Epicrates crassus SNAKE: COMPLETE MITOCHONDRIAL GENOME SEQUENCE

SERPENTE Epicrates crassus*: SEQUÊNCIA COMPLETA DO GENOMA MITOCONDRIAL*

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ABSTRACT

Epicrates crassus Cope, 1862 is a snake distributed in South America and a target species for illegal animal trafficking. In this work, we sequenced and described this species complete mitochondrial genome sequence. The mtDNA of *E. crassus* has 17,379 bp, with 22 transfer RNA genes, 13 protein-coding genes, two ribosomal RNA genes, and two non-coding control regions. Phylogenetic reconstruction, based on the complete mitogenome sequence of *E. crassus* and 18 other mitogenomes, confirmed the best grouping of this species with other members of the Boidae family. These new data can contribute to correctly identifying *E. crassus*, which would represent one more tool in the fight against the illegal trafficking of this species.
KEYWORDS: biodiversity, animal trafficking, biosystematics

RESUMO

Epicrates crassus Cope, 1862 é uma serpente distribuída na América do Sul e espécie-alvo do tráfico ilegal de animais. Neste trabalho, sequenciamos e descrevemos a sequência completa do genoma mitocondrial desta espécie. O mtDNA de *E. crassus* possui 17.379 pb, com 22 genes de RNA de transferência, 13 genes codificadores de proteínas, dois genes de RNA ribossômico e duas regiões de controle não codificantes. A reconstrução filogenética, baseada na sequência completa do mitogenoma de *E. crassus* e de outros 18 mitogenomas, confirmou o melhor agrupamento desta espécie com outros membros da família Boidae. Estes novos dados podem contribuir para a correta identificação de *E. crassus*, o que representaria mais uma ferramenta no combate ao tráfico ilegal desta espécie.
PALAVRAS-CHAVE: biodiversidade, tráfico de animais, biossistemática

INTRODUCTION

The snake *Epicrates crassus* Cope, 1862, popularly known as "jiboia arco-íris" (Figure 1), occurs in South America – Argentina, Bolivia, Brazil, and Paraguay – and feeds mainly on mammals[1,2]. This species belongs to the Boidae family, commonly traded as a pet[2]. For this reason, this can be a target species for the illegal animal trade. Here we present the complete sequence of the mitochondrial genome of *E. crassus*.

Figure 1. Specimen of the Boidae snake *Epicrates crassus*. The photograph was kindly provided by Criatório Jiboias Brasil, located in Betim, Minas Gerais, Brazil.

MATERIALS AND METHODS

The *E. crassus* specimen was provided by Criatório Jiboias Brasil, Minas Gerais, Brazil (Brazilian Institute for the Environment and Renewable Natural Resources Registration: CTF 5520052) and deposited at the Taxonomic Collections Center (CCT-UFMG), under registration UFMG–3430. DNA was

obtained by extracting and purifying total DNA from muscle tissue. The genomic library was constructed using Nextera DNA Sample Preparation protocol, and the NGS sequencing was performed on a MiSeq (Illumina) with Reagent Kit v3 (600 cycles) for paired-end strategy. The obtained reads were filtered (>50 pb) and trimmed with a value ≥ Q30, and subsequently, mapped using other snake mitochondrial genomes as a reference, with Geneious Prime 2022.0.2[3]. Furthermore, a *de novo* assembly was performed to confirm the mitogenome sequence. Annotation was performed by MITOS Web Server[4]. The mitochondrial genome was mapped with OGDRAW[5], and the phylogenetic analysis was performed using MEGA11[6]. Another 17 complete mitogenomes of snakes from seven different families were used for the phylogenetic tree construction. The mitogenome of *Leiolepis guttata* (Reptilia, Agamidae) was used as an outgroup.

RESULTS AND DISCUSSION

The complete mtDNA sequence of *E. crassus* has 17,379 pb (GenBank accession number OQ679742). As found in other snakes[7–9], *E. crassus* mitogenome presents 22 tRNA genes, 13 protein-coding genes (PCGs), two rRNA genes, and two non-coding control regions instead of one, as typically found in vertebrates and invertebrates[10–12]. The mitochondrial genome map of *E. crassus* is shown in Figure 2. The landscape of nitrogenous bases was: A–T = 61.1% and C–G = 38.9%, maintaining the pattern found in other vertebrates with a higher A–T base ratio than G–C bases[13].
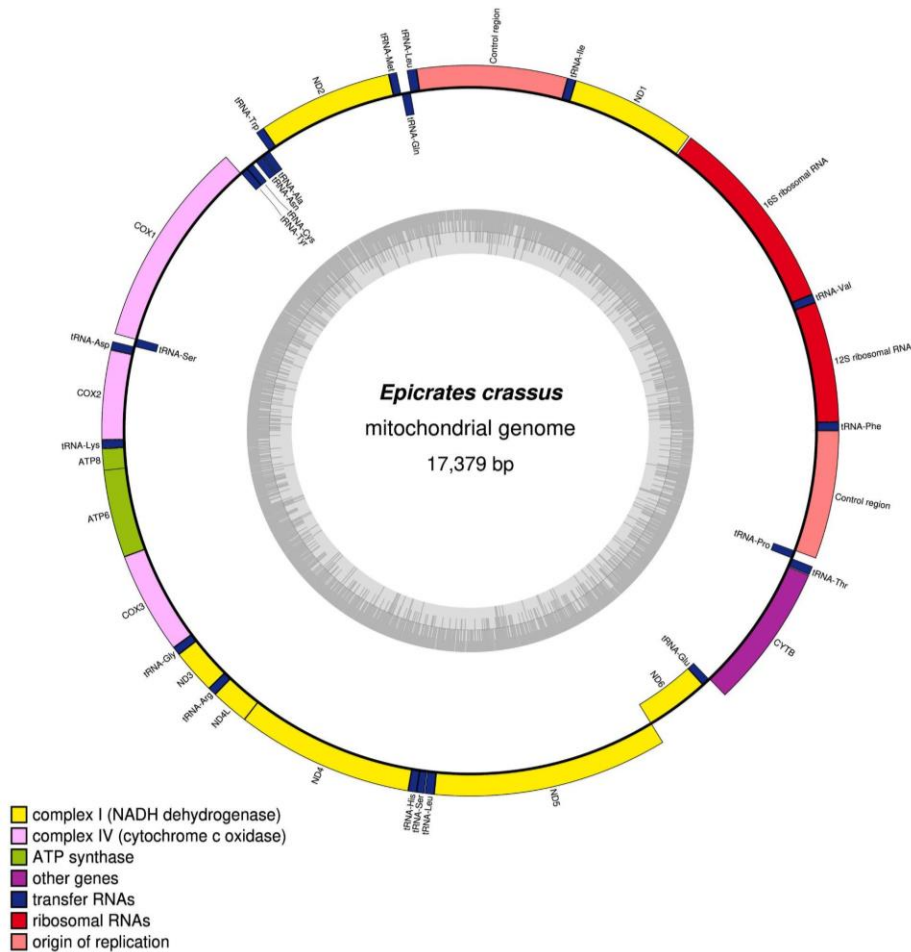
Figure 2. Map of the complete mitogenome of *Epicrates crassus*, with its 22 tRNA, 13 protein-coding genes, two rRNA, and two control regions (D-loops), distributed along its 17,379 bp. The map shows the location of genes on the light and heavy strands (inside and outside the ring, respectively) of the mitochondrial genome. The inner graph, in gray, shows the GC content.

The mitogenome of the snake *E. crassus* contains nine protein-coding genes (ND1, ATP8, ATP6, COX3, ND4L, ND4, ND5, ND6, and CYTB) whose start codon is ATG. Moreover, it has two genes (COX1 and COX2) that starts with GTG as the start codon, one gene (ND2) that starts with ATA, and another (ND3) that starts with ATT. Regarding the stop codon, three of the 13 genes (ND1, COX3, and ND3) have an incomplete T-- stop codon, which is completed for TAA by post-transcriptional polyadenylation[14]. Five genes (COX2, ATP8, ATP6, ND4L, and ND5) have complete TAA as a stop codon, while two genes

(ND2 and CYTB) have TAG as a stop codon. Two genes (COX1 and ND4) end with AGG; the remaining gene (ND6) has AGA as a stop codon.

Eight of the 13 protein-coding genes have overlapping regions (ATP8/ATP6, ATP6/COX3, ND4L/ND4, and ND5/ND6), and all protein-coding genes are present on the heavy strand, except for ND6, which is located on the light strand. Eight of the 22 transfer RNA (tRNA) genes are present in the light strand (trnQ, trnA, trnN, trnC, trnY, trnS2, trnE and trnP); the other 14 tRNAs and the two ribosomal RNA (rRNA) genes, are found in the heavy strand. We also registered the two control regions (D-loops) and the light-strand replication origin of *E. crassus*.

Phylogenetic reconstruction using the mitochondrial genome of *E. crassus* with another 18 mitogenomes (17 snakes and one outgroup) separated the seven snake families into their respective clades (Figure 3). *Epicrates crassus* was inserted into the clade of species belonging to the Boidae family and was phylogenetically closer to the *Chilabothrus argentum* species.
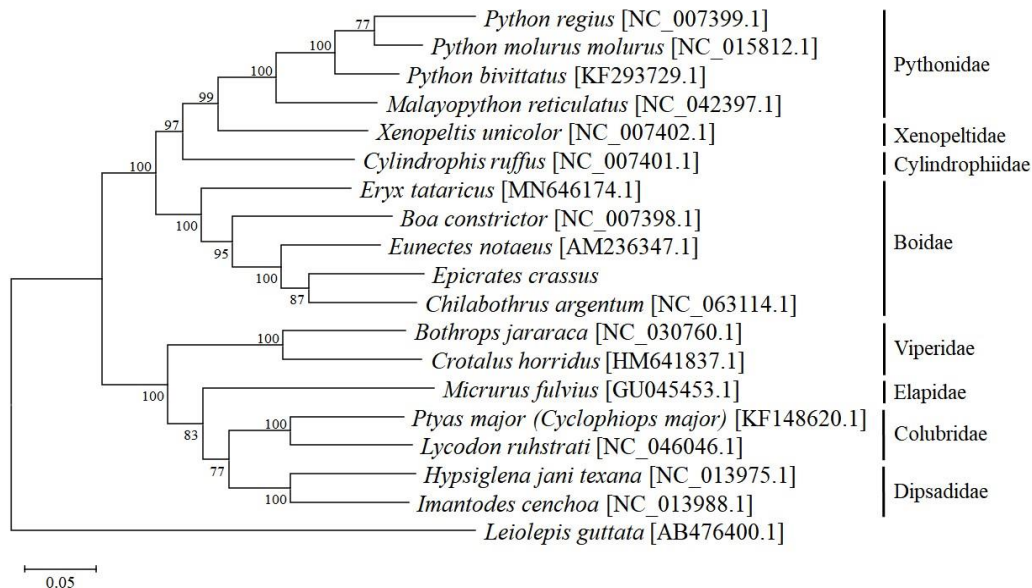

Figure 3. Maximum likelihood phylogenetic reconstruction of *Epicrates crassus* complete mitochondrial genome (GenBank accession OQ679742) with 18 other mitogenomes, using 1000 bootstrap replicates (numbers near the nodes). Accession numbers are in square brackets. The family of each snake species is shown to the right of each clade. The mitogenome of *Leiolepis guttata* was used as an outgroup.

Here we described the complete mitogenome of the snake *E. crassus*, a species traded as a pet. These new data can contribute to understand the evolution of this group and correctly identify this species. Moreover, these data can help adopt public policies aimed at combating the illegal trafficking of these animals.

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