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Complete mitochondrial genomes of the African clawless (*Aonyx capensis*) and spotted necked (*Hydrictis maculicollis*) otter: structure, annotation, and interspecies variation

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ABSTRACT

Otters are flagship species for pristine habitats and their southernmost distribution in Africa includes two species; *Aonyx capensis* and *Hydrictis maculicollis*. Here, we present novel full mitochondrial genomes of these otter species. The comparable mitogenomes consist of 36 genes including 13 protein-coding genes, 2 ribosomal RNAs, and 22 tRNAs including a hypervariable region. Only 19 out of the 36 genes showed some level of variation between species with the smallest being *trnV* (68 bp difference) and the biggest being *nad5* (1830 bp difference). Such variations may provide guidance in selecting gene regions during marker development for phylogenetic assessments.

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Mitogenomes; otter; Illumina sequencing; phylogenetic analysis

Otters (*Lutrinae*) belong to the largest family of carnivores: Mustelidae (Koepli et al. 2008). In South Africa, there are two species of otter; spotted-necked (*Hydrictis maculicollis*) and African clawless (*Aonyx capensis*). The International Union for Conservation of Nature (IUCN) list these otters as Near Threatened (Jacques et al. 2015; Reed-Smith et al. 2015). In this study, we for the first time sequenced, annotated, and compared mitogenomes of two South African otters.

Specimens were obtained from opportunistic sampling for *A. capensis* (approximate coordinates: 34°02'06.5''S 23°01'27.5''E) and *H. maculicollis* (approximate coordinates: 28°43'43.3''S 20°59'09.5''E) and are stored at the National Zoological Garden (NZG), South African National Biodiversity Institute (SANBI) Biobank at –20 °C. DNA was extracted using the ZymoResearch Quick-DNA Tissue kit (Zymo Research Corp., Irvine, CA). Sequencing was performed on an Illumina HiSeq 2500 (Illumina Incorporated, San Diego, CA). Sequencing produced 2738699 and 2840359 reads for *A. capensis* and *H. maculicollis*, respectively, which were assessed for quality using FastQC version 0.11 (BaseSpace Labs App., Illumina Incorporated, San Diego, CA). Editing and trimming of data were done using Trimmomatic version 0.36 (USADL LAB, Aachen, Germany). Single end read assembly of both genomes was done De Novo, using CLC Genomics Workbench version 6 (CLC Bio, Aarhus, Denmark). The number of reads successfully mapped were 3545 (38 × coverage) and 82244 (850 × coverage) for *A. capensis* and *H. maculicollis*, respectively. Annotation was performed using MITOS web-server version 806 (University of Leipzig, Leipzig, Germany)

and the BLAST Ring Image Generator (Alikhan et al. 2011) was used to perform circular alignments.

The complete mitochondrial genomes of *A. capensis* (16,188 bp) and *H. maculicollis* (16,308 bp) are comparable in length to other otter species. The arrangement of genes is also similar to otters and other mustelid species, 36 genes including 13 protein-coding genes, 2 ribosomal RNAs, and 22 for tRNAs including a hypervariable region. Of these, 27 are codons on the sense (+) strand with the remaining nine on the antisense (–) strand for both species. The 13 protein-coding genes share similar start/stop codons between species with variations only observed at gene *nad2* (start codon), *cox2* (stop), and *nad3* (start) where the codons are ATT, TAA, and ATT for *H. maculicollis* and ATC, TAG, and ATA for *A. capensis*, respectively. Only 19 out of the 36 genes showed some level of variation between the two species with the smallest being *trnV* with a 68 bp difference and the biggest being *nad5* with an 1830 bp difference. Individual GC content calculations for both *A. capensis* and *H. maculicollis* gave 42.7 and 41.3%, respectively which are comparable to a variety of mammals, such as brown bears (41.3%), Eurasian otter (42.1%), and sea otter (41.1%) (Ki et al. 2010). To explore phylogenetic relationships, a maximum likelihood (ML) tree was constructed using available mitogenomes from mustelid species. This phylogenetic reconstruction was performed using the Molecular Evolutionary Genetics Analysis (MEGA) phylogenetic software version 7.0.9 (Pennsylvania State University, State College, PA) (Kumar et al. 2016). The topological structure strongly supports the

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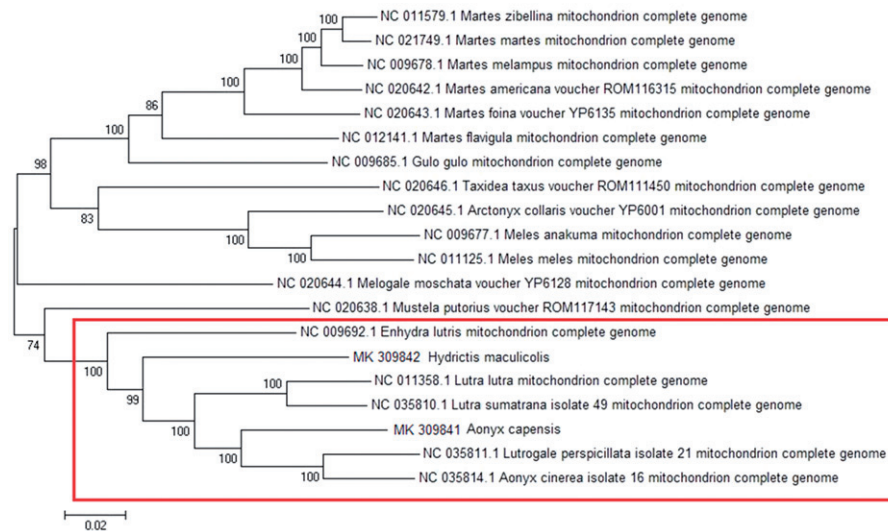


Figure 1. Phylogenetic reconstruction of full mitogenomes from mustelid species. Mitogenomes from this are provided in Genbank with accession numbers MK 309841 (*A. capensis*) and MK 309842 (*H. maculicollis*).

published taxonomy (Koepfli et al. 2008) of the *Lutrinae* family of mustelids with a bootstrap probability of 100% in almost all clades (Figure 1). The two mitogenomes will provide invaluable genetic resources for further studies.

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