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First complete mitogenome sequence of Korean *Gloydius ussuriensis* (Viperidae: Crotalinae)

Hye Sook Jeon, Min Seock Do, Jung A Kim, Yoonjee Hong, Chae Eun Lim, Jae-Hwa Suh* and Junghwa An*

National Institute of Biological Resources, Incheon 22689, Republic of Korea

**Correspondent: amphibia@korea.kr (J.H. Suh), safety@korea.kr (J. An)*

The first complete mitogenome sequence of the Red-tongue Pit Viper (*Gloydius ussuriensis*) from Korea was characterized using next-generation sequencing. The mitogenome is a circular molecule (17,209 bp) with a typical vertebrate mitogenome arrangement, which consists of 2 ribosomal RNA genes (rRNA), 22 transfer RNA genes (tRNA), two non-coding regions (D-loop), and 13 protein-coding genes (PCGs). The base composition of the mitogenome is 32.7% of A, 27.5% of C, 13.9% of G, and 25.9% of T, with a slight AT bias (58.6%). This phylogenetic analysis infers that *G. ussuriensis* is in the same group as the Chinese *G. ussuriensis* (Accession No. KP262412) and is closely related to *G. blomhoffi* and other species of the genus *Gloydius*. In our study, the complete mitogenome sequence of Korean *G. ussuriensis* was characterized and we provided basic genetic information on this species.

Keywords: *Gloydius ussuriensis*, mitochondrial genome, next-generation sequencing

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Introduction

The Red-tongue Pit Viper (*Gloydius ussuriensis*) lives in Asia (China, Russia, and Korea) and is one of the four species of venomous snakes native to the Korean Peninsula (Do *et al*., 2022a). Unlike other species belonging to the family Viperidae, Korean pit vipers have a red tongue (NIBR, 2010). It is distributed throughout the country, including Jeju Island, and is known to be more widely distributed in lowlands than in highlands (Slevin, 1925; Shannon, 1956; Do *et al*., 2016). The Red-tongue Pit Viper was known as *Agkistrodon blomhoffi ussuriensis* (Emelianov, 1929), but was promoted to a species by Toriba (1986) and classified as *Agkistrodon ussuriensis*. Afterwards, in the 1990s, as a result of phylogenetic study using mitochondrial DNA (ND4 and 16s rRNA genes) for the genus *Agkistrodon*, eight species of pit vipers (*A. blomhoffii*, *caliginosus*, *halys*, *rhesus*, *monticola*, *strauchi*, *tsushimaensis*, and *ussuriensis*) were changed to the genus *Gloydius* (Knight *et al*., 1992; Kraus *et al*., 1996). Phylogenetic studies since the 2000s have shown that the genus *Gloydius* contains at least 23 species worldwide and is divided into two groups: brevicaudus and intermedius (Guo and Zhang, 2002; Shi *et al*., 2018). *Gloydius ussuriensis*, belonging to the brevicaudus group, which has four palatine teeth and 21 dorsal scales, is morphologically different from the intermedius group, which has three palatine teeth and 23 dorsal scales (Gloyd and Conant, 1982; Guo and Zhang, 2002). *Gloydius ussuriensis* is the most common of the pit vipers in Korea, but its population is rapidly decreasing due to poaching, habitat destruction, and climate change (Kim *et al*., 2011; Do and Nam, 2021; Do *et al*., 2022b). Moreover, it is listed as "Least Concern" in the IUCN Red List though, biological information of this species is deficient, including genetic data (NIBR, 2020; Lee *et al*., 2022); only one mitogenomic sequence of a Chinese individual is available data from NCBI. In comparison, other species of the genus *Gloydius* have been studied genetically, including their mitogenomes. In this study, the complete mitogenome of Korean *G. ussuriensis* was sequenced and characterized for the first time, enriching the basic genetic information on this species and genus.

Materials and Methods

The *Gloydius ussuriensis* (NIBRGR0000665344) individual used in the present study was collected from Buan-gun, Jeollabuk-do, South Korea (35°42′9.6″, 126°36′38.72″) and stored in the National Institute of Biological Resources (NIBR: https://www.nibr.go.kr) in

Incheon, South Korea. Sample collection was approved by a local government sampling permit (approval number: Buan-gun, 2020-02). This study was also approved by the NIBR animal ethics committee (NIBR IACUC 20220001). Total genomic DNA was isolated from tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol, and a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was checked using for the amount and purity of the extracted DNA. Next-generation sequencing was performed using an llumina NovaSeq 6000 platform at DNA Link (Seoul, South Korea). GetOrganelle version 1.6.4 (Jin *et al*., 2020) was used for the mitogenome assembly. GeSeq (Tillich *et al*., 2017) annotated protein coding regions and rRNA, and ARAGORN version 1.2.38 (Laslett and Canback, 2004) and tRNEscan-SE version 2.0.5 (Lowe and Chan, 2016) predicted tRNA detection. The complete mitogenome of *G. ussuriensis* (accession number OR680782) has been deposited in GenBank. The maximum likelihood (ML) method was used for constructing phylogenetic tree with 100 bootstrap replicates in MEGA ver. 11 (Tamura *et al*., 2021) and genetic distances was determined using the Kimura 2-parameter (Kimura, 1980). The best-fit-model was a general time-reversible model (GTR) with a gamma distribution (+G) using MEGA. *Achalinus meiguensis* (GenBank accession no. FJ424614) was used as an outgroup.

Results and Discussion

The complete circular *G. ussuriensis* mitogenome was 17,209 bp in length and composed of two rRNA genes, 22 transfer RNA genes, two putative control (D-loop) region, and 13 protein-coding genes (PCGs). The base composition of the mitogenome was 32.7% of A, 27.5% of C, 13.9% of G, and 25.9% of T, with a slight AT bias (58.6%). In the mitogenome of *G. ussuriensis*, one PCG (ND6) and eight tRNAs (tRNA-Pro, Gln, Ala, Asn, Cys, Tyr, Ser, and Glu) genes were located on the light strand (L-strand), and other genes were located on the heavy strand (H-strand) (Table 1). The start codon of 10 PCGs was ATG, with the exception of three COX1 (GTG), ND1 (ATA), and ND3 (ATC). There were only five stop codon-terminated PCGs in intact vertebrate mitochondria: TAA (ATP6, ND4L, and ND5), AGG (ND6), and AGA (COX1). The other eight genes ended with incomplete stop codons: AAT (ND1 and COX2), CCT (COX3 and CYTB), TAT (ND2), ATA (ATP8), AGT (ND3), and AAG (ND4). For the phylogenetic analysis (Fig. 1), we selected the 13 protein-coding genes (11,367 bp) of *G. ussuriensis* from South Korea, and 36 mitogenome sequences of 27 Crotalinae species obtained from GenBank. This phylogenetic analysis infers *G. ussuriensis* being in the same group as the Chinese *G. ussuriensis* (Accession No. KP262412) with 0.037 of genetic distance and is closely related to *G. blomhoffi* and other species of the genus *Glyodius*. This result was similar to that presented in Lee *et al*. (2021) which indicated that South Korea *G. saxatilis* mitogenome closely related to

Table 1. Mitogenome structure of *Gloydius ussuriensis*.

Gene/elements	Position		Size (bp)	Strand
	Origin	Stop		
$tRNA^{Phe}$	1	64	64	H
12S rRNA	65	971	907	H
$tRNA^{Val}$	972	1,034	63	H
16S rRNA	1,035	2,512	1,478	H
ND1	2,513	3,473	961	H
$tRNA^{Ile}$	3,474	3,542	69	H
$tRNA^{Pro}$	3,547	3,609	63	L
d-loop	3,610	4,637	1,028	H
$tRNA^{Leu}$	4,638	4,710	73	H
$tRNA^{Gln}$	4,711	4,779	69	L
$tRNA^{Met}$	4,780	4,842	63	H
ND2	4,843	5,866	1,024	H
$tRNA^{TRP}$	5,867	5,932	66	H
$tRNA^{Ala}$	5,933	5,997	65	L
$tRNA^{Asn}$	5,998	6,070	73	L
$tRNA^{Cys}$	6,109	6,163	55	L
$tRNA^{Tyr}$	6,170	6,230	61	L
COX1	6,232	7,833	1,602	H
$tRNA^{Ser}$	7,834	7,891	58	L
$tRNA^{Asp}$	7,892	7,954	63	H
COX2	7,955	8,639	685	H
$tRNA^{Lys}$	8,639	8,702	64	H
ATP8	8,702	8,865	164	H
ATP6	8,857	9,537	681	H
COX3	9,537	10,320	784	H
$tRNA^{Gly}$	10,321	10,381	61	H
ND3	10,382	10,724	343	H
$tRNA^{Arg}$	10,725	10,788	64	H
ND4L	10,789	11,079	291	H
ND4	11,079	12,415	1,337	H
$tRNA^{His}$	12,420	12,479	60	H
$tRNA^{Ser}$	12,480	12,534	55	H
$tRNA^{Leu}$	12,536	12,607	72	H
ND5	12,609	14,396	1,788	H
ND6	14,392	14,913	522	L
$tRNA^{Glu}$	14,927	14,975	49	L
Cyt *b*	14,976	16,089	1,114	H
$tRNA^{Thr}$	16,090	16,153	64	H
d-loop	16,154	17,209	1,056	H

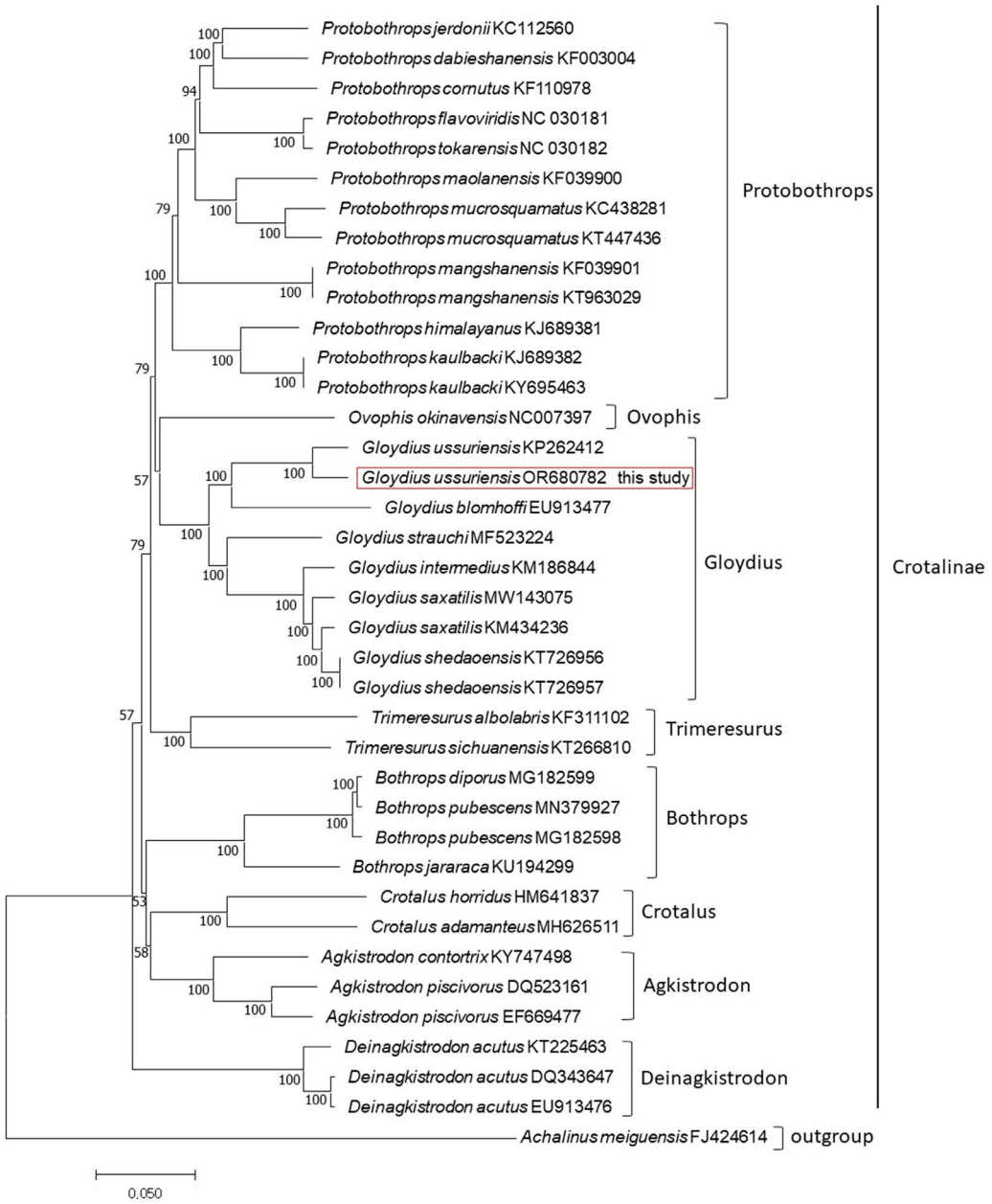

Fig. 1. Maximum likelihood (ML) phylogenetic tree constructed with 37 viper mitogenomes, based on 13 concatenated protein-coding genes. *Achalinus meiguensis* (FJ424614) was used as an outgroup species and bootstrap values are shown at the nodes. The mitogenome of *Gloydius ussuriensis* (OR680782) was determined in this study.

G. intermedius and *G. shedaoensis*. Our results present here a complete mitogenome of *G. ussuriensis*, delivering important data for molecular species identification and further evaluating phylogenetic relationships within the Crotalinae.

Conflicts of Interest

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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References

Do, M.S., J.W. Lee, H.J. Jang, D.I. Kim and J.C. Yoo. 2016.

Interspecific Competition and spatial Ecology of three Species of Vipers in Korea: An Application of Ecological niche-based Models and GIS. Korean Journal of Environment and Ecology 30(2):173-184.

Do, M.S. and K.B. Nam. 2021. Distribution Patterns and Ecological Niches of the Red-tongued Pit Viper (*Gloydius ussuriensis*) and the Central Asian Pit Viper (*Gloydius intermedius*) in Cheonmasan Mountain, South Korea. Russian Journal of Herpetology 28(6):348-354.

Do, M.S., S.J. Son, G. Choi, N. Yoo, D.I. Kim, K.S. Koo and H.K. Nam. 2022a. The establishment of ecological conservation for herpetofauna species in hotspot areas of South Korea. Scientific Reports 12(1):1-13.

Do, M.S., S. Choi, H.J. Jang and J.H. Suh. 2022b. Predicting the Distribution of Three Korean Pit Viper Species (*Gloydius brevicaudus*, *G. ussuriensis*, and *G. intermedius*) under Climate Change. Russian Journal of Herpetology 29(5):262-274.

Emelianov, A.A. 1929. Snakes of the Far Eastern District [In Russian]. Mem. Vladivostok Sect. Russian State Geogr. Soc. 3(20):1-208.

Gloyd, H.K. and R. Conant. 1982. The classification of the Agkistrodon halys complex. Japanese Journal of Herpetology 9(3):75-78.

Guo, P. and F.J. Zhang. 2002. Phylogenetic and biogeographic studies on *Deinagkistrodon* and *Gloydius* in China. Journal-Sichuan University Natural Science Edition 39(2):378-381.

Jin, J.J., W.B. Yu, J.B. Yang, Y. Song, C.W. dePamphilis, T.S. Yi and D.Z. Li. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):1-31.

Kim, J.B., M.S. Min, D.S. Bark and J.Y. Song. 2011. Red data book of endangered birds in Korea (NIBR). National Institute of Biological Resources, Incheon.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Knight, A., L.D. Densmore and E.D. Rael. 1992. Molecular systematics of the Agkistrodon complex. Biology of Pit vipers. 49-69.

Kraus, F., D.G. Mink and W.M. Brown. 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. Copeia. 1996(4):763-773.

Laslett, D. and B. Canback. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 32(1):11-16.

Lee, Y.S., M.S. Do, H.S. Jeon, S.C. Lee, J.H. Jung, J.H. Suh and J. An. 2021. Complete mitochondrial genome of Gloydius saxatilis (Viperidae: Crotalinae) from Korea. Mitochondrial DNA Part B. 6(2):645-647.

Lee, Y.S., M.S. Do, W. Kim, H.S. Jeon, S.C. Lee, J.H. Jung and J. An. 2022. Phylogenetic relationships between three Korean pit viper *Gloydius* (Serpentes: Crotalinae) species using mitochondrial DNA genes. Genes & Genomics. 44(5):517-526.

Lowe, T.M. and P.P. Chan. 2016. tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 44(W1): W54-57.

NIBR. 2010. NIBR. https://www.nibr.go.kr. National Institute of Biological Resources, Incheon.

NIBR. 2020. Red data book of Republic of Korea (Amphibians and Reptiles). Incheon, pp. 110-117.

Shannon, F.A. 1956. The reptiles and amphibians of Korea. Herpetologica 12(1):22-49.

Shi, J.S., D.C. Yang, W.Y. Zhang, L.F. Peng, N. Orlov, F. Jiang, L. Ding, M. Hou, X.L. Huang, S. Huang and P.P. Li. 2018. A new species of the *Gloydius strauchi* complex (Crotalinae: Viperidae: Serpentes) from Qinghai, Sichuan, and Gansu, China. Russian Journal of Herpetology 25(2):126-138.

Slevin, J.R. 1925. Contributions to oriental herpetology II. Korea or Chosen. Proc. Calif. Acad. Sci. 14:89-100.

Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7):3022-3027.

Tillich, M., P. Lehwark, T. Pellizzer, E.S. Ulbricht-Jones, A. Fischer, R. Bock and S. Greiner. 2017. GeSeq-versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45 (W1): W6-W11.

Toriba, M. 1986. The classification of the genus *Agkistrodon* with 21 scale rows in Eastern Asia. Acta Herpetol. Sinica. 5:61-62.

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