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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of *Rana coreana* (Anura: Ranidae)

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ABSTRACT

Rana coreana is a brown frog species native to the Korean Peninsula. We characterized the complete mitochondrial genome of the species. The mitochondrial genome sequence of *R. coreana* is 22,262 bp and comprises 13 protein-coding genes, two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and two control regions (CRs). The CR duplication and gene organization were identical to those observed in *Rana kunyuensis* and *Rana amurensis*. A total of 13 protein-coding genes were used to examine the phylogenetic relationships between this species and the genus *Rana*. *R. coreana* living on the Korean Peninsula, formed a cluster with *R. kunyuensis* and *R. amurensis*, with *R. coreana* showing the closest phylogenetic affinity for *R. kunyuensis*.

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Rana coreana; brown frog; mitochondrial genome; CR duplication

Introduction

Rana coreana (Okada, 1928) is one of three brown frog species found in Korea, along with *Rana uenoi* and *Rana huanrenensis* (Kim et al. 2002; Song et al. 2006; Yang et al. 2017). *R. coreana* is also known as *Rana amurensis*; however, according to Song et al. (2006), it demonstrates morphological and genetic differences from *R. amurensis*. *R. coreana* differs from other brown frogs in having a continuous white line along the upper lip and distinct dark speckling extending from behind the eardrums to the snout tip (Figure 1). *R. coreana* is an endemic species restricted to Korea. In 2015, *R. kunyuensis*, which inhabits the Kunyu Mountains of the Shandong Peninsula in China, was pronounced as a junior synonym of *R. coreana* (Zhou et al. 2015). The mitochondrial genome sequence of *R. kunyuensis* has been reported by Li, Yin, et al. (2016). However, there is no complete mitochondrial genome of *R. coreana*. This study aimed to present the complete mitochondrial genome, which contributes to the genetic diversity of brown frogs.

Materials

The *R. coreana* specimen was collected from Boeun-gun, Chungcheongbuk-do, Republic of Korea (N32°34′15.46″, E124°52′24.38″). Tissue biopsy was performed at the National Institute of Biological Resources (NIBR, Incheon, Republic of Korea; https://www.nibr.go.kr/cmn/main/enMain.do; contact Jung A Kim (jakim21@korea.kr) under voucher number NIBRGR0000654507). The sampled frog was released immediately after sampling and bleeding was stopped using an alcohol swab.

Methods

DNA was extracted from 2 mm frog toe samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) per the manufacturer's protocol. The MGIEasy DNA Library Prep Kit was obtained from MGI (Shenzhen, China), and next-generation sequencing (NGS) reads were generated from the DNBSEQ-T7RS (MGI, Shenzhen, China) platform with a read length of 150 bp. The mitochondrial genome was assembled using MitoZ (v.2.3) (Meng et al. 2019) and two gaps were assembled using long-PCR analysis and Sanger sequencing (Table S1 and Figure S1). Sanger sequencing was used to build an assembly using CAP3 (version date 02/10/15) (Figure S2) (Huang and Madan 1999). Small gaps in the assembly were re-mapped using MITOBim (v.1.9.1) (Hahn et al. 2013) and error correction was conducted using PILON (v.1.24) (Walker et al. 2014). The final assembly was manually curated (Table S2). Gene prediction of *R. coreana* mitogenome was performed using GeSeq (Tillich et al. 2017) and MITOS (Bernt et al. 2013). A mitogenome map of *R. coreana* was generated using CGView (https://proksee.ca) (Grant and Stothard 2008).

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Figure 1. *Rana coreana* specimen. *Rana coreana* is a brown frog found in South Korea. This species differs from other brown frogs in having a continuous white line along its upper lip, as shown in the figure. Photograph was taken of National Institute of Biological Resources (NIBR) immersion specimens (NIBR AM0000000711).

We constructed a phylogenetic tree for the 16 *Rana* species using 13 protein-coding genes with the *R. sylvatica* as an outgroup. To construct the phylogenetic tree, we used the neighbor-joining algorithm with the Poisson model in MEGA11 (Tamura et al. 2021) and conducted bootstrap analysis with 500 replicates to assess the tree robustness. Tree information was visualized using FigTree (ver. 1.4.4) (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The entire mitochondrial genome (mitogenome) sequence of *R. coreana* (GenBank accession: ON920705) was 22,262 bp in length, including 13 protein-coding genes, two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes (Figure 2, Table S2). The mitogenome contained two control regions (CRs) that increased the genome size of *R. coreana* (Figure S3). Phylogenetic relationships among the mitogenomes of 15 East Asian *Rana* species revealed the closest affinity between *R. coreana* and *R. kunyuensis* (Figure 3).

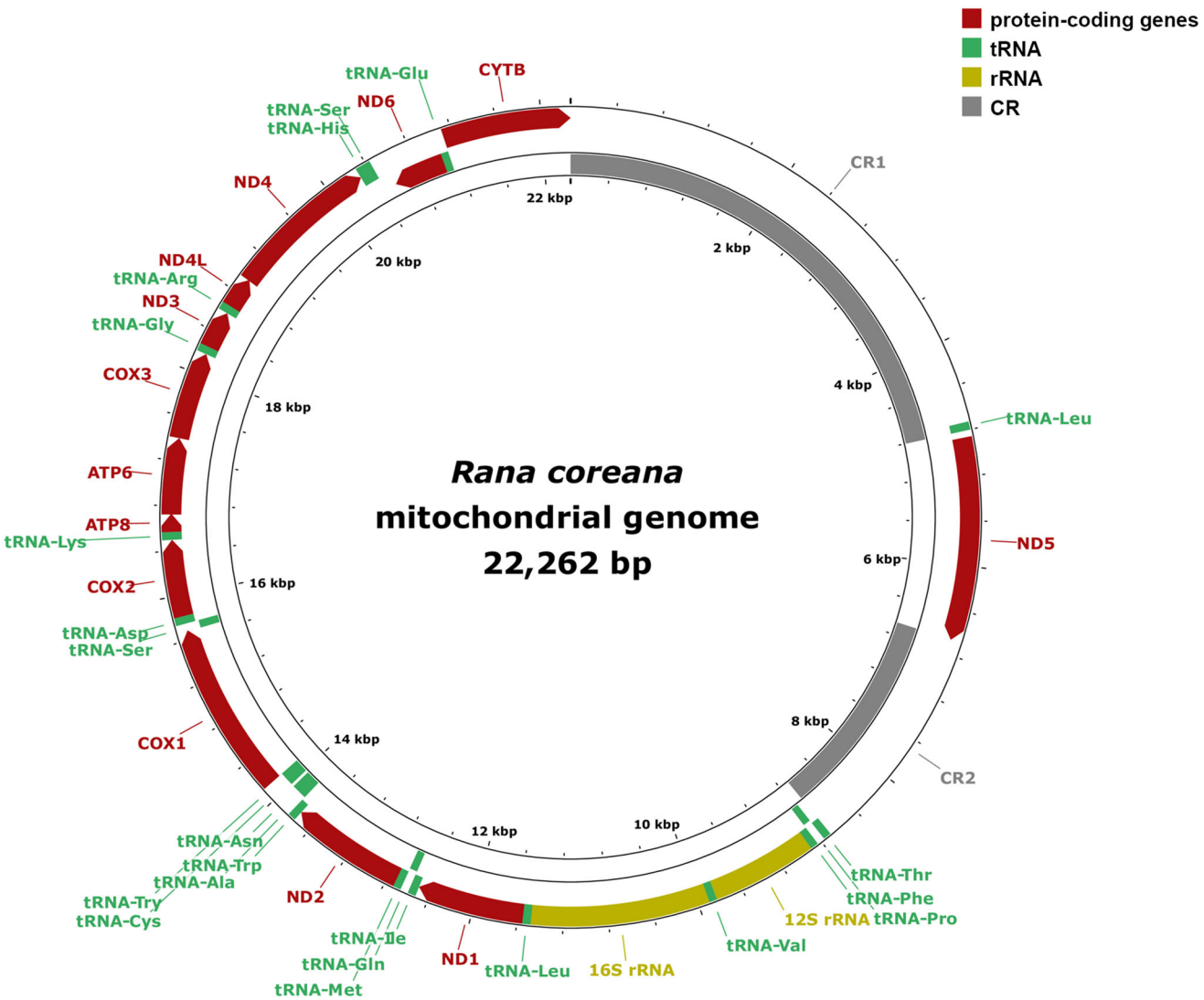

Figure 2. Mitochondrial genome map of *Rana coreana*. Mitochondrial genome map. Genes located outside the circle are transcribed in the heavy-strand direction, whereas genes inside the circle are transcribed in the light-strand direction. The genomic coordinates of *R. coreana* mitochondrial genes are summarized in Table S2.

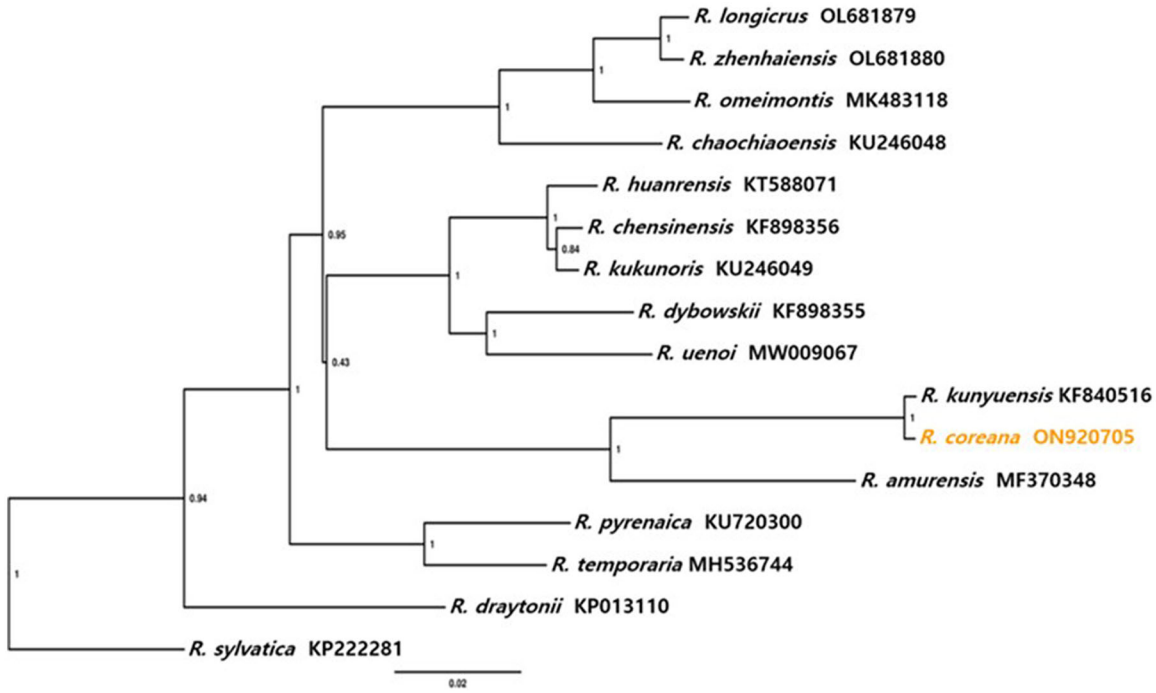

Figure 3. Phylogenetic tree relationship. The phylogenetic tree of 16 species in *Rana* based on mitochondrial genome, including *R. longicrus* (Chen et al. 2022), *R. zhenhalensis* (Chen et al. 2022), *R. omeimontis* (Jiang et al. 2020), *R. chaochiaoensis*, *R. huanrensis* (Dong et al. 2016), *R. chensinensis* (Li, Lei, et al. 2016), *R. kukunoris* (Wang et al. 2020), *R. dybowskii* (Li, Lei, et al. 2016), *R. uenoi* (Suk et al. 2021), *R. kunyuensis* (Li, Yin, et al. 2016), *R. amurensis* (Liu et al. 2017), *R. pyrenaica* (Peso-Fernãndez et al. 2016), *R. temporaria* (Chen 2018), *R. draytonii* (Li, Lei, et al. 2016), and *R. sylvatica* (Ni et al. 2016). *R. coreana* (orange) is available under NCBI GenBank accession number ON920705. The GenBank accession numbers for the sequences are indicated next to the species names. Bootstrap values right to the nodes.

Discussion and conclusions

We assembled the mitogenome of *R. coreana*, which was 22,262 bp in length, with CR duplication (Figure 2 and Figure S3). It is considerably larger than the standard vertebrate mitogenomes (Formenti et al. 2021). Moreover, the *R. coreana* mitogenome shares CR duplication and rearrangement with two other East Asian brown frogs, *R. kunyuensis* and *R. amurensis* (Figure S4) (Li, Yin, et al. 2016; Liu et al. 2017). These three taxa form a separate clade in the phylogenetic tree obtained for the 16 *Rana* species using 13 protein-coding genes (Figure 3). This finding supports both the common ancestry and evolutionary conservation of CR duplication and the unique gene order (Chen et al. 2022). The sister relationship between *R. coreana* and *R. kunyuensis* and the small nucleotide divergence of their mtDNAs suggest that nuclear genes should be studied to verify their taxonomic status. These findings support those of previous studies and highlight the evolutionary conservation of this genomic change in closely related *Rana* species. Overall, our study contributes to the understanding of the genetic diversity and evolution of the genus *Rana* and provides valuable insights for future studies in this field.

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Author contributions

All authors contributed to the conception and design of this study. M. M. and J. A. K. conducted material preparation and species identification. J. K., J. C., and M. S. K. conducted the bioinformatics data processing and analyses. J. B. and J. A. obtained funding, conceptualized the study, and provided supervision. J. K., J. A. K., and M. M. wrote and revised the first draft of the manuscript. All authors commented on the previous versions of the manuscript. All the authors have read and approved the final version of the manuscript.

Ethical approval

Sample collection protocols were approved by the Institutional Animal Care and Use Committee (approval number: NIBR IACUC 20220001).

Disclosure statement

J. B. is the CEO of Clinomics, Inc. The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this study.

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Data availability statement

Genome sequencing data supporting the findings of this study are available in the NCBI GenBank database at ncbi.nlm.nih.gov. The genome sequence data that support the findings of this study are openly

available in GenBank (https://www.ncbi.nlm.nih.gov/) under accession no. ON920705. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA909043, SRR22542064, and SAMN32064903, respectively.

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