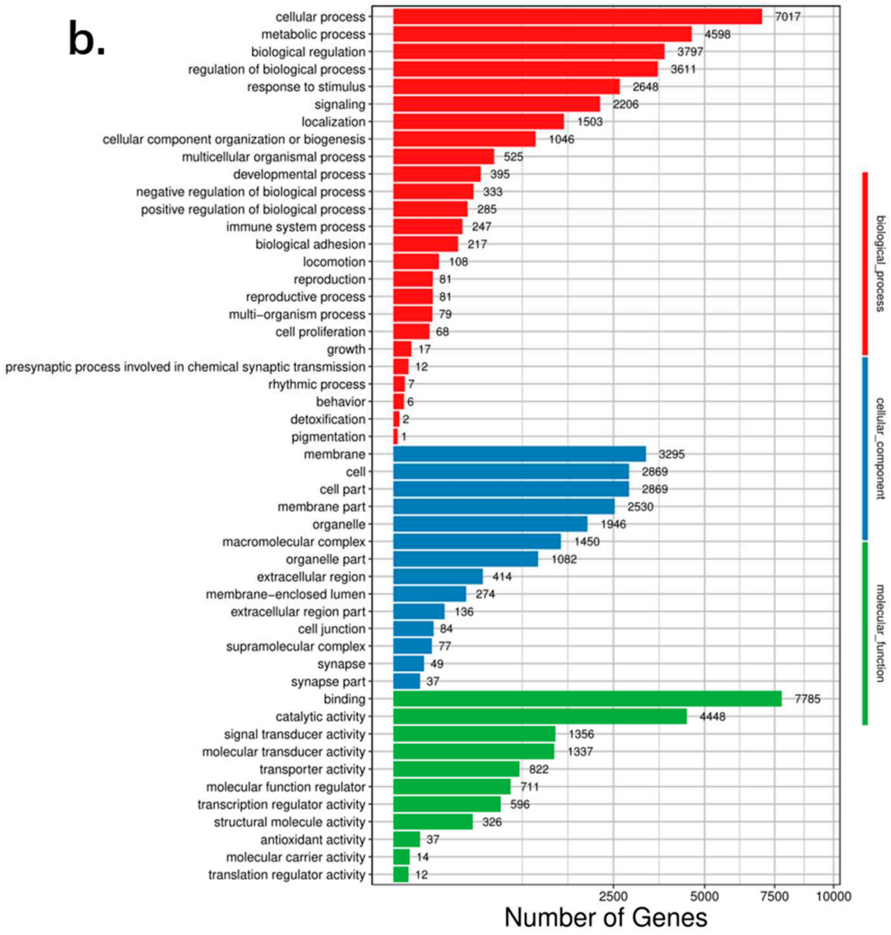


Figure 5. Gene annotation information of *P. mucosa*. (a) KEGG enrichment of *P.mucosa*. **(b)** GO enrichment of *P. mucosa*.

Reuse Potential

P. mucosa is a species of snake belonging to the species rich Colubrid family. Therefore, assembling the genome of the *P. mucosa* genome helps to understand the development process and origin of the Colubrids. Alongside this, as an economically important species, understanding the genome of the *P. mucosa* can potentially helpful for the breeding and breeding of the mouse snake in the future and can provide guidance for its breeding.

Author Contributions: Tianming Lan designed and initiated the project. Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences collected the samples. Jiangang Wang performed the DNA extraction, library construction and data analysis. Jiangang Wang wrote the manuscript. All authors read and approved the final manuscript.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0004141. Raw reads are in the SRA [accession] and additional data is in the GigaDB repository[20].

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Conflicts of Interest: The authors declare no conflict financial interests.

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