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MITOGENOME REPORT

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The complete mitochondrial genome of Meller's mongoose (*Rhynchogale melleri*)

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ABSTRACT

Meller's mongoose (*Rhynchogale melleri*) is a member of the family Herpestidae (Mammalia: Carnivora) and the sole species in the genus *Rhynchogale*. It is primarily found in savannas and open woodlands of eastern sub-Saharan Africa. Here, we report the first complete mitochondrial genome for a female Meller's mongoose collected in Tanzania, generated using a genome-skimming approach. The mitogenome had a final length of 16,644 bp and a total of 37 annotated genes. Phylogenetic analysis validated the placement of this species in the herpestid subfamily Herpestinae. Ultimately, the outcomes of this research offer a genetic foundation for future studies of Meller's mongoose.

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Introduction

Meller's mongoose, *Rhynchogale melleri* (Gray 1865) is a mesocarnivore in the family Herpestidae, subfamily Herpestinae. It is the only known species in its genus and demonstrates unique physical characteristics from other solitary mongooses in the Herpestinae, such as flatter molars and a snub nose (Veron et al. 2022). It is native to the eastern portion of sub-Saharan Africa, specifically Tanzania, Zimbabwe, Zambia, Mozambique, and northeastern South Africa. Within this region, Meller's mongoose can be found in savannas, open woodlands, and bamboo forests (Stuart and Stuart 2014). While many mongoose species include insects in their diets, Meller's mongoose is unusual in that its diet largely consists of termites in the genera *Hodotermes* and *Macrotermes*. It is mainly nocturnal and therefore elusive, but has been observed in protected areas (Veron et al. 2022). Two subspecies have been described: *R. m. melleri* (Gray 1865) and *R. m. langi* (Roberts 1938), which respectively occupy the northern and southern parts of the species distribution (Gilchrist et al. 2009). The subspecies were described to differ in several morphological characteristics, with *R. m. langi* suggested to have smaller teeth, lighter gray fur, and darker feet relative to *R. m. melleri* (Roberts 1938). However, the validity of the two subspecies has not yet been evaluated using genetic data. The size and trends of the Meller's mongoose population are currently unknown due to a lack of sufficient data, but as of 2015, the species was listed as Least Concern on the IUCN Red List of Threatened Species (White et al. 2015). Meller's mongoose has been incorporated into multiple molecular phylogenetic investigations of the Herpestidae, based on complete or partial mitochondrial gene sequences and nuclear exons and introns (Patou et al. 2009; Perez et al. 2006; Veron et al. 2004), as well as studies on the relationships and timing of the diversification among the families of the order Carnivora (Eizirik et al. 2010). To further elucidate the phylogenetic history of this monotypic lineage, we sequenced, assembled, and annotated the first complete mitogenome for a Meller's mongoose.

Materials

A sample of skeletal muscle tissue from a juvenile female Meller's mongoose (*Rhynchogale melleri melleri*) that had been freshly killed by road traffic was collected on 16 September 1994 by M. Roelke-Parker and G. A. Machange approximately 10 km west of Karatu and 27 km east of the Ngorongoro Conservation Area Authority gate in Tanzania (3° 21.151′ S 35° 36.441′ E). Based on the location of where this specimen was found, we infer that this Meller's mongoose belonged to the nominate subspecies *Rhynchogale melleri melleri*. Care was taken to minimize bacterial contamination

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Table 1. List of species and GenBank accession numbers for the mitogenomes used for the phylogenetic analysis (Figure 3).

Species Name	Family	GenBank Accession Number	References
Cryptoprocta ferox	Eupleridae	MW257203	(Hassanin et al. 2021)
Fossa fossana	Eupleridae	MW257201	(Hassanin et al. 2021)
Galidia elegans	Eupleridae	MW257200	(Hassanin et al. 2021)
Salanoia concolor	Eupleridae	MW257198	(Hassanin et al. 2021)
Atilax paludinosus	Herpestidae	MW257238	(Hassanin et al. 2021)
Bdeogale nigripes	Herpestidae	MW257221	(Hassanin et al. 2021)
Crossarchus platycephalus	Herpestidae	MW257212	(Hassanin et al. 2021)
Cynictis penicillata	Herpestidae	MW257211	(Hassanin et al. 2021)
Galarella sanguinea	Herpestidae	MW257224	(Hassanin et al. 2021)
Helogale parvula	Herpestidae	OP485294	N/A
Herpestes ichneumon	Herpestidae	MW019668	(Boukhdoud et al. 2021)
Ichneumia albicauda	Herpestidae	MW257213	(Hassanin et al. 2021)
Mungos mungo	Herpestidae	MW257205	(Hassanin et al. 2021)
Suricata suricatta	Herpestidae	MW257236	(Hassanin et al. 2021)
Urva auropunctata	Herpestidae	MW401772	N/A
Urva brachyura	Herpestidae	KY117547	(Mohd Salleh et al. 2017)
Urva javanica	Herpestidae	MW257214	(Hassanin et al. 2021)
Xenogale naso	Herpestidae	MW257235	(Hassanin et al. 2021)

Taxonomic names follow the recent classification proposed by Veron et al. (2022). N/A = sequence only published in GenBank.

by sampling an interior portion of one of the thigh muscles, using gloves and a sterile scalpel blade to collect the sample, and then depositing it into a clean glass vial. The sample (ID = RME_94-248) was frozen and initially deposited in the cryobank collection of the former Laboratory of Genomic Diversity, National Cancer Institute, in Fort Detrick, Maryland, USA. It was eventually transferred to the frozen collection of K-P. Koepfli at the Smithsonian-Mason School of Conservation, George Mason University (https://smconservation.gmu.edu/people/klaus-koepfli/, Klaus-Peter Koepfli, kkoepfli@gmu.edu).

Methods

We used a genome-skimming approach to generate sequencing reads to assemble the Meller's mongoose mitogenome to high coverage. A ~0.5 cm^3 piece was sliced from an interior section of the skeletal muscle sample using a sterile scalpel blade to avoid introducing possible bacterial or other biological contaminants in the DNA extraction, since the sample had been collected from a specimen killed on the road. This sample was delivered to Psomagen, Inc. (Rockville, Maryland, USA), where DNA extraction, library preparation, and sequencing were conducted. The Mag-Bind Blood and Tissue Kit (Omega Bio-Tek Inc., Norcross, GA) was used for genomic DNA extraction following the manufacturer's protocol. Picogreen and Victor X2 fluorometry (Life Technologies, Carlsbad, CA) along with an Agilent 4200 Tapestation (Agilent Technologies, Santa Clara, CA) and 1% TBE gel electrophoresis were used to evaluate the concentration and quality of genomic DNA. A Covaris S220 ultrasonicator (Woburn, MA) was used to shear the DNA into fragments of 350 bp, which were used to prepare a genomic library using the TruSeq DNA PCR-free library kit (Illumina, San Diego, CA). The library was quality checked with an Agilent 4200 Tapestation and then quantitated by quantitative PCR using a Lightcycler (Roche Life Science, St. Louis, MO). The library was paired-end sequenced (2 × 150 bp) on an Illumina NovaSeq 6000 instrument to a depth of 5x, resulting in 102,153,502 reads, of which 90.3% had a Q-score of 30.

Raw reads were checked using FastQC (Andrews 2010) and then subsampled to 40 million reads with BBMap version 38.96 (Bushnell 2014). AdapterRemoval (Lindgreen 2012) in PALEOMIX version 1.3.6 (Schubert et al. 2014) was used to trim and filter the subsampled reads, which were subsequently imported into Geneious Prime version 2022.1.1 (https://www.geneious.com). The Geneious mapper was then used to map the reads to the black-footed mongoose (*Bdeogale nigripes*) reference mitogenome (GenBank accession: MW257221; Hassanin et al. 2021) with the settings Sensitivity: medium-low and Fine-tuning: 5 iterations. A consensus sequence was extracted to obtain the final mitogenome assembly. The MITOS2 webserver (Donath et al. 2019) was used to annotate the mitogenome.

The Meller's mongoose mitogenome was aligned to the mitogenomes of 14 other species of Herpestidae and four species of Eupleridae (Table 1), downloaded from the NCBI GenBank database, using MAFFT version 7.450 (Katoh and Standley 2013) in Geneious Prime version 2022.1.1. We used default settings (algorithm = AUTO, scoring matrix = 200 PAM/k = 2, gap open penalty = 1.53, offset value = 0.123) to generate a multiple sequence alignment with a length of 17,422 bp. Because of alignment ambiguities in the tandem repeat region, the control region was removed prior to phylogenetic analysis, resulting in a final alignment of 15,444 bp. We then used the RAxML version 8.2.11 (Stamatakis 2014) plugin in Geneious Prime with the rapid hill-climbing algorithm and the GTR GAMMA model of nucleotide substitution to reconstruct a maximum-likelihood phylogenetic tree. Node support was estimated using 1000 bootstrap replicates and the four species from the Eupleridae were used to root the tree.

Results

A photograph of a Meller's mongoose shows features typical of this species, including the grayish-brown coloration of the body, the long bushy tail with black-colored guard hairs and the blunt muzzle (Figure 1). The consensus sequence length for the Meller's mongoose mitogenome was 16,644 bp with a mean coverage of 1,588x obtained by alignment of 227,813 reads against the *Bdeogale nigripes* reference mitogenome.

Figure 1. Photograph of Meller's mongoose (*Rhynchogale melleri*) taken on June 29, 2015 at 1:04 am in Levubu, South Africa. Based on the location, this animal likely represents the subspecies *R. melleri langi*. The photograph was obtained by a camera trap and provided by Lourens Swanepoel, University of Venda, South Africa.

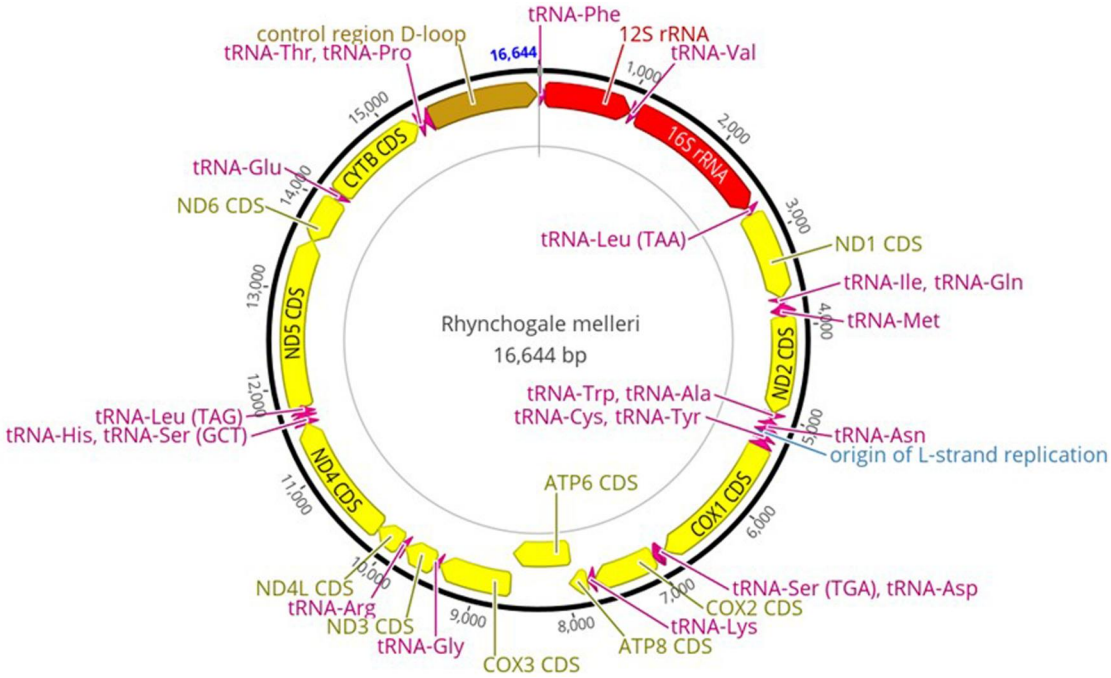

Figure 2. Annotated mitochondrial genome for Meller's mongoose (*Rhynchogale melleri*). Yellow colored bars represent coding sequences for protein-coding genes, red bars represent rRNA genes, magenta bars represent tRNA genes, and the orange bar represents the control region. The direction of transcription is indicated by gene arrow orientation: right direction indicates a plus strand and left direction indicates a minus strand. Relative nucleotide position of each gene in the mitogenome is shown via the black outer ring.

Annotation of the mitogenome assembly produced 13 protein-coding genes, two rRNAs, 22 tRNAs, an origin of replication, and the control region containing the D-loop (Figure 2). Phylogenetic analysis supported the placement of *Rhynchogale melleri* within the Herpestinae and as the sister taxon to the genus *Bdeogale*, represented here by the black-footed mongoose, *B. nigripes* (Pucheran 1855), both with 100% bootstrap support (Figure 3). The mitochondrial genome demonstrates approximately 90-93% similarity compared to other Herpestinae species and 88-89% similarity to species in the Mungotinae included in this study. Our phylogeny also strongly supports the fundamental split between the social

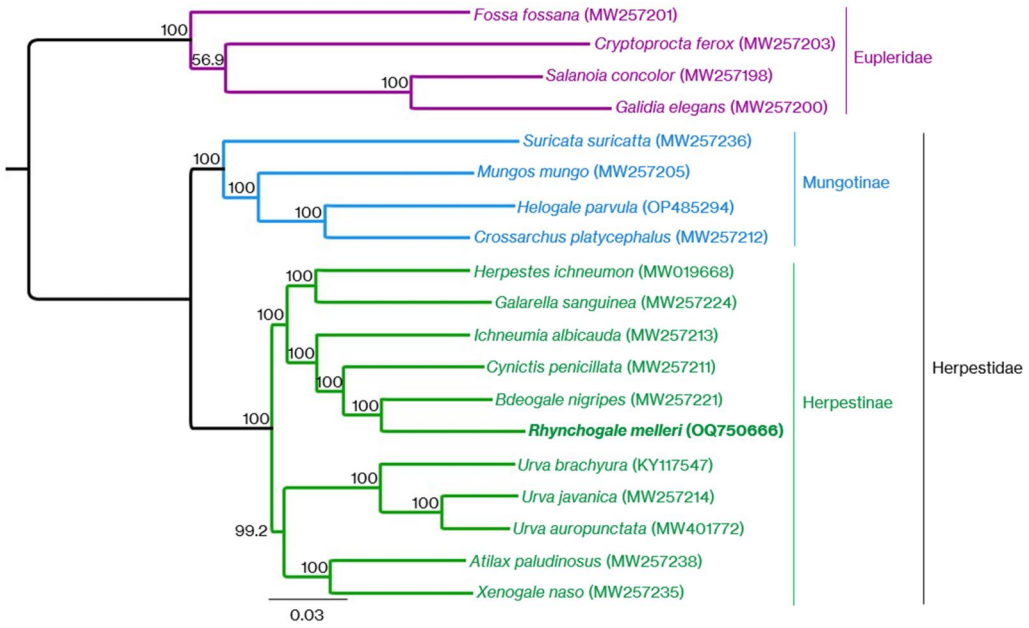

Figure 3. Maximum-likelihood phylogenetic tree demonstrating the relationship of the *Rhynchogale melleri* mitogenome to other species in the Herpestidae. The tree was rooted using the four species of the Eupleridae, shown in purple. The subfamilies Herpestinae (green branches) and Mungotinae (blue branches) are indicated. The numbers on each branch are bootstrap support values out of 1000 replicates. The parentheses contain GenBank accession numbers for each species, and the vertical lines on the side indicate families and subfamilies. The following sequences were used: MW257201 (Hassanin et al. 2021), MW257203 (Hassanin et al. 2021), MW257198 (Hassanin et al. 2021), MW257200 (Hassanin et al. 2021), MW257236 (Hassanin et al. 2021), MW257205 (Hassanin et al. 2021), OP4985294 , MW257212 (Hassanin et al. 2021), MW019668 (Boukhdoud et al. 2021), MW257224 (Hassanin et al. 2021), MW257213 (Hassanin et al. 2021), MW257211 (Hassanin et al. 2021), MW257221 (Hassanin et al. 2021), KY117547 (Mohd Salleh et al. 2017), MW257214 (Hassanin et al. 2021), MW401772, MW257238 (Hassanin et al. 2021), MW257235 (Hassanin et al. 2021).

mongooses (Mungotinae) and the largely solitary mongooses (Herpestinae), in accordance with Veron et al. (2004), Perez et al. (2006), and Patou et al. (2009).

Discussion and conclusion

Through our research, we sequenced, assembled, and analyzed the first-ever complete mitochondrial genome of *Rhynchogale melleri*. We confirmed the validity of this mitogenome by conducting a phylogenetic analysis whose results align with previous DNA-based studies of the Herpestidae. These findings are valuable as they now provide the first and only complete mitochondrial genome for the genus *Rhynchogale*, which only contains the Meller's mongoose. As a result of this research, mitogenomes for 15 of the 34 species (44%) have now been sequenced across both the Herpestinae (11 species) and Mungotinae (4 species) subfamilies, which includes 12 of the 25 species found exclusively or mostly in Africa, and 3 of the 9 species found in Asia (genus *Urva*). Overall, these results provide an important baseline for future studies on the evolutionary history and phylogeography of this elusive mongoose.

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Ethical approval

The sample used in this study was collected from a road-killed Meller's mongoose, and therefore no ethical approval was required.

Authors' contributions

The study was developed by K-P.K., C.W.E., and H.V.F. The sample was collected by M.R-P. and G.A.M. Data analysis and initial manuscript drafting were completed by M.S.S. All authors contributed to manuscript edits and finalization, approved final publication of the manuscript, and are willing to assume responsibility for all aspects of this research.

Disclosure statement

We declare no potential conflict of interest.

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Data availability statement

Raw reads for the Meller's mongoose genome were deposited via the BioProject PRJNA847318 into the NCBI Short Read Archive (BioSample ID: SAMN35218670, SRA accession: SRR24951203). The sequenced and annotated mitogenome for Meller's mongoose was submitted to NCBI GenBank (accession: OQ750666).

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