

Genome Resources

A chromosome-level genome assembly and annotation for the beauty snake *Elaphe taeniura*

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Abstract

The genus *Elaphe* Fitzinger, a large species clade within Colubridae, comprises 18 non-venomous snake species. Among them, *Elaphe taeniura* serves as a representative species due to its wide distribution and strong adaptability. However, genomic studies on this group remain limited. Here, we present a chromosome-level, high-quality reference genome for this species. The genome is assembled by integrating high-accuracy PacBio HiFi long-read sequencing and Hi-C chromatin conformation capture technologies and comprehensively annotated with the aid of transcriptomic data. The genome of *E. taeniura* spans 1.62 Gb, with a scaffold N50 of 206.4 Mb and the longest scaffold reaching 344.4 Mb. The genome exhibits a BUSCO completeness score of 98.2% and contains 22,246 protein-coding genes. Comparative analysis between the assembled genome and three other colubrid species revealed high synteny. In addition, several chromosomal fusion and fission events were observed. This reference genome provides a valuable resource for studying the taxonomy, conservation, and evolutionary history of the widely distributed *Elaphe* species.

Key words: *Elaphe taeniura*, genome assembly, Colubridae, snake, comparative genomics

Introduction

Snakes are distributed across all continents except the polar regions and have undergone extensive adaptive evolution, resulting in over 30 families and more than 4,000 species (Zug et al. 2001; Feldman et al. 2015). In the long evolutionary process, various branches have occupied unique ecological positions through significant adaptive differentiation: the Elapidae and Viperidae have independently evolved efficient venom delivery systems (Kerkkamp et al. 2017); the Scolecophidia have evolved regressive vision and specialized body scales adapted for burrowing (Avila et al. 2006); Hydrophiinae have evolved salt-excreting glands and paddle-like tails among other marine adaptations (Shine 2005). This kind of cross-ecological phenotypic differentiation makes snakes a typical species for parsing the adaptive radiation of vertebrates (Pyron et al. 2013). In recent years, some high-quality chromosome-level genomes of snakes have been published, providing valuable insights into the evolution of snakes and even vertebrates (Peng et al. 2023). Despite their wide distribution and extensive species differentiation, snakes still lack high-quality reference genomes, which hinders the study of conservation and molecular biology of this group. High-quality reference genomes are crucial for studying genetic variation in a species, especially when combining re-sequencing data with high-quality annotation, which can accurately characterize structural variation (Ejigu and Jung 2020).

Elaphe taeniura (Cope, 1861), commonly known as the beauty snake, belongs to the genus *Elaphe* within the Colubridae family and is widely distributed across Southeast



Fig. 1. Photograph of *Elaphe taeniura* taken by Songwen Tan in Yibin, September 2023.

and East Asia. This nonvenomous snakes typically prey on rodents and birds and their lengths can reach up to 2 meters (Asato et al. 2022). They inhabit a variety of environments, including plains, hills, and mountainous regions, at elevations ranging from sea level to 3,000 m. Due to dramatic population decline, this species has been classified as Vulnerable (VU) on the IUCN Red List in 2021 (Li et al. 2021). *E. taeniura* once belonged to the genus *Orthriophis* and was named *O. taeniurus* (Wang et al. 2020). With the advance of molecular taxonomy, the intricate phylogenetic relationships among numerous species have been rigorously reexamined, resulting

Received on 16 June 2025; revised on 14 August 2025; accepted on 14 August 2025

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Fig. 2. Circos plot showing the distribution of genomic features. a) Chromosomes, with numbers indicating their lengths in Mb; b) repetitive sequences; c) GC ratio; d) gene density; the innermost track is synteny among chromosomes.

in the reclassification of New World and most Old World taxa into newly established or resurrected genera (Helfenberger 2001; Lenk et al. 2001; Chen et al. 2017). Therefore, *Orthrioph* was synonymized with the genus *Elaphe*, and *O. taeniurus* has accordingly been named as *E. taeniura*. According to the most recent entries in the Reptile Database (<http://www.reptile-database.org/>), the genus *Elaphe* consists of 18 species, *E. taeniura* includes nine recognized subspecies, most of which are distributed in Asia (Utiger et al. 2002). However, this widely distributed and highly adaptable species lacks a reference genome, posing challenges for genomic research on this group.

Here, we present a chromosome-level and high-quality genome assembly of *E. taeniura*, derived from *Elaphe taeniura* in mainland China. We employed PacBio Revio System for long-read sequencing and integrated Hi-C data to achieve a chromosome-level assembly. Genome annotation was conducted using RNA-seq data from multiple tissues, published genomes of closely related species, and de novo prediction methods. We assessed the quality of the genome assembly using BUSCO. Overall, this study ultimately produced a high-quality reference genome for the genus *Elaphe*.

Methods

Biological materials

A single adult male *E. taeniura* (Fig. 1) was captured in Yibin City, Sichuan Province, in September 2023. After

several months of captivity, we extracted muscle, blood, heart, pancreas, spleen, kidney, liver, Duvernoy's gland, and lung samples, which were rapidly frozen at -80°C . Muscle tissue was used for PacBio HiFi long-read sequencing and Hi-C sequencing, while the remaining eight tissues were used for RNA-seq.

HiFi library preparation and sequencing

High-quality genomic DNA (gDNA) was extracted using the QIAGEN Genomic Kit. The quantity and quality of the extracted DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and a Qubit fluorometer (Invitrogen). DNA integrity was evaluated by 1% agarose gel electrophoresis. After the detection, DNA purification was performed using AMPure PB beads (Pacbio Cat #100-265-900). The library of PacBio SMRTbell was constructed using SMRTbell Express Template Prep Kit 3.0. The size and concentration of library fragments were detected with Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Qualified libraries were evenly loaded on SMRT Cell and sequenced for 30 h using Sequel II/Ie system (Pacific Biosciences, CA, USA).

Hi-C library preparation and sequencing

Muscle tissue was fixed at room temperature under vacuum in MS buffer (10 mM potassium phosphate, pH 7.0; 50 mM sodium chloride; 0.1 M sucrose) containing 1% formaldehyde

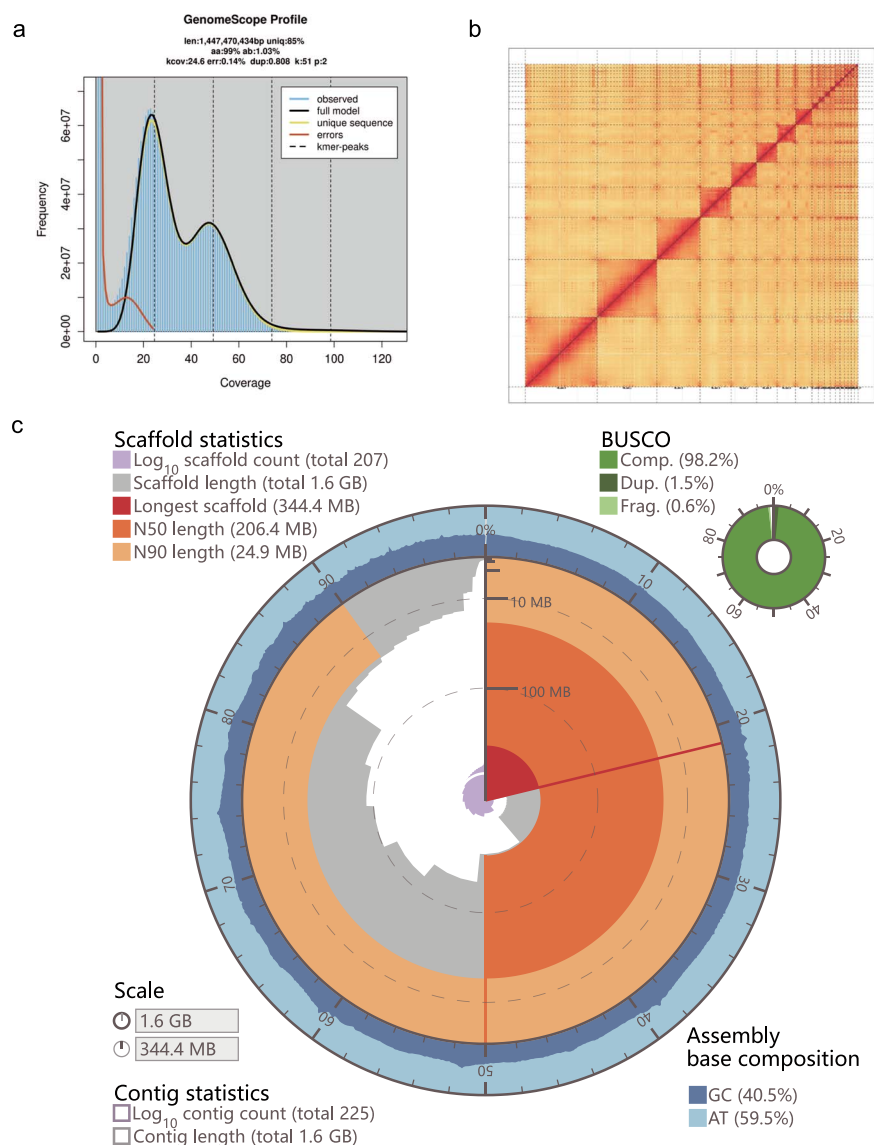


Fig. 3. Visual overview of genome assembly metrics. a) K-mer ($K = 51$) spectrum generated using GenomeScope. The bimodal distribution observed is characteristic of a diploid genome. b) The Hi-C contact map is based on a genome assembly partitioned into 500 kb bins. The number of Hi-C read pairs connecting any two bins was used to represent interaction intensity. Eighteen chromosome groups can be clearly distinguished. Interaction signals are stronger along the diagonal, reflecting high contact frequency between adjacent genomic regions. c) The BlobToolKit Snail plot provides a graphical summary of the *E. taeniura*-related quality metrics presented in Table 2. The plot circle represents the full size of the assembly. From the inside out, the central plot covers length-related metrics in percentage of the assembly. The line indicating the longest scaffold is highlighted within the central plot. All other scaffolds are arranged clockwise in descending size and are shown in contrast, starting from the outer edge of the central plot.

to cross-link DNA and proteins. The crosslinked DNA was subsequently digested with the Hind III restriction enzyme. Sticky ends were repaired, and biotin was simultaneously added to label the oligonucleotide ends. T4 DNA ligase was added to the end-repaired DNA, followed by incubation at 16 °C for 6 h to facilitate ligation. Proteinase K was added to uncross-link the DNA, followed by overnight incubation at 65 °C. The unligated ends were then removed, and the purified DNA was fragmented to a size of 300 to 500 bp, with subsequent end repair. The labeled DNA fragments of biotin were finally isolated on Dynabeads M-280 Streptavidin (Life Technologies). The obtained DNA fragments were used to construct a second-generation sequencing library, followed by PE150 sequencing on the Illumina NovaSeq 6000 platform.

RNA sequencing

Total RNA was extracted from multiple tissues of the same individual. mRNA was purified using poly-T oligo-attached magnetic beads. After cDNA synthesis and purification, library quality control was performed, with the 5' end of each library undergoing phosphorylation and circularization. Circular amplification was then carried out to generate DNA nanoballs, which were loaded onto a flow cell for sequencing on the DNBSEQ-T7 platform.

Predicting genome size and heterozygosity

Prior to genome assembly, the genome size and heterozygosity of *E. taeniura* were estimated using Jellyfish

Table 1. Assembly pipeline and software used.

Procedures	Software and options	Version
Initial assembly		
Quality control of the raw polymerase reads	PacBio SMRT-Analysis	
K-mer counting	Jellyfish ($k = 51$)	2.3.0
	Genomescope	2.0
ccs reads generate	SMRTLink (–min-passes = 3 –min-rq = 0.99)	9.0
De novo assembly	Hifiasm	0.19.7
Hi-C		
quality controlled	fastp (-q 20)	0.21.0
SAM/BAM processing	samtools	1.15.1
Map to contigs	hicup	0.8.0
contigs clustering, sorting and orienting	ALLHiC	0.9.8
fine-tune clustering results	JuiceBox	1.11.08
Genome quality assessment		
assembly quality assess	BUSCO (-m geno -l vertebrata_odb10)	5.5.0
Repeat annotation		
Repetitive element identification	RepeatMasker	4.1.2
Predict LTR	LTR_FINDER	1.07
	PILER	
	RepeatScout	1.0.5
	RepeatModeler	2.0.5
	TRF	4.09.1
Structural annotation		
Homology-based gene prediction	GenblastA	1.0.4
Transcriptome-based gene prediction	Tophat	2.1.1
	cufflinks	
De novo gene prediction	PASA	2.5.1
	Augustus	3.3.1
Integration of gene prediction	EVidenceModeler	1.1.1
Update of gene annotation	PASA	2.5.1

Table 2. Sequencing and assembly statistics.

BioProjects	NGDC BioProject	PRJCA037373
	NGDC Biosample	SAMC5075381
		SAMC5075382
		SAMC5075383
		SAMC5075384
		SAMC5075385
		SAMC5075386
		SAMC5075387
		SAMC5075388
		SAMC5091416
		SAMC5091417
	NGDC GSA	CRA025507
		CRA025653
Genome Sequence	PacBio HiFi reads	PacBio_SMRT (Revio) cell: 6.6 million reads, 113.95Gbases
	Hi-C reads	DNBSEQ-T7: 2.06 billion reads, 309.40 Gbases
	RNA-seq reads	ILLUMINA (Illumina NovaSeq 6000):190.57 Gbases from eight organizations
Genome Assembly	Number of contigs	225
Quality Metrics		
	Contig N50	202,669,265
	Contig N50 number	3
	Longest contigs	344,398,495
	Number of Scaffolds	207
	Scaffold N50	206,420,752
	Scaffold N50 number	3
	Longest Scaffolds	344,398,495
	Size of final assembly	1,624,145,665
	BUSCO completeness (tetrapoda_odb10)	C:98.2% [S:96.7%, D:1.5%], F:0.6%, M:1.2%, n:3354, E:3.7%

Table 3. Statistical outcomes regarding repetitive sequences in the *E. taeniura* genome.

	Number of elements	Length occupied	Percentage of sequence (%)
Retroelements	1,697,456	439,096,101	27.04
SINEs	399,563	76,710,039	4.72
LINEs	874,204	240,920,889	14.83
L2/CR1/Rex	617,430	158,486,427	9.76
R2/R4/NeSL	30,844	8,008,372	0.49
RTE/Bov-B	89,297	24,099,611	1.48
L1/CIN4	59,224	38,024,486	2.34
LTR elements	423,689	121,465,173	7.48
BEL/Pao	227	114,525	0.01
Ty1/Copia	95,590	25,086,546	1.54
Gypsy/DIRS1	168,229	59,529,893	3.67
Retroviral	4,954	3,409,141	0.21
DNA transposons	39,730	11,501,646	0.71
hobo-Activator	7,601	1,735,049	0.11
Tc1-IS630-Pogo	30,643	9,225,237	0.57
PiggyBac	238	102,634	0.01
Rolling-circles	1,461	357,801	0.02
Unclassified	1,376,183	236,186,661	14.54
Total interspersed repeats		686,784,408	42.29
Small RNA	34,986	4,062,456	0.25
Satellites	14	3,655	
Simple repeats	555,220	33,743,591	2.08
Low complexity	67,423	7,543,176	0.46

(Marçais and Kingsford 2011) and GenomeScope2 (Ranallo et al. 2020). Jellyfish was employed to generate k -mer ($k = 51$) (Peng et al. 2023) frequency distribution tables from clean short reads, which were then analyzed by GenomeScope2 to derive genome prediction information.

De novo genome assembly

Raw polymerase reads were quality controlled using the PacBio SMRT Analysis software suite (<https://www.pacb.com>). HiFi reads were generated using SMRT Link v9.0 and subsequently assembled into contigs using Hifiasm (Cheng et al. 2021). Hi-C data were quality controlled using fastp (Chen et al. 2018). The clean reads were aligned to the contigs using HiCUP software (Wingett et al. 2015). Contigs were then clustered, ordered, and oriented using ALLHiC software (Zhang et al. 2019). Finally, the orientation results were fine-tuned with JuiceBox (Durand et al. 2016) to generate a chromosome-level genome. To assess the assembly quality, BUSCO (Simão et al. 2015) was used with the vertebrata_odb10 database for evaluation. Snail plot (Challis et al. 2020) was generated using assembly-stats to visualize the assembly quality. To assess assembly accuracy, short reads were mapped to the assembled genome using BWA (Li and Durbin 2009).

Genome annotation

The known repeat sequences were identified using RepeatMasker (Chen 2004). The de novo prediction was performed by constructing a repeat library with tools LTR_FINDER (Xu and Wang 2007), PILER (Edgar and Myers 2005), RepeatScout (Price et al. 2005), RepeatModeler (Flynn et al. 2020), and TRF (Benson 1999). Then, RepeatMasker was adopted to annotate repeat sequences based on the constructed repeat library.

In genomic structural annotation of *E. taeniura*, three methods were used to predict protein-coding genes, including homology prediction, transcriptome prediction, and de novo prediction. The results were then integrated to obtain the final comprehensive annotation. In homologous prediction, the genomes of five related species (*Pantherophis guttatus*, *Ahaetulla prasina*, *Thamnophis elegans*, *T. sirtalis*, *Eryx carinatus*) were compared using the GenBlastA tool (She et al. 2009). Nonredundant gene structures predicted by PASA (Haas et al. 2008) were used to train gene model parameters, and then Augustus (Stanke et al. 2008) was used for de novo gene prediction. Transcriptome prediction was conducted by mapping clean RNA-seq reads to the assembly sequences using Tophat (Trapnell et al. 2009), followed by transcript assembly with Cufflinks (Trapnell et al. 2012). Finally, we used EVIDENCEModeler (Haas et al. 2008) to merge the prediction results. Genomic structural information was visualized with Circos (Fig. 2) (Krzywinski et al. 2009).

Functional annotation of protein-coding genes was performed by aligning the predicted protein sequences to the NR (Non-Redundant protein Database) and Swiss-Prot databases (Bairoch and Apweiler 1999) using Diamond (Buchfink et al. 2021). eggNOGmapper (Cantalapiedra et al. 2021) was used to predict gene function by aligning protein sequences against the eggNOG database (Huerta-Cepas et al. 2019). InterProScan (Hunter et al. 2009) was used to classify proteins and annotate functional domains and functional sites. Both InterProScan and eggNOGmapper performed GO (Gene Ontology) (Ashburner et al. 2000) annotation of the sequences. In addition, eggNOGmapper also produces the KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa and Goto 2000) pathway annotation.

For noncoding RNAs, tRNAs were identified using tRNAscan-SE (Chan et al. 2021), rRNAs, miRNAs, and snRNAs were predicted using the Infernal (Nawrocki and

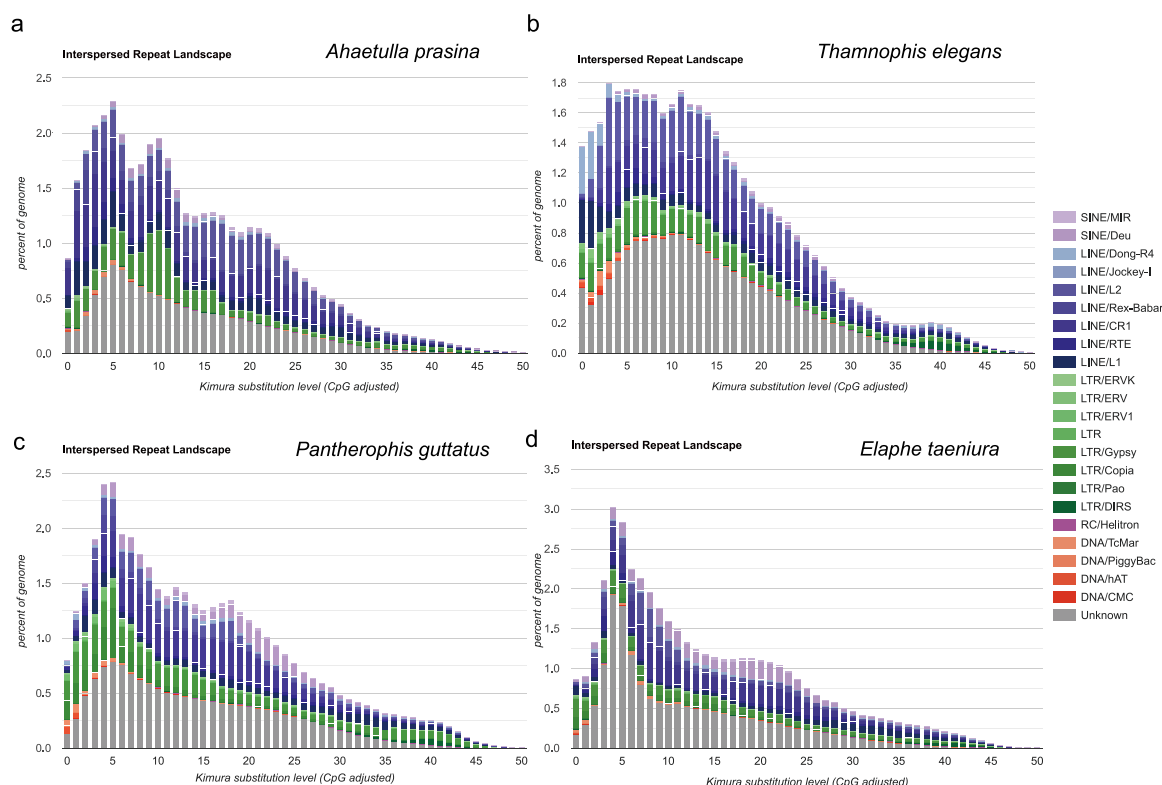


Fig. 4. Interspersed repeat sequence landscape. The x-axis represents the mutation rate of transposon sequences relative to their consensus sequences; higher values indicate earlier insertion times. a) *Ahaetulla prasina*. b) *Thamnophis elegans*. c) *Pantherophis guttatus*. d) *Elaphe taeniura*.

Eddy 2013) software with Rfam database (Ontiveros-Palacios et al. 2025).

Genome synteny and gene family analysis

Peptide sequences of *E. taeniura* were compared with the chromosome-level genomes of other Colubridae species, including *A. prasina* (NCBI accession GCF_028640845.1), *T. elegans* (NCBI accession GCA_009769535.1), and *P. guttatus* (NGDC accession GWHBOZR000000000). Collinearity analysis was conducted using MCScanX (Wang et al. 2012). Protein family identification was carried out using OrthoVenn3 (Sun et al. 2023), and the species divergence time was inferred using the Mmctree (Reis and Yang 2011). Two additional species from different families, *Naja naja* (NCBI accession GCA_009733165.1) and *Argyrophis diardii* (NGDC accession GWHBWDT000000000), were also included in the analysis. The species tree was constructed using OrthoFinder (Emms and Kelly 2019).

Results

Genome assembly and annotation

For genome survey analysis, a total of 76.24 Gb high-quality data were obtained from Illumina sequencing. K-mer distribution analysis estimated the genome size of 1,447.47 Mb, heterozygous ratio of 1.03% (Fig. 3a). The software and parameters used in the assembly are listed in Table 1. PacBio HiFi sequencing generated 113.95 Gb of data, with approximately 6.6 million reads and N50 length of 17,244 bp. The Hi-C sequencing library yielded 309.49 Gb clean data with a Q30 rate of 96.64% and an average GC content of 41.38%.

RAN-seq produced 190.57 Gb clean data from eight tissues (Table 2).

The Hi-C data was aligned to contigs to facilitate chromosome-level scaffolding, successfully anchoring sequences to 18 chromosomes (Fig. 3b), with a chromosome anchoring rate of 98.27%. The final assembly of *E. taeniura* consisted of 207 scaffolds, with a total genome size of approximately 1.62 Gb, with scaffold N50 of 206.4 Mb, N90 of 24.9 Mb, and the longest scaffold of 344.4 Mb (Fig. 3c). BUSCO evaluation identified 98.2% complete single-copy genes, indicating high assembly completeness (Table 2).

For repeat annotation, we identified 6,574,206 repeat elements with a total length of 732.5 Mb accounting for 45.1% of the assembled genome. Among these, long interspersed nuclear elements (LINEs, 14.83%) and long terminal repeat sequences (LTRs, 7.48%) have the largest number of family members (Table 3). In the interspersed repeat landscape, *E. taeniura* exhibits a substitution level peak at 3–5, indicating recent transposable element activity (Fig. 4a). *P. guttatus* and *A. prasina* show similar peak patterns to *E. taeniura*, but with slightly more gradual activity (Fig. 4b and c). *T. elegans* displays a broader peak between 5 and 15, dominated by LINEs (Fig. 4d). The results indicate that *E. taeniura* has recently experienced a transposon expansion event, with a more pronounced expansion trend compared with other species in the same family.

A total of 22,246 protein-coding genes were predicted (Table 4), functional annotation against major protein databases resulted in successful annotation of 85.88% of these genes (Fig. 5). For noncoding RNA annotations, we have detected 255 miRNAs, 552 tRNAs, 451 rRNAs, and 525 snRNAs from the assembled genome (Table 5).

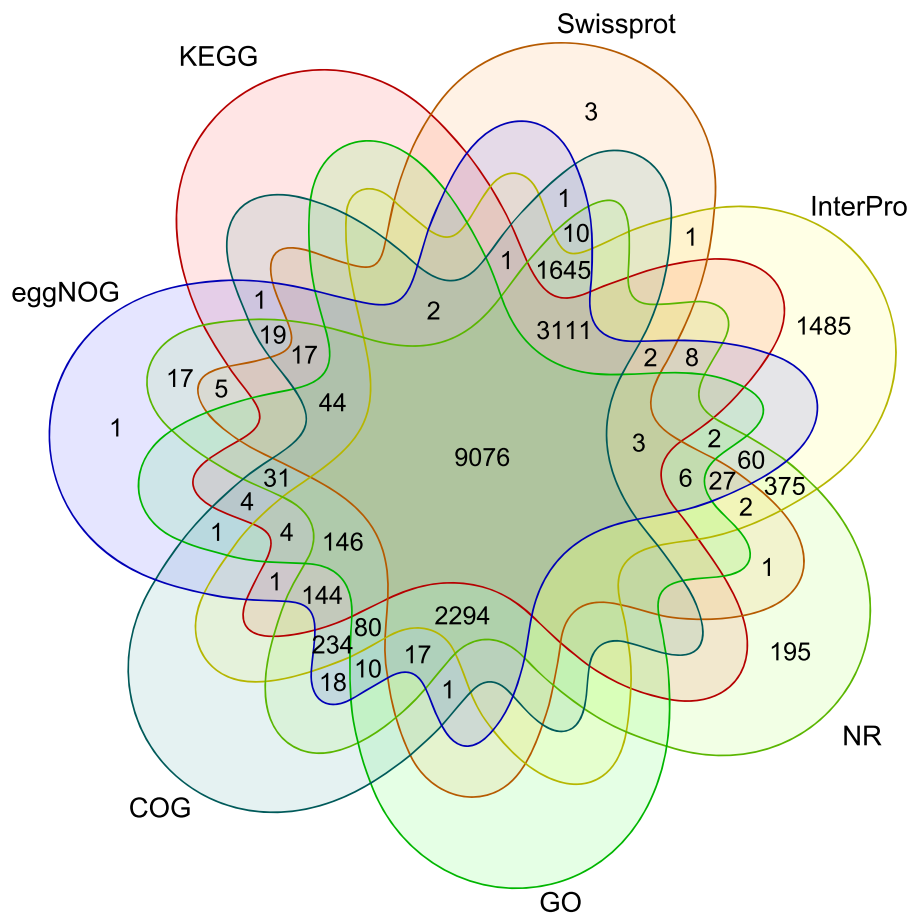


Fig. 5. Functional annotation of protein-coding genes across multiple databases.

Table 4. Summary of the functionally annotated protein-coding genes.

	Database	Number	Percent (%)
Annotation	Swissprot	16,269	73.13
	NR	17,599	79.11
	KEGG	12,614	56.70
	GO	11,721	52.69
	eggNOG	17,043	76.61
	COG	16,912	76.02
	Interpro	18,709	84.10
Total	Annotated	19,105	85.88
	All	22,246	100

Table 5. Statistical results of noncoding RNA annotation of the *E. taeniura* genome.

Type	Number	Total length (bp)
tRNAs	552	57,581
miRNAs	255	20,546
rRNAs	451	64,6790
snRNAs	525	66,044
Total	1,783	79,0961

Orthologous cluster identification

Among the 22,246 protein-coding sequences of *E. taeniura*, 15,064 were assigned to orthologous gene families. A total of

10,466 orthologous clusters were shared among all six species. Notably, *E. taeniura* exhibited the highest number of singleton genes (5,986) compared with the other five species (Fig. 6).

Chromosomal synteny analysis

Among species of the same genus, *E. taeniura* shows strong chromosomal collinearity with other species, although some chromosomal rearrangements are also present. The syntenic correspondence between chromosome 4 of *E. taeniura* and chromosome 12, 16 of *P. guttatus* suggests the occurrence of chromosomal fusion. In contrast, *T. elegans* exhibited more extensive chromosomal rearrangements. The longer chromosomes (>99 Mb) in *E. taeniura*, including chr1, chr2, chr3, chr5, and chr6, appear to have formed through the fusion of 2 to 3 chromosomes from *T. elegans*, while shorter chromosomes (<30 Mb) (chr9-16) resulted from chromosomal fission events (Fig. 7a). To further characterize these structural changes, synteny dot plots were generated and confirmed the identified rearrangement patterns (Fig. 7b, c, and d). These extensive chromosomal rearrangements may have contributed to the genetic diversification within the family Colubridae.

Discussion

In the Genebank database (accessed on 26 April 2025), 26 snake genome assemblies at the chromosome level are available, with genome sizes ranging from 1.3 Gb in *Crotalus viridis viridis* (NCBI accession GCA_003400415.2) (Pasquesi et al. 2018) to 2.1 Gb in *Hydrophis major* (NCBI accession

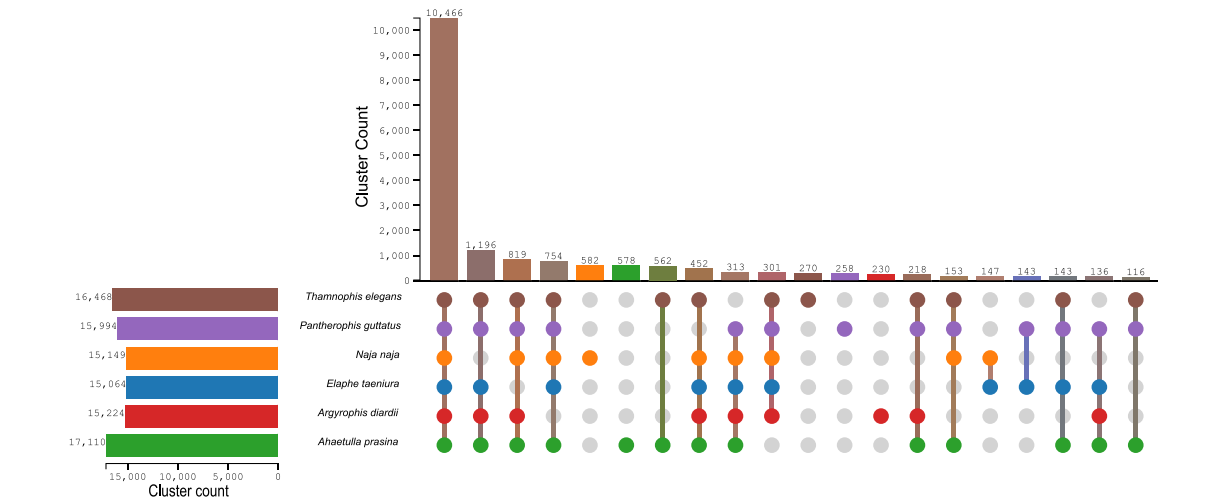


Fig. 6. Orthologous cluster identification. Homology clustering of protein sequences across selected species. Bars represent the number of gene families per species; dots indicate the presence of each gene family.

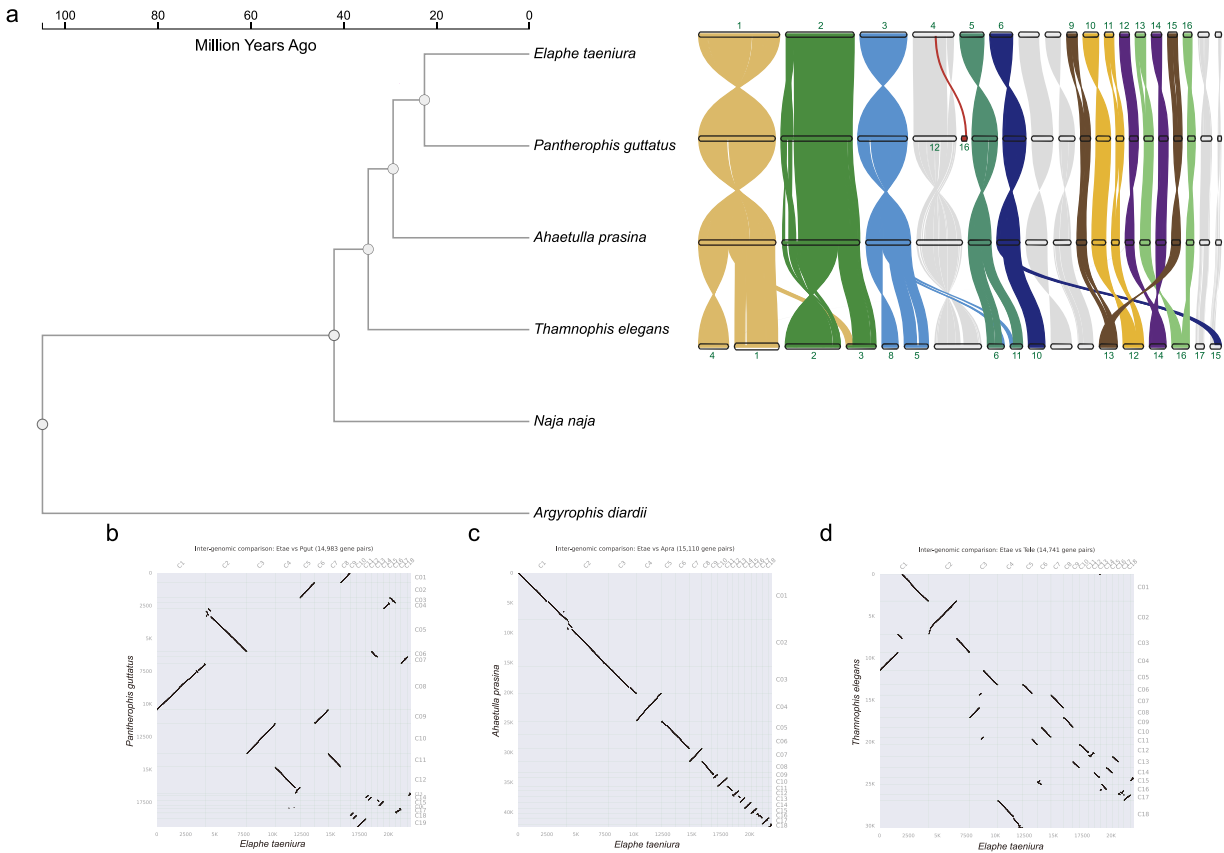


Fig. 7. Species tree and chromosomal synteny. Divergence times (in millions of years) are shown above the species tree. a) Pairwise alignment of four Colubridae genomes (*E. taeniura*, *P. guttatus*, *A. prasina*, *T. elegans*). b) Collinearity plot for *E. taeniura* and *P. guttatus*. c) Collinearity plot for *E. taeniura* and *A. prasina*. d) Collinearity plot for *E. taeniura* and *T. elegans*.

GCA_033807585.1) (Ludington et al. 2023) and scaffold N50 values varying from 100.9 Mb in *T. elegans* to 268.3 Mb in *H. major*. The *E. taeniura* genome spans 1.62 Gb with a scaffold N50 of 206.4 Mb, placing it well within the range of existing chromosome-level snake genomes. This study presents a chromosome-level, high-quality genome assembly for the genus *Elaphe*. Although a genome for the *Elaphe carinata* was previously published (Fan et al. 2023), it was not assembled at the chromosome level. Its

genome size of 1.56 Gb is comparable to that of *E. taeniura* 1.62 Gb. Compared with reference genomes of species in the family Colubridae in GeneBank, which range from 1.7 to 1.9 Gb, the genomes of the *Elaphe* genus appear to be relatively smaller. *Elaphe taeniura* comprises nine subspecies, and its high level of differentiation makes it a promising model for investigating adaptive evolution within the genus *Elaphe*. Moreover, the availability of a high-quality reference genome facilitates

further studies on adaptive morphology, extreme sex determination, and the inheritance of traits relevant to conservation (Brandies et al. 2019; Paez et al. 2022). The genome presented here provides a valuable resource for future research on the evolutionary history and conservation of this genus.

Author contributions

Jiahao Chen (Formal analysis, Visualization, Writing—original draft), Qin Liu (Data curation, Resources), Songwen Tan (Data curation, Investigation), Peng Guo (Conceptualization, Funding acquisition, Project administration, Supervision), and Lianming Du (Funding acquisition, Methodology, Software, Writing—review & editing)

Funding

This work was supported by the National Natural Science Foundation of China (32200525, 32370486) and the second Tibetan Plateau Scientific Expedition and Research Program (2024QZKK0200).

Data availability

Data generated for this study is available under NGDC (National Genomics Data Center) BioProject PRJCA037373. The dataset includes the final assembled genome, PacBio HiFi sequencing data, Hi-C sequencing data, and RNA-seq data. No custom scripts were employed in this study. All analyses were conducted using standard bioinformatics tools following their official manuals and protocols.

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