

Whispers of the Wild: Genetic Diversity of Carnivores Across Southern Saudi Arabia Through Multilocus DNA Barcoding

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INTRODUCTION & AIM

Carnivores play a crucial ecological role as apex predators, regulating prey populations and maintaining ecosystem stability. In Saudi Arabia, 14 carnivore species from six families (Felidae, Hyaenidae, Mustelidae, Canidae, Herpestidae, and Viverridae) have been reported. However, many populations are declining due to hunting, poisoning, illegal trade, and habitat degradation. Despite their ecological importance, carnivore diversity and distribution in the region remain poorly documented, partly because traditional identification methods based on morphology, field observations, or camera trapping are often limited for rare, nocturnal, or cryptic species.

Recent molecular tools provide new opportunities for biodiversity monitoring. Environmental DNA (eDNA) and DNA barcoding enable species identification from trace genetic material, while portable sequencing technologies allow rapid analysis directly in the field.

This study aims to: Assess carnivore diversity in Saudi Arabia using molecular approaches.; Apply DNA barcoding targeting mitochondrial markers (COI, Cytb, 16S) from environmental samples.; Evaluate the efficiency of portable sequencing with the MinION developed by Oxford Nanopore Technologies for rapid species identification.; Provide molecular data to support monitoring and conservation strategies for carnivores in the region.

METHOD

Sample collection: eDNA samples (scats, tissues, decomposed cadavers) were collected across Saudi Arabia by MRC field teams. Samples were preserved in ethanol (70%) or sterile bags and processed in the laboratory.

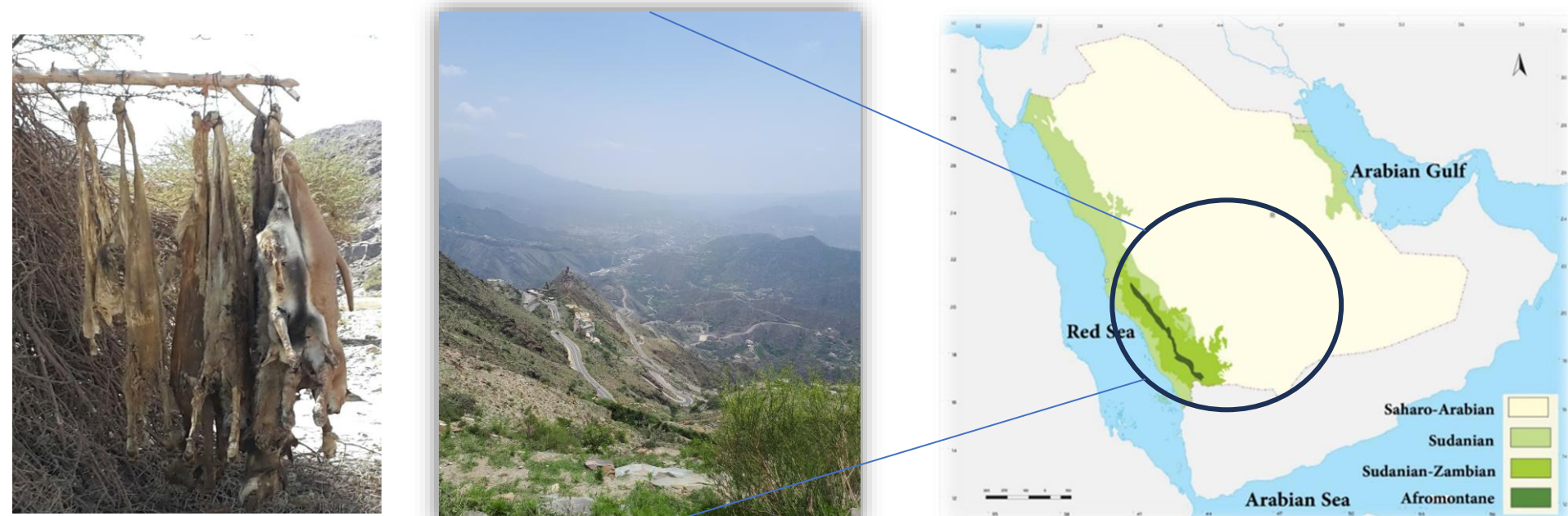


Figure1: eDNA sampling locations across southern Saudi Arabia.

DNA extraction & barcoding: Genomic DNA was extracted using the appropriate kit. We amplified three mitochondrial markers (COI, Cytb, 16S) from 155 samples using tagged primers. Amplicons were sequenced with the MinION platform by Oxford Nanopore Technologies following a metabarcoding workflow. Representative samples were validated by Sanger sequencing.

Bioinformatics analyses: Basecalling and barcode reconstruction were performed using ONTbarcode2. Species identification relied on GBIF SequenceID and BLAST comparisons with GenBank references. Genetic diversity indices, haplotype networks, and pairwise distances were calculated. Phylogenetic relationships (concatenated dataset, 1,608 bp) were inferred using Bayesian inference and Maximum Likelihood approaches.



Figure2: General eDNA workflow with NGS sequencing. a) eDNA sampling and extraction; b) amplification, library preparation and NGS sequencing; c) bioinformatic analysis.

RESULTS & DISCUSSION

Species identification: DNA barcoding successfully identified 152/155 samples (98%). BLAST analyses revealed 149 carnivore individuals + 3 **Procaviidae:** hyraxes *Procavia capensis*.

Carnivores (Five families) : **Felidae:** *Panthera pardus* (10), *Caracal caracal*(8), *Acinonyx jubatus*(3), *Felis lybica*(1) **Viverridae:** *Genetta genetta*(2), **Hyaenidae:** *Hyaena hyaena*(13), **Herpestidae:** *Ichneumia albicauda*(2), *Herpestes javanicus*(1). **Canidae:** *Vulpes vulpes*(33), *Canis lupus*(55), *Canis lupus familiaris*(24).

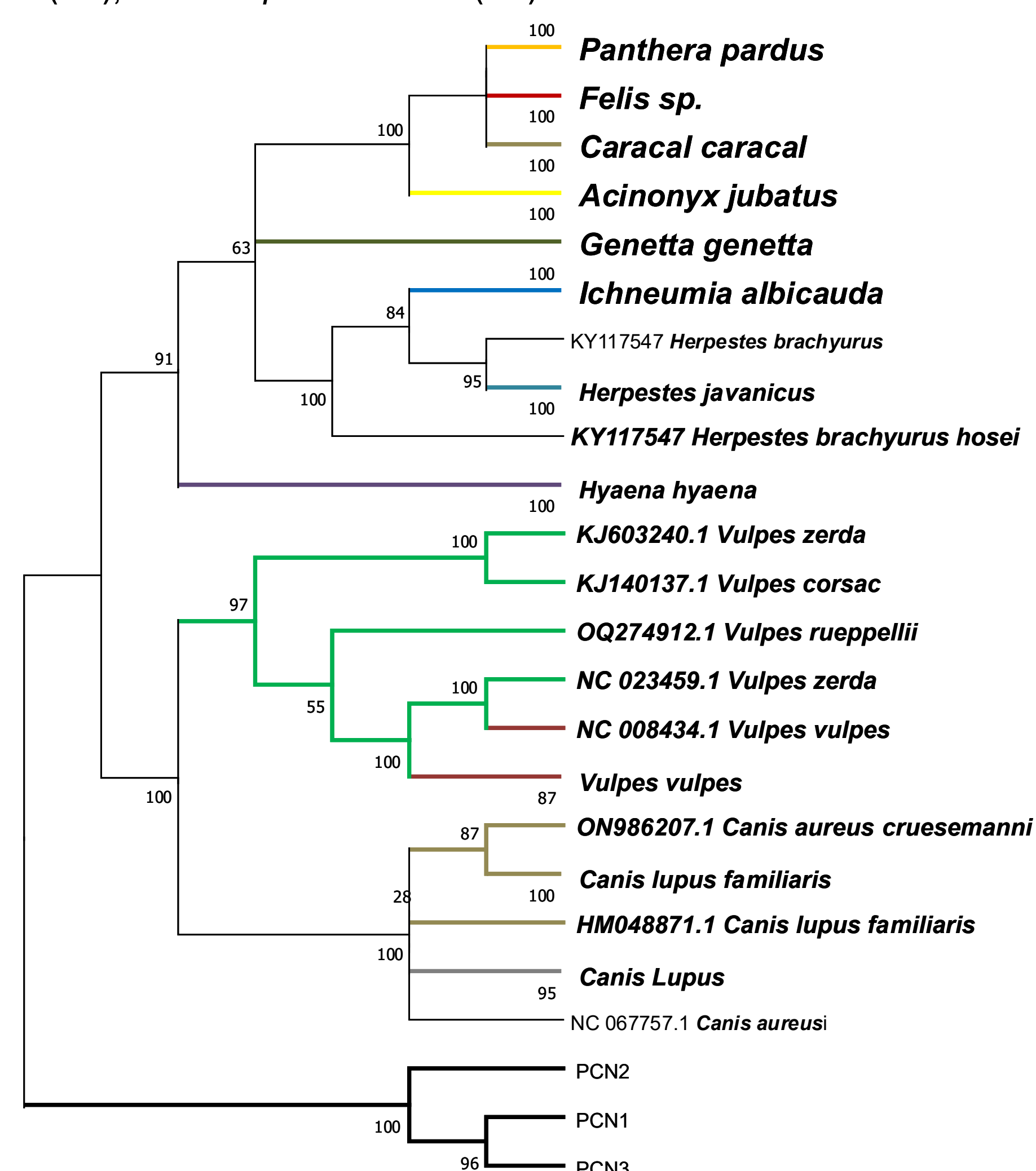


Figure: Phylogenetic analyses based on COI, 16S, Cytb, and concatenated datasets (1,608 bp) recovered nine well-supported clades corresponding to the identified species.

Implications: This study demonstrates that portable sequencing with the MinION developed by Oxford Nanopore Technologies provides rapid and reliable species identification from eDNA samples.

The approach is particularly effective for monitoring elusive species such as the Arabian leopard *Panthera pardus nimr*, supporting biodiversity assessment and conservation in Saudi Arabia.

CONCLUSION

- Oxford Nanopore sequencing enables high-throughput eDNA analysis in harsh environments. Using ONTbarcode2, species IDs matched Sanger results, confirming reliability.
- Detection of the endangered Arabian leopard (*Panthera pardus nimr*) and other carnivores from five families (Felidae, Canidae, Hyaenidae, Herpestidae, Viverridae) revealed unique haplotypes, highlighting conservation priorities.
- eDNA sequencing offers a non-invasive tool to monitor biodiversity, genetic divergence, and threats like habitat loss and wildlife trade.

REFERENCES

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