



Review Paper

Applications of Biochemistry and Molecular Biology in Wildlife Health Assessment, Disease Surveillance, and Conservation

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Abstract

Wildlife constitutes a fundamental component of the ecosystem integrity. Wild animals play a crucial role in the maintenance and transmission of infectious agents and may act as reservoir hosts for several emerging and re-emerging zoonotic diseases. Wildlife biochemical investigations are indispensable for understanding disease dynamics, identifying physiological stressors, monitoring health status, and developing strategies for disease prevention, preparedness, and response in wildlife populations. This review synthesizes available literature on wildlife hematology, serum biochemistry, molecular diagnostics, and conservation genomics, with an emphasis on Indian wildlife species and relevant global studies. It also exhibits the importance of application of advanced omics technologies, including genomics, proteomics, metabolomics, and metagenomics, in understanding wildlife physiology, disease resistance, dietary adaptation, and host-microbiome interactions. Many molecular markers like mt marker, AFLP, RAPD, Sex chromosome markers, microsatellites are contributing for the wildlife conservation to the greatest extent. The utility of these approaches in conservation management, assisted reproductive technologies, and genetic resource banking is discussed in the context of preserving endangered and vulnerable species. Collectively, this review is an attempt to emphasize the urgent need for coordinated, multidisciplinary efforts to generate species-specific biochemical and molecular reference data and to integrate modern biotechnological tools into wildlife health monitoring and conservation strategies in India and beyond.

1. Introduction

Wildlife constitutes a fundamental component of the ecosystem integrity. Wild animals play a crucial role in the maintenance and transmission of infectious agents and may act as reservoir hosts for several emerging and re-emerging zoonotic diseases. Wildlife biochemical investigations are indispensable for understanding disease dynamics, identifying physiological stressors, monitoring health status, and developing strategies for disease prevention, preparedness, and response in wildlife populations.

India is home to approximately 27,875 Asian elephants (2018 census), 9,593 leopards (2015 census), 3,682 tigers (2022 census), and 674 Asiatic lions (2020 census). However, population estimates for many other wildlife species remain unavailable due to the logistical challenges of conducting comprehensive censuses across diverse habitats, compounded by the presence of migratory species. Similarly, biochemical profiling of wildlife populations is limited, as blood collection and chemical immobilization are constrained by regulatory frameworks, ethical considerations, and safety concerns.

Despite its critical importance, systematic biochemical information on wildlife species remains

limited, fragmented, and often inaccessible. Available data are largely derived from opportunistic or case-based investigations rather than structured population-level studies. Although attempts have been made by several researchers to establish reference ranges for hepatological and biochemical parameters in selected wildlife species, these values exhibit considerable variability. Such variability arises due to a multitude of intrinsic and extrinsic factors, including geographic location, climate, seasonal variations, nutritional availability, and subspecies differences, free-ranging versus captive conditions, immobilization protocols, and pharmacological agents used during restraint, analytical techniques, and laboratory methodologies.

Therefore, there is a pressing need for comprehensive, systematic, and region-specific investigations to establish baseline biochemical profiles for wildlife species. In the Indian context, this requirement is particularly critical given the country's extraordinary biodiversity and ecological heterogeneity. Synthesizing biochemical data generated by researchers across India—and, where relevant, globally—would provide a robust foundation for wildlife health assessment, conservation planning, and epidemiological surveillance.

2. Wildlife Biochemistry

Wildlife biochemistry in Indian scenario differs with reference to other countries data where in the serum biochemistry of the wild animals are relatively normal to their physiological status except for the variations due to chemical immobilization. Whereas the parameters values ranges might differ in the data collected in India as in the same is often performed only in the cases of illness or disease spread. Thus, many of the parameters we observe may or may not be of the healthy individual. Blood is collected majorly for the various haematological and serum biochemical analysis which includes, includes CBC, Live function tests, Kidney function tests, Cardiac wellness and very often tests for the musculoskeletal system etc.

2.1 Complete Blood Count

A complete blood count refers to the estimation of complete RBC, WBC, Platelets, Haemoglobin and serum protein levels. As CBC plays important role in the domestic animals to assess the status of inflammation or any disease conditions, the same is implicated for the wild animals. The normal haematology and serum biochemistry is thus summarised as below

2.2 Physiological and Environmental Variations: Case studies

Wildlife Biochemistry is a complicated endeavour as demonstrated by the following case studies,

Reptiles

In snakes the total leukocyte levels are found to be higher in female snakes, but the levels of haemoglobin, haematocrit, and MCV values are higher in male snakes. MCH and MCHC values are higher in female snakes. Mean protein and cholesterol contents differ significantly between male (45.32 ± 1.76 and 3.76 ± 0.06 mg/mL) and female (12.47 ± 0.82 and 4.72 ± 0.2 mg/ml) snakes. (Sanath et al., 2014)

Large felids

A study on the Asiatic lions WBCs has revealed that there is a positive correlation between the numbers of WBCs with the body masses of animals: large cats had more WBC, which may be due to greater risks of infection associated with larger body surface, lifespan and home range size. There are differences in the number of WBC types also. Large cats also have more neutrophils and monocytes but fewer lymphocytes than smaller cats. The ratio of neutrophils to lymphocytes is greater in large felids. This phenomenon may be related to diet (relative prey size and kill utilization time), which suggests regular contact of large cats with bacterial and protozoa pathogens in contrast to the small cats. (Sergey et al., 2020).

Sloth Bears

Serum lipid parameters and age have no correlation in sloth bear. However, there is a positive correlation of body weight to both triglyceride and HDL cholesterol levels in bears. Seasonal trends in physiological serum lipid values, potentially due to variations in the sloth bear diet, can be identified. Serum triglyceride levels were found higher during post-monsoon season and cholesterol levels were higher during winter compared to other seasons. (Arun et al., 2011)

Cervids

Although there are some studies which show that during antler growth in the spotted deer, there will be some changes in the blood parameters like altered calcium levels with some electrolytes and minerals with high alkaline phosphatase. However, studies have also revealed that, a stable and constant feeding habit will not induce any changes even during the antler growth. (Jeon et al., 2006)

2.3 Serum biochemistry

Increased levels of serum enzymes like ALT, AST, Bilirubin, Creatinine, BUN, LDH, CK, Troponin, Cholesterol, Triglyceride, HDL and LDL due to stress-linked muscle and organ damage especially in spotted deer is very common. Thus, in the field conditions capturing and treating any animals of the Cervidae family is generally avoided especially the spotted deer, as most of the time it acts as threat to the life of the animal due to hypothermia and multiple organ failure.

2.4 Disease Diagnosis

Altered haematological and serological values have always been a foundation for the diagnosis of many infectious and metabolic diseases in domestic animals. The same can be extrapolated into the wildlife field where the parameters can be used as an indicator of various infectious and metabolic ailments. Anaemia, leucocytosis, hypercholesterolemia, hypophosphatemia are the common incidences in the Hepatozoan infection in the leopards. Leucocytosis ($15.9 \times 10^3/\mu\text{l}$) associated with marked absolute neutrophil ($15\ 000/\mu\text{l}$). High serum enzyme activities of ALT (136 U/L) and AST (1159 U/L), high level of urea and creatinine 79 mg/dl and 1.7 mg/dl compared to the reference range in canines (15–45 mg/dl and 0.54–1.4 mg/dl, respectively) indicating kidney injury is a common picture in the sloth bears (*Melursus ursinus*) infected with Infectious Canine Hepatitis. Total protein (3.9 g/dl: albumin 1.94 g/dl, globulin 1.96 g/dl) and alkaline phosphates (306 U/L) values also will be found altered (Singh et al., 2010).

Table 1: Normal hematological parameter of some important wild animals of India

Species	Total RBC ($10^6/\text{mm}^3$)	PCV%	Hb (g/dl)	Total leucocyte ($10^3/\text{mm}^3$)	Neutrophils%	Lymphocyte%	Monocytes%	Eosinophils%	Basophils%	ESR (mm at 1hr)
Asiatic lion ^a	7.97	40.66		13.05		25.83				
Bengal tigers ^b	7.9	38	13.96 $\pm$ 0.54	8.5	75.69 $\pm$ 1.57	20.45 $\pm$ 1.52	0.64 $\pm$ 0.14	3.09 $\pm$ 0.41	0.15 $\pm$ 0.08	21
White rumped vultures ^c	2.86 $\pm$ 1.01	44.69 $\pm$ 3.63%	12.86 $\pm$ 1.67	14.75 $\pm$ 6.01						
Cobra ^d	1.21 $\pm$ 0.14	33.10 $\pm$ 1.89	8.23 $\pm$ 0.39	10.26 $\pm$ 0.61	45.60 $\pm$ 4.05	52.30 $\pm$ 4.00	0.70 $\pm$ 0.34	0.30 $\pm$ 0.21	1.20 $\pm$ 0.79	-
Asiatic elephant ^e	3.46	36.02 $\pm$ 1.47	11.94 $\pm$ 0.2	9.25 $\pm$ 1.11	40.5	36	8.7	14.3	0.21	
Sloth bear ^f	6.56 $\pm$ 0.19	52 $\pm$ 1.03	16.92 $\pm$ 0.63	11.72 $\pm$ 0.32	70.3	24.3	3	1.7	0.59	6.0 $\pm$ 0.45
Leopard ^g	8.9 $\pm$ 2.2	38 $\pm$ 3.2	12.3 $\pm$ 2	7.8 $\pm$ 5.4	66.1 $\pm$ 2.4	23.6 $\pm$ 3.5	5.1 $\pm$ 1.3	4.6 $\pm$ 1.8	0.1 $\pm$ 0.4	33.5 $\pm$ 3.5
Sika deer ^h	7	42	14							
Goral ⁱ	5.05		10							
Barking deer ^j	6.25	37.5	12.5	8.83						
Spotted deer ^k	13.05 $\pm$ 0.83	34.5 $\pm$ 0.65	9.62 $\pm$ 0.97	4.36 $\pm$ 0.34	51.75	44	2.25	2	0	-
Sambar deer ^l	5.8 $\pm$ 0.2	29.3 $\pm$ 0.7	9.6 $\pm$ 0.3	3.6 $\pm$ 0.2	47	-	6	-	-	-

(a Bharathidasan et al., 2016, b Srivastava et al and Sunil Kumar et al., 2019., 2012, c Bikalpa karki et al., 2020, d Sanath et al., 2014, e Barkha et al., 2020 and Divya et al., 2018, f Veeraselvam et al., 2014, g Srivatsav et al 2016, h, i, j Nagappa et al., 2005, k Sahoo et al., 2002, l Joju et al., 2014)

Table 2: Normal serological parameters of some important wild animals of India

Species	ALT(I/U)	AST(I/U)	Total serum protein (g/dl)	Blood glucose(mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	Total bilirubin (mg/dl)	Total cholesterol (mg/dl)
Asiatic lion ^a	38.17 $\pm$ 2.32	31.00 $\pm$ 3.06	8.00 $\pm$ 0.58	92.17 $\pm$ 3.77	33.67 $\pm$ 1.36	2.17 $\pm$ 0.39				
Bengal tigers ^b	27.52 $\pm$ 2.18	19.76 $\pm$ 1.55	7.25 $\pm$ 0.12	84.48 $\pm$ 1.54	123.22 $\pm$ 4.45	1.58 $\pm$ 0.04	3.89 $\pm$ 0.10	3.35 $\pm$ 0.09	0.75 $\pm$ 0.10	159.85 $\pm$ 7.27
Cobra ^d	4.70 $\pm$ 0.73	27.10 $\pm$ 3.58	8.30 $\pm$ 0.48	-	3.08 $\pm$ 0.49	0.52 $\pm$ 0.05	3.05 $\pm$ 0.19	-		
Asiatic elephant ^e	2.9 $\pm$ 0.75	-	6.71 $\pm$ 0.42	79 $\pm$ 2.66	15.89 $\pm$ 0.98	1.29 $\pm$ 0.28	2.49 $\pm$ 0.55	4.22 $\pm$ 0.17	0.73 $\pm$ 0.03	58 $\pm$ 5.25
Sloth bear ^f	103.56 $\pm$ 1.90	137.74 $\pm$ 3.11	6.98 $\pm$ 0.30	98.82 $\pm$ 1.96	19.08 $\pm$ 0.55	1.24 $\pm$ 0.09	3.89 $\pm$ 0.19	3.09 $\pm$ 0.15	0.15 $\pm$ 0.04	268.63 $\pm$ 5.22
Leopard ^g	53.8 $\pm$ 23.9	32.8 $\pm$ 31.4	6.0 $\pm$ 2.0	95.8 $\pm$ 20.4	30.5 $\pm$ 15.0	1.1 $\pm$ 0.7	4.2 $\pm$ 1.6	-	0.8 $\pm$ 0.4	-
Sika deer ^h	-	-	7.61	-	18.12	1.7	3.32	4.27	-	-
Goral ⁱ	-	-	7.24	66.26	20.30	2.71	2.91	4.3	-	-
Barking deer ^j	-	-	5.56	94.26	21.07	2.41	3.03	2.5	-	-
Spotted deer ^k	39.67 $\pm$ 3.43	66.35 $\pm$ 4.89	8.18 $\pm$ 0.15	62.16 $\pm$ 6.90	10.11 $\pm$ 0.83	-	4.74 $\pm$ 0.33	3.44 $\pm$ 0.19	-	100.03 $\pm$ 10.14
Sambar deer ^l	71.25 $\pm$ 32.95	57.03 $\pm$ 21.65	64.58 $\pm$ 6.95	6.75 $\pm$ 1.89	9.01 $\pm$ 4.04	171.11 $\pm$ 46.90	33.69 $\pm$ 5.98	30.98 $\pm$ 5.26	10.17 $\pm$ 2.51	2.92 $\pm$ 0.91

3. Wildlife Genomics

PCR (Polymerized Chain Reaction) has been a boon to the ecologists and biologists as their contribution is very wide and remarkable in the various field including wildlife. Several diseases are easily diagnosed and treated in the early stages which contribute for the conservation of many endangered species. Diseases like Herpes virus, Salmonella infections in Elephants are easily diagnosed with PCR. The differences in the strains of the blood protozoans affecting the big cats are also being recorded with the help of PCR technology. Non-invasive technologies which involve DNA and RNA extractions from the animal excreta like hair, faeces, tissues will assist the molecular biologists to understand the physiological status of the animals like sex, age, population which will assist not only in the conservation but also will help in disease diagnosis and treatment.

Polymerase chain reaction of 18S rRNA with genomic DNA amplified 1775 bp and 1665 bp segments for Hepatozoon species and Babesia species of Asiatic lion respectively with further sequencing of PCR amplicon and BLAST analysis indicated that Hepatozoon spp. in Asiatic lion was 99% similar to Hepatozoon felis isolate of Spain and Babesia spp. like organism was 95% similar to Babesia canis 18S ribosomal RNA gene of Israeli cat and Babesia canis canis of domestic dogs. Thus, helping the scientists to conclude that haemoprotezoa are not restricted to their respective hosts, and more than one genotype can be found in the same habitat. Cryptic babesiosis and hepatozoonosis can flare up in immune-compromised animals and may result into fatal consequences. (Shafiya et al., 2018). Real time PCR and high nucleotide sequence homology with the human virus also helped to diagnose SARS-COV 2 in the Indian leopards (*Panthera pardus fusca*) which showed a spillage of infection from the humans. This diagnosis throws light on the importance of disease screening in the wild animal population also whenever there is a pandemic outbreak in order to understand the disease spread and epidemiology. (Mahajan et al., 2022)

Agar gel immune diffusion test and dot immune binding assay are used to detect the Canine distemper viruses, Feline Pan Leukopenia in big cats. Indirect ELISA is also employed in the diagnosis of Toxoplasmosis, FIV antibody, Fe LV antigen detection in Bengal Tiger. (Abd-eldaim et al., 2009). Screening of the big cats for these diseases is very important to prevent the death of the animals, restrict their movements to avoid spread of the disease and timely vaccination to conserve the species as these viral diseases are major threat to the felines in the wild. Despite the wide range of genomic application in the wildlife, the data availability on the same of various genes of the India wild animals as enlisted under department of forestry, government of India (Table 3) is very minimal. This disparity underscores the urgent need of the national initiatives aimed at generating

comprehensive genomic studies on par with the human genomic project which would assist in the conservation process at the genetic level.

Table 3: Number of genomic entries in the NCBI website of the wild animals commonly seen in India

Asiatic Elephant	14
Bharal	50
Andaman wild pig	0
Binturong	74
Black buck	0
Brow antlered deer	0
Himalayan Brown Bear	0
Capped langur	0
Caracal	37
Catecean specils	0
Cheetah	33507
Chinese Pangolin	31308
Indian Gazelle	37
Clouded Leopard	36903
Crab-eating Macaque	40694
Desert cat	7
Desert fox	8
Dugong	13
Ermine	34310
Fishing Cat	30895
Four-horned antelope	37
Gangetic dolphin	0
Indian bison	2
Golden Cat	137
Golden Langur	0
Giant squirrel	53
Himalayan Ibex	0
Himalayan Tahr	37
Hispid Hare	1
Hog badger	37
Hoolock	148
Indian Lion	6
Indian Wild Ass	0
Indian Wolf	0
Kashmir Stag	0
Leaf Monkey	157
Leopard	163121
Leopard Cat	30741
Red Panda	56
Lion-tailed Macaque	0
Loris	38993
Little Indian Porpoise	0
Lynx	58400
Malabar Civet	0
Sun Bear	13
Marbled Cat	37
Markhor	37
Mouse Deer	132305
Musk Deer	30034
Nilgiri Langur	37
Nilgiri Tahr	0
Great Tibetan Sheep	0

Pallass Cat	11189
Pangolin	65125
Pygmy Hog	37
Ratel	37
Rhinoceros unicornis	13
Rusty spotted Cat	37
Serow	28988
Clawless Otter	37
Sloth Bear	13
Slow Loris	39004
Small Travancore Flying Squirrel	0
Snow Leopard	31172
Snubfin Dolphin	37
Spotted Linsang	287788
Swamp Deer	0
Mishmi Takin	28788
Tibetan Antelope	13
Tibetan Fox	40
Tibetan Gazelle	0
Tibetan Wild Ass	0
Tiger	36612
Urial or Shapu	13
Wild buffalo	40
Wild Yak	96
Tibetan Wolf	0

3.1 The markers

Molecular markers have proved to be the most reliable and accurate advanced technology which can be adopted in the various fields of biological sciences. The markers are used in the human medicine as well as in the veterinary field for various diagnosis, treatment, investigations, ecological study, conservations, toxicological study etc (Arif et al., 2009). Various markers have wide range of application which can be summarised in the table as below:

Molecular Marker	Field of application
mt Marker	Used to study evolutionary relationship and biodiversity
RAPD and AFLP	Do not require prior information
AFLP	Has greater differentiation ability than RAPD
RAPD	Simple and least expensive
Microsatellite and AFLP	Powerful to determine genetic diversity
Microsatellite	Best for parentage and strain analysis

3.2 Restriction Fragment Length Polymorphisms (RFLP)

The non- invasive sample collection and genetic technique standardization was done in the Indian felids and canids with the use of PCR amplification and restriction enzyme digestion of scat collected in the

field. Using the primers from 16S r RNA region of the mitochondrial genome on ten felid and five canid species produced potential differentiation of species based on fragment patterns. The subsequent sequencing confirmed the species and showed that the RFLP method is both reliable and economical genomic technology for wildlife species identification and in turn conservation. The species identified were Jungle cat, caracal, leopard cat, fishing cat, rusty spotted cat, lynx, manual, marbled cat, Asian golden cat, domestic cat, wild cat group, clouded leopard, snow leopard, lion, tiger (Mukherjee et al., 2010).

3.3 Direct Nucleotide Sequencing

New Generation Sequencing (NGS) is the advanced technology adopted in human genomic study and the same has been extended to domestic and wild animals. After the sequencing of the human genome under Human genome project, the first non-human primate genome sequenced was of chimpanzee (*Pan troglodytes verus*) from West Africa. They used cloned primary blood leucocyte DNA in 2005. Followed by Sumatran Orangutan, Rhesus Macaque (*Macaca mullata*). Among the felids, Amur tiger, Bengal tiger, African lion, white African lion and snow leopards were also sequenced.

3.4 Microsatellite DNA

Microsatellite DNA consists of 2-6 nucleotides that repeat several times and they are widely distributed throughout the genome which can be potentially used for the PCR amplification and thus ensuring accuracy in genetic purity assessment, inbreeding, genotype - phenotype correlation, identification of unique alleles. They are advantageous as they have known genome localization and have incredible polymorphism ability. They provide us the road map for the breeding and conservational studies. (Kulibaba et al., 2025). Study of microsatellites in elephants resulted in the information that cryptic speciation in Asian elephants i.e. in Thai elephants' genetic diversity varied across populations and declined over 50 generations. Similarly, reduction in genetic variation before extinction of about 4000 years ago was found out in woolly mammoth. Domestic cats have found to have higher microsatellite frequency as compared to that of wild cats providing extensive genetic resource for the further study and analysis. (Kulibaba et al., 2025).

3.5 Single Nucleotide Polymorphisms (SNPs)

Here alleles at SNPs loci are characterized by either single nucleotide substitution or single nucleotide insertion / deletion events (Primmer et al., 2002). The large percentage of variations among the humans is widely due to the SNPs and this helps in the identification of the variations and the deviations from the normal population. However, the SNPs cannot be widely used in the wildlife due to the following reasons listed as heterozygous individuals make the detection of alleles at a locus difficult, as alleles will appear to

have a similar peak height on an automated gel. Second is SNPs are of not greater help for the parentage and relatedness study. Third its time consuming and expensive. (Stoneking 2001, Belfiore et al 2003). At present only used in species that are closely related to model organisms where the genomic sequence and large number of well characterised loci are available. (Seddon et al., 2005)

3.6 Sex Chromosome Markers

The mammalian sex chromosomes are X and Y. The male carrying XY and the female carries XX. The avian species have Z and W. Where females are ZW and males are ZZ. The Y linked markers often provide information about the differences that exist among the ethnic groups as well as helps to understand the unravelling historical relationship among the humans (Shen et al., 2000, Bosch et al., 2001). The same can be used in the wildlife species for better comparison.

DNA markers play a crucial role in the wildlife conservation and propagation in many ways. The genetic markers help us to understand the ecological deviations and derivations with accurate classification of the population. The molecular understand also helps us to prevent the illegal poaching and hunting.

3.7 Mitochondrial DNA markers

It has special feature of maternal inheritance, low recombination, high replication rate and ease of use as from samples like hair, bone which makes an additional advantage in wildlife as non-invasive method of sample collection is always a choice in wildlife conservation and any kinds of analysis. Sequence information of the mt DNA can be best utilised for the conservational studies. The mitochondrial genome has control region (CR) which is the non-coding region but involved in replication, Cytochrome C oxidase subunit (COI) and Cytochrome b (Cyt b) which are commonly used as the markers for the genetic study. mt DNA has less repair ability and thus have high mutation rate. The main uses of mt DNA sequences in conservation genetics include population structuring, resolving taxonomies, establishing interspecific hybridization and the detection of illegal hunting and poaching of endangered animals. However, the selection of appropriate bioinformatics tool plays an important role for reliable phylogenetic inference using mitochondrial markers (Khan et al., 2008). Table 4 provides quick information on numbers of names of taxa, types of mitochondrial markers used in some relevant studies.

3.8 Ribosomal DNA (12S and 16S rDNA)

Several investigators have used 12S rDNA sequences for wildlife forensic biology (Prakash et al., 2000; Gurdeep et al., 2004). Amplification of 12S rDNA gene using universal primers and then cloned and sequencing the 450 bp fragment for phylogenetic

analysis of endangered species was done in Indian muntjac (*Muntiacus muntjak*). (Shukla et al., 2001). Similarly genetic variation in the endangered spur-thighed tortoise (*Testudo graeca*), an endangered species from broad distribution range (Alvarez et al., 2000; van der Kuyl et al., 2005) was done using 12S rRNA. The study of Pesole et al., 1999 revealed that the 12S fragment of *Testudo graeca* was found to be somewhat less variable than the D-loop fragment, a finding compatible with the situation in mammals, where rRNA genes evolve slower than synonymous sites and the variable parts of the D-loop. 12S rDNA for molecular identification of Indian leopard, which is an endangered species except in Central Africa and India was done by Panday et al. (2007). Chinese antelopes study of 12S observed that the average sequence divergence values for 16S and 12S rDNA genes are 9.9% and 6.3% respectively. (Lei et al., 2003). He also suggested that Phylogenetic analysis revealed that Przewalski's gazelle is more closely related to Mongolian gazelle than Tibetan gazelle suggesting that the critically endangered Przewalski's gazelle should be treated as a species, not a subspecies of the Tibetan gazelle, which clearly warrants more attention from conservationists.

Mitochondrial 16S rRNA has been used to elucidate the pattern of relationships and systematic status of 4 genera, including 9 species of skates living in the Mediterranean and Black Seas (Turan, 2008). For the identification of seven pecora species including Blackbuck, Goral, Nilgai, Hog deer, Chital, Sambar and Thamin deer, a heminested PCR assay based on species-specific polymorphism at the mitochondrial 16S rRNA gene has been used and designed by Guha and Kashyap, 2005. Molecular studies on endangered Pecoran have shown lower sequence diversity in 16S rRNA gene as compared to cytochrome b gene, both between and within species however the 16S rRNA gene harboured a larger number of species-specific mutation sites than cytochrome b gene, suggesting that it could be more useful for species identification (Guha et al., 2006).

3.9 Major Histocompatibility Complex (MHC) Markers

The major histocompatibility markers are the two genes associated with immune response. It has two classes, class I and class II. The MHC genes are polymorphic in nature in most of the vertebrate animals especially in the antigen binding site. The role of MHC genes in pathogen resistance implies a link between the loss of population MHC diversity and increased susceptibility to disease (Arkush et al. 2002; Hedrick 2004). There are some evidence of link between MHC diversity and disease resistance (O'Brien and Evermann 1988). But some populations of bighorn sheep have appeared to high levels of MHC variation but are highly susceptible to domestic livestock diseases (Gutierrez-Espela et al. 2001).

Table 5: Application of Nuclear DNA markers in wildlife

Taxa	Study	Marker	Reference
Australian tiger snake	Marker isolation	SSR	Scott et al., 2001
Protected deer and other taxa	Illegal hunting	SSR	Fang and Wan., 2002
Japanese Quail	Generic Variation	SSR	Ye et al., 1998
Ostrich	Genetic structure	SSR, RAPD	Kawka et al., 2007
Rattlesnake	Genetic diversity	RAPD	Lougheed et al., 2000
Pea fowl	Genetic diversity	RAPD	Chang et al., 2002
Wild mouse	Genetic diversity	RAPD	Neveu et al., 1998
Cattle	Genetic diversity	RAPD	Yu et al., 2004
Plains zebra	Genetic diversity	RAPD	Bowland et al., 2001
Iberian imperial eagle	Genetic diversity	RAPD	Padilla et al., 2000
Asiatic lion	Genetic diversity	RAPD	Shankarnarayan et al., 1997
Snake	Species tree	AFLP	Giannasi et al., 2001
Topmouth cutler	Genetic diversity	AFLP	Wang et al., 2007
Asian arowana	Genetic diversity	AFLP, SSR	Yue et al., 2004
Arabian Oryx	Genetic diversity	SSR	Arif et al., 2010

Table 6: mt DNA markers used for the study in different species

Taxa	Study	Marker	Reference
Sun Bear	Evolutionary significant unit	CR	Onuma et al., 2006
Black muntjac	Population structure	CR	Wu et al., 2006
Chinese water deer	Genetic diversity	CR	Hu et al., 2006
Roe, deer, horse, cow	Illegal hunting	Cyt b	An et al., 2007
Houbara sable	Genetic diversity	CR	Idaghdour et al., 2004
African sable	Genetic structure	CR, Cyt b	Pitr et al., 2002
Rock-wallaby	Population structure	CR	Eldridge et al., 2001
Tibetan gazelle	Genetic diversity	CR, Cyt b	Zhang and Jiang., 2006
Indian leopard	Species identification	12S rRNA	Pandey et al., 2007
Orynx dammah	Genetic diversity	CR	Iyengar et al., 2007
Orynx beisa	Genetic diversity	CR, Cyt b	Masembe et al., 2006
Orynx leucoryx	Genetic diversity	CR	Khan et al., 2023

However European beaver (*Castor fiber*) in Sweden has low levels of MHC variation due to a recent genetic bottleneck, but they displayed high rates of population growth after conservation measures were enacted (Ellegren et al. 1993). Similarly, moose (*Alces alces*) have much lower MHC diversity (e.g., only 6 amino acid substitutions in the DRB1 exon 2) than other Artiodactyls (Mikko and Anderson 1995, Van den Bussche et al. 1999), yet they maintain viable populations. The above evidence would confuse the use of MHC use as marker, yet we have evidence to prove that there is a relationship between variations in MHC gene and components of fitness in individual white-tailed deer like their body size, parasite resistance, antler development etc. (Ditchkoff et al. 2001) and study in feral sheep on parasitic resistance was done by Coltman et al., 1999.

4. Wildlife Proteomics

4.1 Mitochondrial Protein coding genes

Most used mitochondrial Protein coding gene is of Cyt b sequence to understand the genetic variation among the species like Tibetan gazelle (*Procapra picticaudata*), a threatened species on the Qinghai-Tibet Plateau of China (Zhang & Jiang, 2006). The partial Cyt b analysis of genetic distance showed that there is a considerable difference between Chinese goral and Korean goral, but virtually none between Korean and Russian gorals (Min et al., 2004). The Korean gorals possessed two haplotypes with only one nucleotide difference between them, while the Japanese serows showed slightly higher sequence diversity with five haplotypes. NADH dehydrogenase subunit 5 (318 bp) is yet another important gene that has been used for phylogenetic

analysis of multiple individuals of different species from Felidae family (Johnson & O'Brien, 1997). Mitochondrial cytochrome oxidase I (COI) gene currently has gained more attention in developing DNA barcodes for species identification and biodiversity analysis.

5. Understanding the physiology through metabolomics

Biotechnology has wide range of application in the understanding of the physiology of various endangered and vulnerable animals along with biochemistry. Animal like Koala which is classified as "vulnerable" due to the lack of food availability, changing habitats and outbreak of infectious diseases. The Genomic sequencing of koala which feeds only on the eucalyptus leaves have found to contain anti-nutritional hydrolysable tannins (HTs). Genome sequence data revealed that koala's ability to detoxify eucalypt HTs might be due to expansions within a cytochrome P450 gene family, and its ability to smell, taste, and regulate intake of phytometabolites owing to expansions in the vomeronasal and taste receptors (Johnson et al. 2018). Similarly Metagenomic study of the gut microflora of bamboo-eating giant panda (*Ailuropoda melanoleuca*) and red panda (*Ailurus fulgens*) has revealed that there is abundance of cyanide-degrading bacteria (Zhu et al. 2018). The same knowledge can be applied to formulate unconventional feeds which can be fed to the domestic animals. In the African elephant (*Loxodonta Africana*), characterization and phylogenetically conserved and derived transcripts (55,910 expressed sequence tag or ESTs) contigs analysis has revealed that the evolution and variation of eutherian placentation shows that some candidate genes might be important for normal development and functioning of human placenta. Around 2963 genes were found to be expressed commonly in the placenta of the animals like mouse, cattle and humans and any mutations in these genes might impact the human foetal development similarly a detailed study of the genomes of the placenta would be helpful for the better reproductive capacity in wild animals and would assist in the better conservation process. (Hou et al. 2012).

6. Wildlife conservation

Application of proteomics and metabolomics to understand the effect of feed on the performances of various domestic animals is very common in this advanced field of science and technology. The same principles and technologies have been adapted to the wild animals to understand their feeding behaviour, availability of the food and in-turn the biomass and their implications on the health of the wild animals. Assessment of the stress levels in various wild animals using NMR, GC-MS, LS-MS have helped in better understanding of the behaviour patterns of the wild

animals in the wild and in the captivity. GC-MS technology has been adopted for the many studies involving toxicological study of wildlife. The plastic exposure prediction study was done by measuring the phthalate level. Live birds' samples of short, tailed shearwaters and Heron Island in northeast Queensland exhibited presence of DBP and DEHP ranging from 0.02 to 0.22 ng/ μ l of preen oil in which the tissue extraction was processed. (Hardesty et al., 2015). The information obtained thus through the application of various technologies and knowledge of biochemistry has helped the veterinarians, ecologists, scientists to establish a standard protocol for the management practices of the wild animals in the captivity and to adopt many conservational methodologies in the natural ecosystem.

The below table depicts the number of entries of the genetic information data available in the NCBI website of the mammals most commonly seen in India. One can notice that except for few animals, maximum animals' genomic information or data is null which depicts the importance of the implications of the genomic work in wildlife especially in India.

Cellular culture technology has been a boon to the wildlife conservation. The Assisted Reproductive Technologies (ART), tissue culture, cryopreservation (bio banking) are some of the technologies which can be adopted. Under ART, Captive breeding, Seed banking, Somatic Cell Nuclear Transfer (SCNT) and In vitro fertilisation would be of greater technologies to preserve and propagate the wildlife germ plasma. The most famous endangered species across the world are tiger, western gorilla, Asiatic elephant and whooping crane. The attempts have been made to adopt the cell culture technologies to save them. (Abdul et al., 2023)

However, the challenge in the ART is many as one have to thoroughly understand the reproductive physiology of these animals which include the understanding of the oestrus cycle, pattern of ovulation, hormonal surge, semen collection, quality analysis and many.

7. Conclusions

The present review on the collection of various data and studies in the fields of biochemistry and molecular biology in the field of wildlife throughout the world is an attempt to depict the importance and requirement of the extrapolation of knowledge of molecular biology, bioinformatics into the wildlife. A wide opportunity to explore "omics" world of wildlife will be beneficial to the entire world of wildlife conservationists and scientists. As one can see that most of the genomic data available is not from the studies conducted in India which gives a large field of scope to initiate genomic projects similar to that of Human Genomic Project where in the genomic data of all the wildlife species is studied and preserved for the future conservation. A strong collaboration is the need of the hour between the forest department and various

institutes working on the economic projects for the successful implementation of the ideas and the policies. A thorough study of the wild animal physiology, anatomy and biochemistry would be of greater help to treat and conserve the wildlife in India.

8. Statements and Declarations

Ethical Approval: Not applicable

Conflict of Interest: Not applicable

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