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The Genome Assembly of the King Ratsnake *Elaphe carinata*, Helps Reveal Its Biological Characteristics

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Abstract: The king ratsnake (*Elaphe carinata*) of the genus Elaphe is a common large non-venomous snake that is widely distributed in Southeast and East Asia, and is an economically important farmed snake species. As a non-venomous snake, the king snake that is predatory on venomous snakes such as cobras and pit vipers. The immune mechanisms of which has been unclear. Despite their economic and research importance, genomic resources which will benefit studies in toxicology, phylogeography and immunogenetics are lacking. In this study, we use single-tube long fragment read (stLFR) sequencing to display the first complete genome of a King ratsnake from Huangshan City, Anhui province in China. The genome size is 1.56GB with a scaffold N50 of 6.53M, the total length of the genome is approximately 621Mb, and the repeat content is 38.90%. Additionally, we predicted 22,339 protein-coding genes, of which 22,065 had functional annotations. Our genome is a potentially useful addition to those currently available for snakes.

Keywords: genetics and genomics; zoology; animal genetics

Data description

The king ratsnake (*Elaphe carinate*) of family Colubridae and genus Elaphe is a large oviparous snake[1] that is found in many provinces in South-eastern China, the southern edge of the distribution area can reach northern Guangdong, Guangxi and Taiwan, while the northern edge is located in the Beijing-Tianjin area (Figure 1). Also distributed in northern Vietnam and several islands (Ryukyu Islands, including the Senkaku Islands) in Japan [2,3]. *E. carinata* mainly inhabit mountainous and hilly areas and generally feed on rodents, birds, and eggs. Its juveniles differ greatly from adults, and when threatened, can use its anal glands to secrete a foul-smelling fluid [3]. King ratsnakes are farmed in many countries as an important food source as they provide a large amount of protein[4]. According to the China Red Data Book of Endangered Animals [5] (Zhao, 1998), the king snake is listed as a vulnerable species. The common name of "king ratsnake" refers to its habit of eating other snakes, according to reports, due to a special protein in the blood, the non-venomous king snake has a strong antagonistic effect on the venom of some poisonous snakes whose toxins are mainly blood-circulating poisons, such as bamboo leaf green and sharp-nosed viper (*Deinagkistrodon acutus)* snakes. However, the exact immune mechanism for this protection is unknown. As snake antivenom is the

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only treatment that is effective in preventing or reversing the effects of snake venom[6], the genome of the king ratsnake may provide new insight into antivenoms.

Figure 1. An *E. carinate* individual photographed by Diancheng Yang.

In the present study, we assembled the first highly contiguous *E. carinate* genome by using stLFR sequencing data and combined with next-generation sequencing data for correction. The resulting genome, which is comparable in genome size to the previously sequenced corn snake *Pantherophis guttatus* [7] but more contiguous, is valuable for further studies, such as snake evolution and venom immunity.

Main Content

Context

As a snake with a long history of captive breeding, the reproduction and virus carrying of the king ratsnake has been well studied[8,9], but there is insufficient research on its immune resistance and a general lack of genomic resources. Here we demonstrate the de novo assembly of a highly contiguous king ratsnake genome with a genome size of 1.56 Gb based on stLFR sequencing data (Table 1). The maximal length of scaffold is 49.75M and the N50 length is 6.53M. The GC content of *E. carinate* is 40.25%. Based on the characteristics of the published snake genome sequences, the assembled genomes were shown to be highly available and contiguous. Here, we present the draft genome sequence of *E. carinata*. It will be an invaluable resource for understanding snake venom resistance.

Table 1. Summary of the features of the *E.carinata* genome.

	contig	**Scaffold**
Maximal length(bp)	657733	52164798
N90(bp)	3039	4090
N50(bp)	45108	6847971
number>=500bp	187253	134573
Ratio of Ns	0.059	0.059
GC content(%)	40.25	40.25
Genome size(bp)	1574091846	1674021862

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Methods

Experimental procedures and more detailed methods used in this study are available via a protocol collection hosted in protocols.io (Figure 2) [9].

Figure 2. A protocols.io collection of the protocols for sequencing snake genomes [9].

Samples and Ethics Statement

An adult *E.carinata* (NCBI:txid74364) individual from Huangshan City in Anhui province, which was collected for DNA sequencing and RNA sequencing. After the individual died naturally, the samples were transferred to dry ice and quickly frozen, then kept at -80℃ until further use. We used four tissues and organs of liver, stomach, kidney and muscle for RNA sequencing. In addition, single-tube long fragment read (stLFR) sequencing only used muscle samples. Sample collection and experimental studies were both approved by the Institutional Review Board of BGI (BGI-IRB E22017) . All procedures are carried out in accordance with the guidelines of the BGI-IRB.

Nucleic Acid Isolation, Library Preparation, and Sequencing

We extracted DNA according to the method of Wang et al[10]. A stLFR co-barcoded DNA library was constructed using the MGIEasy stLFR Library Prep Kit (MGI, China). Sequencing was performed using a BGISEQ-500 sequencer. The genomic DNA kit (AxyPrep, USA) was used to isolate DNA for WGS sequencing in the meantime. Total RNA was extracted according to manufacturer 's instructions by using TRIzol reagent (Invitrogen, USA). Integrity and concentration of DNA and RNA were assessed using Qubit 3.0 Fluorometer (Life Technologies, USA) and Agilent 2100 Bioanalyzer System (Agilent, USA). Use 200–400 bp RNA fragments for reverse transcription of cDNA libraries.

Genome assembly, annotation and assessment

The stLFR sequencing data were assembled using Supernova software (v2.1.1)[10]. Based on the WGS data, the assembly was gap filled and redundant removed using GapCloser (v1.12-r6)[11] and redundans (v0.14a)[12], respectively.

We first identified de novo repeats using Repeat Finder (TRF) [13] (v. 4.09), LTR finder (v1.0.6) [14] and RepeatModeler [15] (v1.0.8). These repeats were then used together with RepBase in RepeatMasker[16] (v. 3.3.0) as known elements for identifying transposable elements, and known repeat elements were searched using RepeatProteinMask[17] (v. 3.3.0) in genome sequences. For protein-coding gene prediction, we first use Augustus[18] (v3.0.3) for de novo prediction. Based on the RNA-seq data filtered clean by Trimmomatic[19] (v0.30), the transcripts were assembled using

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Trinity[20] (v2.13.2), and compared with the king ratsnake genome through Programto Assemble Spliced Alignments (PASA)[21] (v2.0.2) to obtain the gene structure. For homology-based prediction, we used Blastall[22] (v2.2.26) with an E-value cut-off of 1e-5 to map the protein sequences by comparing four sets of high-quality data of Crotalus tigris, Pseudonaja textilis, Notechis scutatus and Thamnophis elegans from the UniProt database (release-2020_05) with the king ratsnake genome. GeneWise[23] (v2.4.1) was used to analyze alignment results to predict gene models. We used the MAKER pipeline[24] (v3.01.03) to generate final gene set representing RNA-seq, homology, and de novo predicted genes.

Functional annotation was completed by using SwissProt[25], TrEMBL[25], and (KEGG)[26] databases to perform BLAST comparison on structurally annotated gene sets, and the E value cut-off value was 1e-5. InterProScan[27] (v5.52-86.0) was used to count and visualize structural domain information, and Gene Ontology (GO) terms were used for gene enrichment.

The genome integrity was evaluated by Benchmarking Universal Single-Copy Orthologs (BUSCO v5.2.2), with parameters set to genome mode and dataset input set to vertebrata_odb10[28].

We used OrthoFinderv2.3.7 (RRID:SCR_017118)[29] to search for single-copy orthologs in the protein sequences of *Rana temporaria*(GCA_905171775.1), *Gopherus evgoodei* (GCA_007399415.1), *Podarcis muralis*(GCA_004329235.1), *Pseudonaja textilis*(GCA_900518735.1), *Thamnophis elegans*(GCA_009769535.1) *Pantherophis guttatus*(GCA_001185365.2), and to construct phylogenetic trees by orthogroups. A total of 1307 single-copy loci were found.

Results

Usually, genome-wide repetitive elements are important for eukaryotic evolution[30]. In *E. carinata*, the content of repetitive elements in the genome accounted for 38.90%, and the total length reached 621Mb (Tables 2 and 3). Among all repetitive elements, LINE accounted for 38.41%, DNA accounted for 17.11% and unknown types of repetitive elements accounted for 31.93% (Figure 3). This indicates that the content and quantity of repeating elements is one of the sources of species differences.

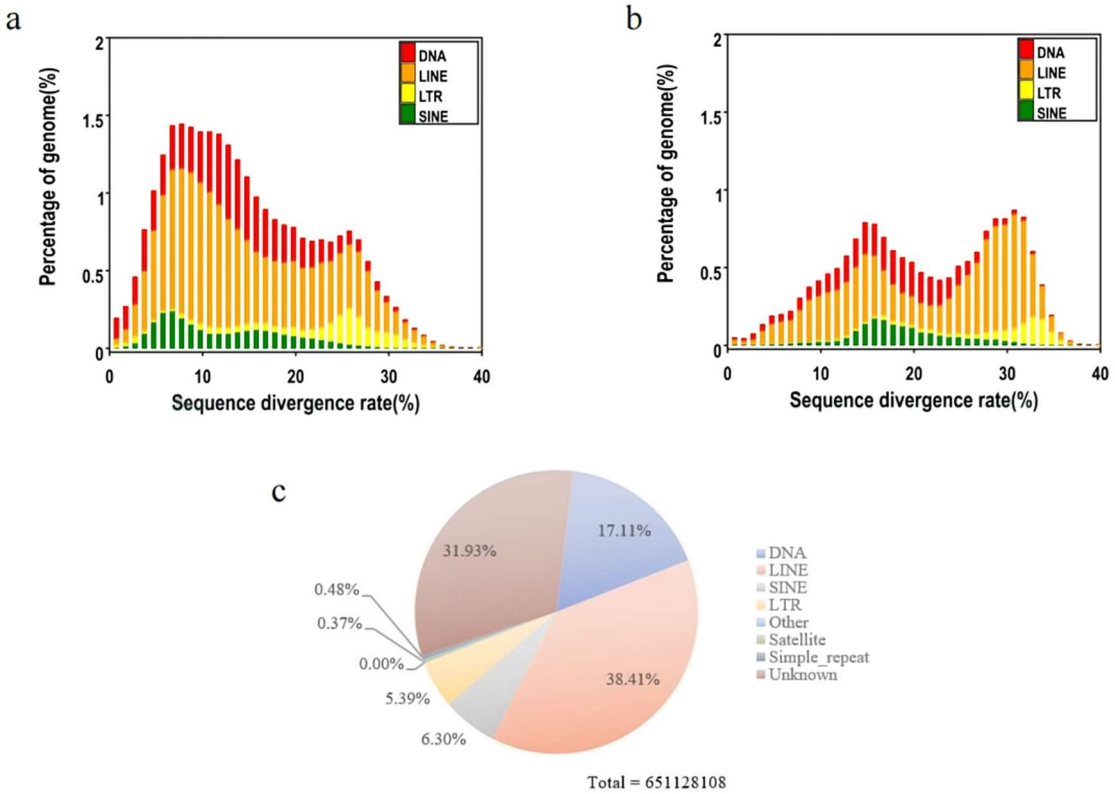

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Figure 3. Distribution of transposable elements (TEs) such as DNA transposons (DNA) and RNA transposons in the E. carinata genome. RNA transposons include DNAs, LINEs, LTRs, and SINEs. (a) Distribution of divergence rates for *De novo* sequences. (b) Distribution of divergence rates for known sequences. (c) Proportion and distribution of repeating elements.

Table 2. Content of various repeat sequences in the *E. carinata* genome.

Type	Length(Bp)	% in genome
DNA	114900759	6.863755
LINE	257937611	15.408258
SINE	42327923	2.528517
LTR	36199886	2.16245
Other	0	0
Satellite	2487376	0.148587
Simple_repeat	3251656	0.194242
Unknown	214450953	12.810523
Total	651128108	38.896034

Table 3. Summary of transposable elements (TEs)in the *E. carinata* 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	44586593	2.663442	3037369	0.181441	114900759	6.863755	137315177	8.202711
LINE	172974640	10.332878	142896461	8.536117	257937611	15.408258	287262246	17.160006
SINE	27330057	1.632599	0	0	42327923	2.528517	52336172	3.126373
LTR	20332067	1.214564	26146398	1.561891	36199886	2.16245	48061022	2.870991
Other	28331	0.001692	291	0.000017	0	0	28622	0.00171
Unknown	0	0	0	0	214450953	12.810523	214450953	12.810523
Total	252872307	15.105675	171980912	10.273516	645389076	38.553205	685733449	40.963231

A total of 22,065 functional genes were annotated, and the annotations associated with the TrEMBL database accounted for the largest proportion, reaching 97.92%(Table 4). In addition, all genes were annotated with KEGG, which showed the highest number in pathways such as Human Diseases, Organismal Systems and Metabolism, and the highest number of Signal Transduction genes in Environmental Information Processing. In Additionally, GO gene enrichment for *E. carinata* revealed that, among 25 biological process pathways, 251 genes were related to immune system processes, and 2 genes were related to detoxification (Figure 4).

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Table 4. Summary of annotation results in the *E. carinata* genome.

Values	Total	Swissprot-Annotated	KEGG-Annotated	TrEMBL-Annotated	Interpro-Annotated	GO-Annotated	Overall
Number	22,339	20,796	19,836	21,874	21,604	15,169	22,065
Percentage	100%	93.09%	88.80%	97.92%	96.71%	67.90%	98.77 %

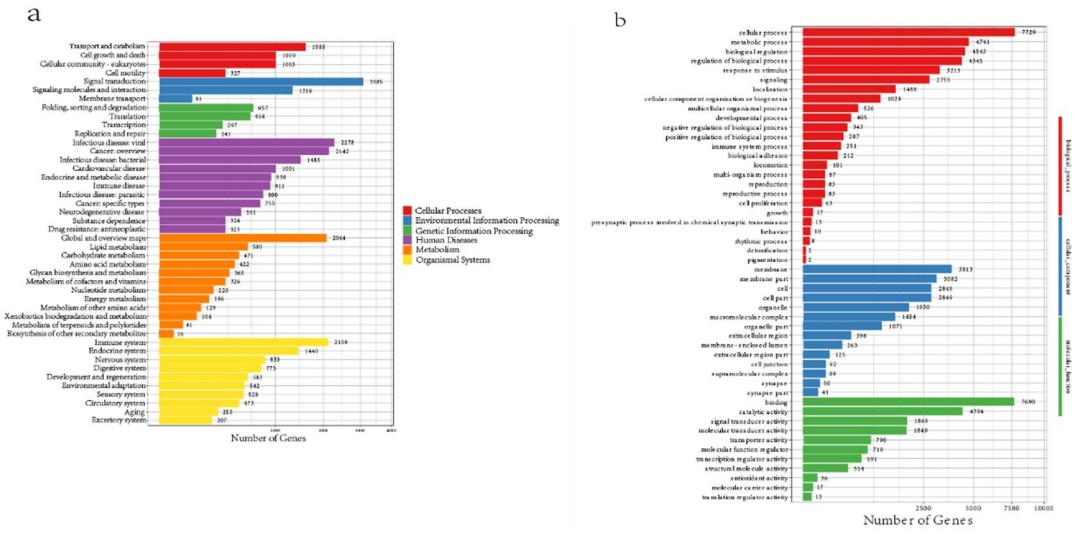

Figure 4. Gene annotation results for *E.carinata*. (a) KEGG enrichment of *E.carinata*. **(b)** GO enrichment of *E .carinata*.

Data validation and quality control

When assessing the quality of the genome, we performed a completeness assessment of the assembly with BUSCO v3.1.0 (RRID:SCR_015008) [31]using the vertebrata_odb10 dataset [31]. This assembly was able to match 83.2% of the complete BUSCOs. (Figure 5) .

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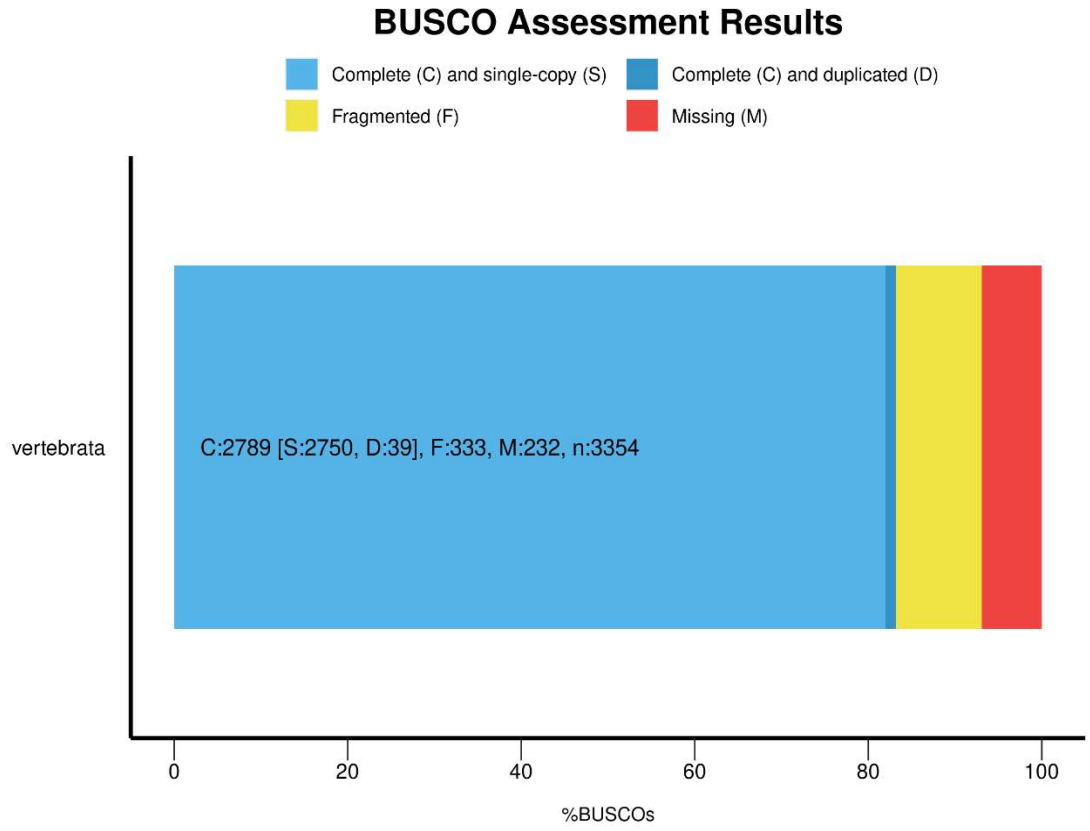

Figure 5. BUSCO Assessment result of the *E.carinata* genome.

By screening closely related species, *Rana temporaria*, *Gopherus evgoodei*, *Podarcis muralis*, *Pseudonaja textilis*, *Thamnophis elegans*, *Pantherophis guttatus* were filtered to construct a phylogenetic tree. Consistent with previous studies[32], our data can construct a phylogenetic trees and cluster closely related species. (Figure 6)

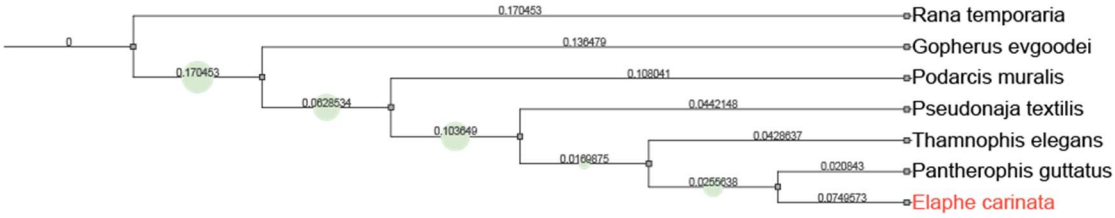

Figure 6. Phylogenetic tree reconstructed using nuclear genome single-copy genes.

Reuse Potential

King ratsnake has both nutritive and medicinal value, and the growth and development of individuals and snake eggs has been widely studied[33]. However, there are insufficient studies and genomics data on its immune system. Only Sun et al. have done relevant research on the development of the immune system in the embryonic stage of the king snake[34].

Our data can be combined with other snake genome data for phylogenetic studies to construct the developmental evolutionary history of snakes and other reptiles. In addition, the genomic data can provide new insights into the study of the immune system, snake venom resistance genes and their mechanisms of action.

Author contribution: Song Huang, He Wang and Tianming Lan designed and initiated the project. Yi Zhang, Tierui Zhang, Zhihao Jiang and Jing Yu collected the samples. Xinge Wang, Zicheng Su and performed the DNA extraction, Diancheng Yang, Yanan Gong and Zhangbo Cui performed genome assembly. Jiale Fan and Ruyi Huang performed data analysis and wrote the manuscript. All authors read and approved the final manuscript.

Data Availability: (Data is uploading). The data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA) [35] of China National GeneBank DataBase (CNGBdb) [36] with accession number CNP0004039. Additional data is available in the *GigaScience* GigaDB repository [37].

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Competing interests: The authors declare no conflict financial interests.

Consent for publication: Not applicable.

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