

First complete mitochondrial genome of the Saharan striped polecat (*Ictonyx libycus*) and its phylogenetic relationships within Mustelidae

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Abstract

The Saharan striped polecat (*Ictonyx libycus*) is endemic to Africa, inhabiting the edges of the Saharan Desert. Little is known about the biology or genetic status of this member of the weasel family (Mustelidae). We present the first complete mitochondrial genome of the Saharan striped polecat, assembled from data generated using a genome skimming approach. The assembled mitogenome is 16,549 bps in length and consists of 37 genes including 13 protein-coding genes, 2 rRNAs, 22 tRNAs, and a control region. Phylogenetic analysis confirmed the placement of the Saharan striped polecat within the subfamily Ictonychinae and supports revision of the taxonomy of the genera *Ictonyx* and *Poecilogale*.

Keywords: *Ictonyx libycus*; mitochondrial genome; evolutionary relationships; phylogeny

The Saharan striped polecat, *Ictonyx libycus* (Hemprich and Ehrenberg, 1833) is a member of the Mustelidae, the most speciose family within the mammalian order Carnivora. This mesocarnivore is endemic to the arid shrublands and grasslands of northern Africa, inhabiting the Mediterranean biome from Egypt to Morocco and the Sahel from Mauritania to Eritrea (Ahmim and Do Linh San, 2015). Although globally listed as ‘Least Concern’ on the IUCN Red List of Threatened Species, it is locally threatened in different countries (Ahmim and Do Linh San, 2015). For example, *I. libycus* is poached in Tunisia for traditional medicine, and is thus protected in that country (Ahmim and Do Linh San, 2015). Like many other animal and plant species that inhabit the Sahara-Sahel, they also likely face threats from habitat loss due to human activities and conflicts (Brito et al., 2014). *Ictonyx libycus* is one of two species belonging to the genus *Ictonyx*, the other being the zorilla, *I. striatus* (Perry, 1810). Here, we present the first

complete mitogenome of a Saharan striped polecat to better understand its evolutionary history within the Mustelidae.

A tissue sample from a road-killed Saharan striped polecat was collected in 2011 near Timokrarin al Hamra in Rio de Oro Province, Western Sahara (24°45'25.4"N 14°52'05.5"W), preserved in ethanol and stored at room temperature and later at -80°C. The sample was deposited in the personal collection of K-P. Koepfli at the Smithsonian's National Zoo and Conservation Biology Institute (kkoepfli@gmu.edu) under the identification number ILI_5724. The sample was delivered to Psomagen, Inc. (Rockville, MD) where DNA extraction, genomic library preparation, and high-throughput sequencing were performed. The Mag-Bind Blood and Tissue Kit (Omega Bio-Tek Inc., Norcross, GA) was used to extract genomic DNA. DNA concentration and quality were assayed using Picogreen and Victor X2 fluorometry (Life Technologies, Carlsbad, CA), an Agilent 4200 TapeStation (Agilent Technologies, Santa Clara, CA), and 1% gel electrophoresis. Genomic fragments of 350 base pairs (bp) were generated using a Covaris S220 Ultrasonicator (Covaris, Woburn, MA), which were used to prepare a library with the TruSeq DNA PCR-free library kit (Illumina, San Diego, CA), which was quality checked on an Agilent 4200 TapeStation and quantified using a Lightcycler qPCR assay (Roche Life Science, St. Louis, MO). The library was paired-end sequenced (2 x 150 bp) to a depth of 10x on an Illumina NovaSeq 6000 instrument, resulting in a total of 172,687,004 reads (% reads with \geq Q30 score = 92%).

We used FastQC (Andrews, 2010) to evaluate raw reads, which were then downsampled to 40 million reads using BBMap version 38.96 (Bushnell, 2014). Reads were trimmed and filtered

using AdapterRemoval (Lindgreen, 2012) within the PALEOMIX version 1.3.6 pipeline (Schubert et al., 2014). Processed reads were imported into Geneious Prime version 2022.1 (<https://www.geneious.com>) and mapped to the reference mitogenome of the zorilla (GenBank accession: MW257237) using the Geneious mapper set to medium-low sensitivity and five iterations of fine-tuning. A 16,549 bp consensus sequence with an average coverage of 832x was extracted and then annotated in MITOS (Bernt et al., 2013). We obtained 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and the control region. Multiple sequence alignment and phylogenetic analyses were performed in Geneious Prime version 2022.1. We aligned the mitogenome of the Saharan striped polecat to the mitogenomes of 25 other species of mustelids and two species of Procyonidae as outgroups using MAFFT version 7.450 (Katoh and Standley, 2013) with the following settings: algorithm = AUTO, scoring matrix = 200 PAM/k=2, gap open penalty = 1.53, offset value = 0.123. The alignment was visually inspected, and the control region was trimmed. The 15,564 bp alignment was then used to estimate a maximum-likelihood tree with RAxML version 8.2.11 (Stamatakis, 2014) using the GTRGAMMA model of substitution and rapid hill-climbing algorithm. Nodal support was quantified with 100 bootstrap replicates.

Phylogenetic analysis (Figure 1) placed the Saharan striped polecat with other members of the Ictonychinae and sister to a clade containing the zorilla and African striped weasel, *Poecilogale albinucha* (Gray, 1864), with 100% bootstrap support, rendering the genus *Ictonyx* paraphyletic. This arrangement is congruent with previous phylogenetic analyses using multilocus datasets (Koepli et al., 2008; Sato et al., 2012; Law et al., 2018). These results along with our results based on mitogenomes, strongly argue for reclassifying *P. albinucha* in the genus *Ictonyx* (Kaup,

1835). As previously noted, (Koepfli et al., 2008), *I. libycus*, *I. striatus* and *P. (Ictonyx) albinucha* exhibit a pattern of diversification from northern to southern Africa. Our mitogenome of the Saharan striped polecat provides a resource for future phylogeographic studies of this wide-ranging and enigmatic species.

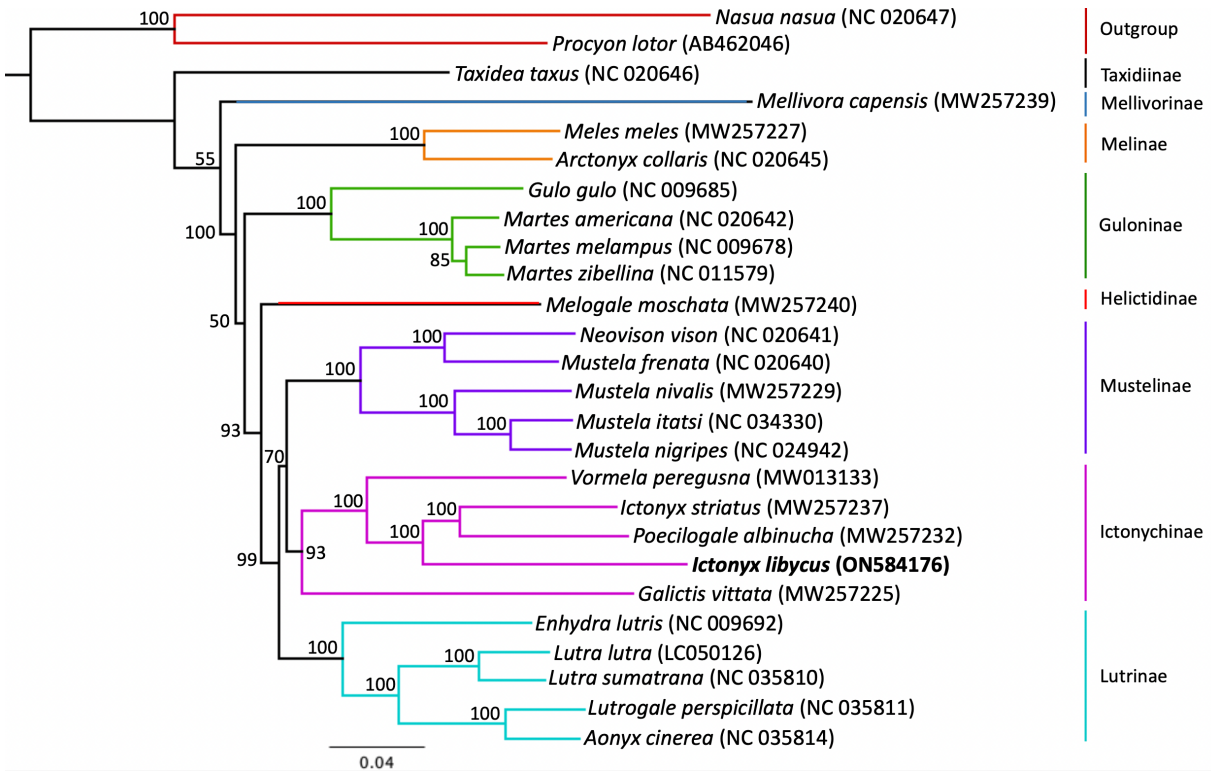


Figure 1. Maximum-likelihood phylogenetic tree showing the relationship of *Ictonyx libycus* within the Mustelidae. Numbers on branches are bootstrap support values (100 replicates). Branch colors highlight each lineage within Mustelidae. GenBank accession numbers are included in parentheses next to the species.

Acknowledgements

We would like to thank Dr. Anneke DeLuycker and Dr. Joshua Davis for supporting our research during the Spring 2022 Endangered Species Conservation course at the Smithsonian-Mason School of Conservation.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Data Availability Statement

The mitochondrial genome sequence is available on GenBank of NCBI at www.ncbi.nlm.nih.gov with the accession number of ON584176. The associated raw reads generated by genome skimming can be found under the BioProject “Lesser-known carnivores”, accession number PRJNA847318.

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