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Evaluating DNA Barcoding as a Molecular Approach to Wildlife Identification and Conservation in Madhav National Park, India

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KEYWORDS

Madhav National Park (MNP), cytochrome c oxidase subunit I (COI), BOLD (Barcode of Life Data System), GenBank, BLAST

ABSTRACT

Wildlife conservation within protected areas requires precise species identification for biodiversity monitoring, ecological research, and law enforcement. In National Parks (NP), remains of animals are frequently discovered in degraded, dismantled, or processed forms due to natural predation, anthropogenic pressures, and illegal poaching. Such conditions severely limit traditional morphological identification. This study evaluates DNA barcoding as a molecular forensic and biodiversity monitoring approach in MNP, focusing on its ability to identify species using mitochondrial COI and 16S rRNA markers, from highly compromised samples, including decayed carcasses, bone fragments, and processed animal products. Results demonstrated that in phase I, the 3 endangered species of MNP *Cuon alpinus*, *Panthera tigris* and *Manis crassicaudata* were identified for the BOLD repository and in phase II, 3 vulnerable species *Cervus unicolor*, *Tetracerus quadricornis*, and *Panthera pardus* might be achieved for the potential of DNA barcoding to strengthen law enforcement, detect illegal wildlife trade, and enhance ecological understanding in MNP. Recommendations are provided for integrating this molecular tool into park management protocols.

Introduction

Madhav National Park (MNP) in Shivpuri district, Madhya Pradesh, is a mosaic of dry deciduous forests, grasslands, and aquatic habitats supporting a rich array of fauna, including apex predators, ungulates, and avian species. Effective conservation management depends on accurate species identification, which is vital for biodiversity assessments, ecological research, and the prosecution of wildlife crimes.

Traditional morphological identification relies on visible traits such as pelage patterns, skeletal features, and dentition (Mishra *et al*., 2003). However, in many cases—particularly when remains are decayed, dismantled, scavenged, or processed—these identifiers are absent or compromised. This situation is common in National Parks, where illegal poaching, human–wildlife conflict, and natural predation often result in severely degraded remains.

DNA barcoding, based on short standardised genetic markers such as mitochondrial cytochrome c oxidase subunit I (COI), has emerged as a powerful molecular forensic and biodiversity

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monitoring approach capable of providing accurate identifications even from compromised samples (Hajibabaei *et al*., 2007; Hebert *et al*., 2003; Janzen *et al*., 2005; Wilson-Wilde, 2010). This study assesses its applicability in the challenging field conditions of MNP, aiming to support both conservation science and enforcement operations.

Study Area

Madhav National Park (latitude 25°25′N to 25°35′N, longitude 77°40′E to 77°55′E) spans approximately 375 km² in Shivpuri district, Madhya Pradesh. The Park features mixed dry deciduous forests interspersed with grasslands, wetlands including Sakhya Sagar and Madhav lakes, and rocky outcrops. The faunal elements include *Panthera tigris* (tiger), *Panthera pardus* (leopard), *Axis axis* (chital), *Boselaphus tragocamelus* (nilgai), *Sus scrofa* (wild pig), and a variety of avian species. The Park experiences a tropical climate with pronounced summer heat and a monsoon season that rejuvenates vegetation and replenishes water bodies.

Materials and Methods

Forest patrols and anti-poaching squads collected samples. Each sample was collected with sterile tools, preserved in 95% ethanol or silica gel, and assigned a GPS location.

1. Sample Collection Materials- Tissue Sampling Tools, vials, ethanol, gloves, centrifuges, sterile water and pipettes.

Laboratory Reagents for DNA Extraction- DNA Extraction Kits, Gel Electrophoresis, DNA Sequencing Kit, Capillary Electrophoresis System, Computational Tools for Sequence Analysis- internet, alignment software and BOLD.

Methodology

1. Sample Collection: Tissue samples (e.g., muscle, fin, skin, or bone fragments) are collected from wildlife specimens, ideally covering diverse taxonomic groups such as fish, reptiles, and mammals (Gupta *et al*., 2011). These included Decayed carcasses, Chopped meat, Processed remains (smoked, dried, or cooked), Bone fragments, horns, antlers, Predator kill remains (partially consumed)

2. DNA Extraction and Amplification: DNA was extracted using a modified silica-based protocol optimised for degraded tissue (Tobe *et al*., 2010). For bone and horn, demineralisation with EDTA was preceded by extraction. DNA is isolated using commercial kits, and the cytochrome c oxidase I (COI) gene is amplified via polymerase chain reaction (PCR)—a widely accepted molecular marker for animal barcoding (Hebert *et al*., 2003; Ward *et al*., 2005). 16S rRNA supplementary marker for degraded samples (Bucklin *et al*., 2011). Mini-barcode primers (<200 bp amplicons) were employed for highly degraded samples (Kress & Erickson, 2012).

Sequencing and Species Identification: The amplified DNA is sequenced and matched against global barcode databases such as BOLD (Barcode of Life Data System) and NCBI GenBank using similarity algorithms (usually BLAST) (Ratnasingham & Hebert, 2007; Zhang *et al*., 2012).

Observations and Results

Table 1 - List of documented mammalian species

in Madhav National Park along with their taxonomic classification and IUCN Red List status

S.NO.	Class	Order	Common name	Scientific Name	IUCN Red List Status
1	Mammalia	Artiodactyla	Chital	*Axis axis*	Least Concern
2	Mammalia	Artiodactyla	Sambar	*Cervus unicolor*	Vulnerable
3	Mammalia	Artiodactyla	Nilgai	*Boselaphus tragocamelus*	Least Concern
4	Mammalia	Artiodactyla	Chinkara	*Gazella bennettii*	Least Concern
5	Mammalia	Artiodactyla	Blackbuck	*Antilope cervicapra*	Least Concern
6	Mammalia	Artiodactyla	Chausingha	*Tetracerus quadricornis*	Vulnerable
7	Mammalia	Artiodactyla	Wild boar	*Sus scrofa*	Least Concern
8	Mammalia	Primates	Langurs	*Presbytis entillus*	Least Concern
9	Mammalia	Carnivora	Tiger	*Panthera tigris*	Endangered
10	Mammalia	Carnivora	Leopard	*Panthera pardus*	Vulnerable
11	Mammalia	Carnivora	Wild dog	*Cuon alpinus*	Endangered
12	Mammalia	Carnivora	Wolf	*Canis lupus*	Least Concern
13	Mammalia	Carnivora	Jackal	*Canis aureus*	Least Concern
14	Mammalia	Carnivora	Hyena	*Crocuta crocuta*	Least Concern
15	Mammalia	Carnivora	Jungle Cat	*Felis chaus*	Least Concern
16	Mammalia	Carnivora	Indian Pangolin	*Manis crassicaudata*	Endangered
17	Mammalia	Carnivora	Indian fox	*Vulpes bengalensis*	Least Concern
18	Mammalia	Carnivora	Mongoose	*Herpestes edwardsii*	Least Concern
19	Mammalia	Lagomorpha	Black naped hare	*Lepus nigricollis*	Least Concern

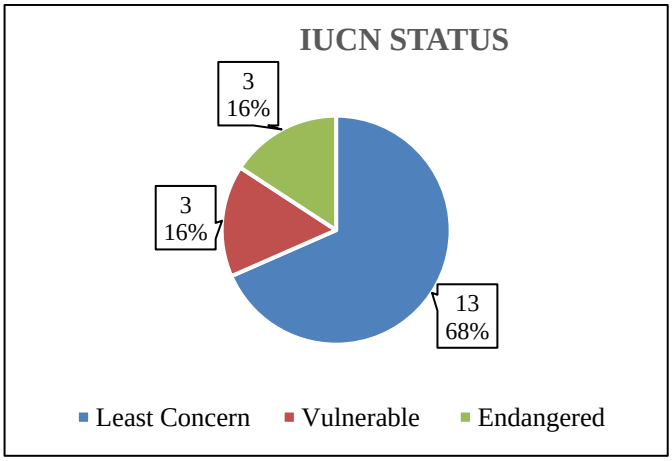

Figure 1. IUCN status of documented species of mammals in MNP

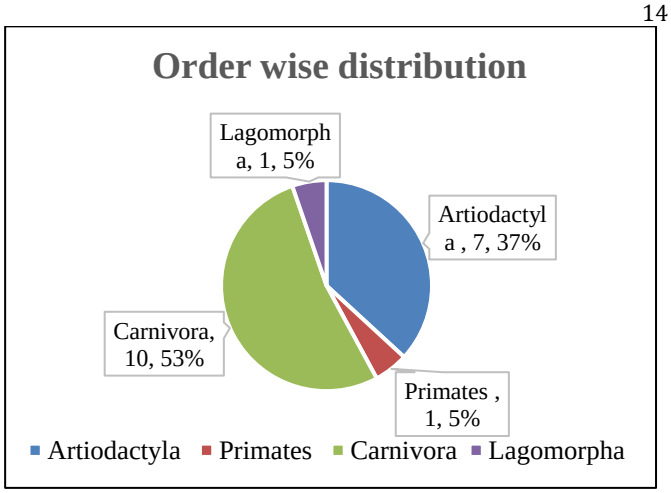

Figure 2. IUCN status of documented species of mammals, order-wise in MNP

MNP faces a range of significant conservation challenges that threaten its ecological integrity and biodiversity. One of the primary issues is illegal poaching pressure, which targets both ungulates and large carnivores, leading to population declines and ecosystem imbalances (Sethi & Chaturvedi, 2011). Additionally, human–wildlife conflict is prevalent due to the proximity of surrounding villages, often resulting in retaliatory killings and disturbances to wildlife behaviour and movement patterns (Mishra *et al*., 2003). Illegal grazing further exacerbates the problem by degrading habitat quality, reducing the availability of natural forage for wild herbivores, and increasing competition between livestock and wildlife. Moreover, habitat fragmentation caused by the expansion of roads and human settlements disrupts animal movement corridors, isolates populations, and increases vulnerability to other threats. Collectively, these pressures result in the frequent occurrence of animal remains in various stages of decay and decomposition.

These pressures contribute to the occurrence of animal remains in varying stages of decay and

processing, making MNP an ideal setting for testing the utility of DNA barcoding.

Results- Based on the IUCN status of MNP documentation following species were identified for the BOLD depository and effective management of conservation (Jhala *et al*., 2020): the identified species included for

Phase I: focused on endangered species *Panthera tigris, Cuon alpinus, and Manis crassicaudata*, and their DNA data should be submitted phase-wise in the BOLD depository, so it could be traced or matched with the samples provided for DNA barcoding when required by the authority of the National Park (Bhargava *et al*., 2019).

Phase II: focused on vulnerable species such as *Axis axis, Boselaphus tragocamelus* and Bushmeat species *Sus scrofa*, and their DNA data should be submitted phase-wise in the BOLD depository (Galimberti *et al*., 2013), so it could be traced or matched with the samples provided for DNA barcoding when required by the authority of the National Park.

Discussion

The ability to accurately identify wildlife species from fragmented, decomposed, or heavily processed remains represents a fundamental challenge in conservation biology and wildlife law enforcement (Mehta *et al.,* 2016). In the context of national parks where biodiversity protection is both an ecological and legal imperative, morphological identification is often rendered ineffective when carcasses are discovered in an advanced state of decay, are partially dismantled due to predation or mechanical trauma, or are deliberately processed to obscure origin, as is common in illegal trade (Galimberti *et al*., 2013). DNA barcoding provides a scientifically robust solution to this challenge by enabling 16% endangered species identification from minimal and degraded biological material (Bhargava *et al*., 2019), thereby bridging the gap between field observation limitations and the need for conclusive forensic evidence (Kumar *et al.,* 2017).

Conclusion

DNA barcoding has been highly effective in identifying species from degraded and processed wildlife remains (Hebert *et al*., 2003; Lahiri *et al*., 2020). It enables forensic-level identification for law enforcement, supports biodiversity assessments, and enhances understanding of ecological dynamics. The integration of this molecular approach into MNP's conservation management framework would significantly improve detection, prosecution, and prevention of wildlife crime.

Recommendations

While highly effective, DNA barcoding's utility is contingent on logistical and infrastructural support. Degraded samples may still yield insufficient DNA if improperly stored or contaminated, and the absence of comprehensive regional reference databases can limit resolution to the genus rather than species level (Srivathsan & Meier, 2012). However, these challenges are surmountable with targeted investments—such as building species reference libraries for local fauna, training field rangers in sterile sample collection, and deploying portable sequencers for rapid in-field analysis

(Ratnasingham & Hebert, 2007). Integrating DNA barcoding into a broader framework of conservation technologies, such as camera traps, acoustic monitoring, and satellite tracking, would amplify its preventive and management benefits (Janzen *et al*., 2005).

1. Integrate DNA Barcoding into Park Protocols

Equip anti-poaching units with preservation kits and train staff in sterile collection methods.

2. Establish a Regional Wildlife DNA Reference Database

Include all mammalian, avian, and reptilian species found in MNP and surrounding landscapes.

3. Adopt GIS-Linked Genetic Monitoring

Link DNA barcoding results to spatial databases to map poaching and predation hotspots.

4. Collaborate with Forensic Laboratories

Develop partnerships with accredited wildlife forensic labs for rapid turnaround in identification.

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