

**MAMMALS FEATURES, STRUCTURE, COMPARATIVE ANATOMICAL
ANALYSIS ON THE EXAMPLE OF TWO REPRESENTATIVES**



JAI PRAKASH SHARMA

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Department of Zoology, B.R.A. Bihar University, Muzaffarpur, India

E-mail: Jps050575@gmail.com

Abstract

Wildlife plays an important role in preserving the ecological balance of nature. The wildlife presence affects the character of its habitat by frequently supporting the evolutionary adaptations of everything in its environment. The quality of the environment can also be assessed by the presence of wildlife. Wildlife serves as a rich resource of food and livelihood in various developing countries. The various plant species in the wild are also useful for the preparation of traditional medicine as well as the presence of wildlife opens the possibility of a thriving tourism industry. Various national parks around the world are developed where people can have an easy look of natural habitat of wild animals and also they have massive incomes every year. Preserving animals' in their natural habitats is one of the most effective ways to protect wildlife. Animals face fears to their habitats, including farming, development and deforestation. Habitat destruction can cause species extinction, and the preservation of natural land is one of the key ways to protect wildlife from destruction. Government ever year make a concentrated effort to protect animals and habitats. But due to various illegal activity specially poaching of animal's parts and skin as well as due to reduce in the habitat there is continuous reduction of wildlife population. For these purpose a new field "Wildlife Forensics

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is emerging for the protection as well as preservation of wildlife prevailing in different countries.

INTRODUCTION

India is the seventh largest country in the world and Asia's second largest nation with an area of 3,287,263 km², a national border of 15,200 km and a coastline of 7516 km. Ecologically, India can be divided into three main regions:

- the Himalayan Mountain system;
- the peninsular India subregion (woodlands and desert); and
- the tropical rain forest region.

A great wealth of biological diversity exists in these regions and in India's wetlands and marine areas. Table 1 represents the number of species present in India and World. The Indian subcontinent include verdant forests, rich coastal fish resources, water-stocked Himalayan ranges, grassy pastures, productive estuaries and bountiful river systems. Abundant rain and fertile soils are also added to this plentitude. However, years of mismanagement, have degraded forests, wounded coastline and poisoned aquifers with devastating results. Today, India contains 172 species (2.9% of the world's total number) of animals that are considered to be globally threatened by the IUCN. These include 69 species of birds, 53 species of mammals, 23 species of reptiles and 3 species of amphibians. Extinction is threatening all species, but smaller animals like bats and rodents, face this threat more as compare to the other animals. The major reasons for extinctions can be attributed, either directly or indirectly, to human demographic and technological expansion, commercialized exploitation of species and human-caused environmental change. These factors further in turn have affected the reproductive rate of endangered species and their adaptability to changing environmental conditions.

Number of species in India and the world.

group	Number of	Number of	SI/SW (%)
	species in India	species in	
	(SI)	the world	
		(SW)	

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Mammals	350	4629	7.6
Birds	1224	9702	12.6
Reptiles	408	6550	6.2
Amphibians	197	4522	4.4
Fishes	2546	21,730	11.7

Wildlife habitat and species around the world are fronting a crisis. It is estimated that global warming may cause the extinction of 15–37% of species by 2050. For the preservation of Wildlife, India has launched an extensive protected area network of research institutions in which legislation, socio-economic factors and wildlife research are playing a great role. The Central Zoo Authority helps in programming research activities related to the conservation and propagation of wild animals. Planned research activities such as studies on wildlife biology, species specific nutritional requirements, genetic variability, epidemiological surveys, disease diagnosis through postmortem examination and animal behaviour. The future rest on interaction between captive and wild animals, preservation of biodiversity and genetic and demographic variations of species. India possess 65% of Asia's tiger population, 80% of the Asian elephant population, 85% of the Asian rhino population and 100% of the Asiatic lion population. These all are highly endangered and poached animals. A large quantity and variety of wildlife products are smuggled from India to international markets. These include live animals such as birds and reptiles, along with skins, bones and other body parts from poached animals. For these reasons a new field is come up known as Wildlife forensics.

Wildlife forensics is the application of science to legal cases involving wildlife. This field uses scientific procedures to investigate wildlife-related crimes involving the exotic pet trade, poaching, other illegal hunting activities as well.

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Wildlife (Protection) Act, 1972

The Wildlife protection was formed by the Government of India in the year 1972 for the protection of wild animals, birds and plants with a vision to protect the ecological and environmental safety of the country.

The Wild life protection Act in relation to wildlife is mention below formed as per the government. As per the government act

THE WILD LIFE (PROTECTION) ACT, 1972 ACT NO. 53 OF 1972

An Act to provide for the protection of wild animals, birds and plants and for matters connected therewith or ancillary or incidental thereto with a view to ensuring the ecological and environmental security of the country. As per the act any captive animal, animal article, trophy, uncured trophy, meat or specified plant buying or selling any such animal or article is illegal. As per the Act Chapter 1 is mention below: CHAPTER I

Short title, extent and commencement.—

This Act may be called the Wild Life (Protection) Act, 1972.

It extends to the whole of India except the State of Jammu and Kashmir.]

It shall come into force in a State or Union territory to which it extends on such date as the Central Government may, by notification, appoint, and different dates may be appointed for different provisions of this Act or for different States or Union territories.

Definitions.—In this Act, unless the context otherwise requires,— “animal” includes amphibians, birds, mammals and reptiles and their young, and also includes, in the cases of birds and reptiles, their eggs;

“animal article” means an article made from any captive animal or wild animal, other than vermin, and includes an article or object in which the whole or any part of such animal [has been used, and ivory imported into India and an article made therefrom];

“Board” means a State Board for Wild Life constituted under sub-section (1) of section 6;

“captive animal” means any animal, specified in Schedule I, Schedule II, Schedule III or Schedule IV, which is captured or kept or bred in captivity;

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“Chief Wild Life Warden’ means the person appointed as such under clause (a) of sub-section (1) of section 4; [(7A) “circus” means an establishment, whether stationary or mobile, where animals are kept or used wholly or mainly for the purpose of performing tricks or manoeuvres;

“Collector” means the chief officer in charge of the revenue administration of a district or any other officer not below the rank of a Deputy Collector as may be appointed by the State Government under section 18B in this behalf;

“commencement of this Act”, in relation to—a State, means commencement of this Act in that State, any provision of this Act, means the commencement of that provision In the concerned State;

“dealer” in relation to any captive animal, animal article, trophy, uncured trophy, meat or specified plant, means a person, who carries on the business of buying or selling any such animal or article, and includes a person who undertakes business in any single transaction;

“Director” means the person appointed as Director of Wild Life Preservation under clause (a) of sub-section (1) of section 3;

(12A) “Forest officer” means the Forest officer appointed under clause (2) of section 2 of the Indian Forest Act, 1927 (16 of 1927) or under any other Act for the time being in force in a State; (12B) “forest produce” shall have the same meaning as in sub-clause (b) of clause of section 2 of the Indian Forest Act, 1927 (16 of 1927);

“Government property” means any property referred to in section 39 4[or section 17H];

“habitat” includes land, water or vegetation which is the natural home of any wild animal;

“hunting”, with its grammatical variations and cognate expressions, includes,—

(a) killing or poisoning of any wild animal or captive animal and every attempt to do so;

(b) capturing, coursing, snaring, trapping, driving or baiting any wild or captive animal and every attempt to do so;

(c) injuring or destroying or taking any part of the body of any such animal or, in the case of wild birds or reptiles, damaging the eggs of such birds or reptiles, or disturbing the eggs or nests of such birds or reptiles;

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“land” includes canals, creeks and other water channels, reservoirs, rivers, streams and lakes, whether artificial or natural, [marshes and wetlands and also includes boulders and rocks]; “licence” means a licence granted under this Act;

(18A) “livestock” means farm animals and includes buffaloes, bulls, bullocks, camels, cows, donkeys, goats, sheep, horses, mules, yaks, pigs, ducks, geese,

poultry and their young but does not include any animal specified in Schedules I to V;

“manufacturer” means a person who manufactures articles from any animal or plant specified in Schedules I to V and VI, as the case may be; “meat” includes blood, bones, sinew, eggs, shell or carapace, fat and flesh with or without skin, whether raw or cooked, of any wild animal or captive animal, other than a vermin;

(20A) “National Board” means the National Board for Wild Life constituted under section 5A; “National Park” means an area declared, whether under section 35 or section 38, or deemed, under sub-section (3) of section 66, to be declared, as a National Park;

“notification” means a notification published in the Official Gazette; “permit” means a permit granted under this Act or any rule made thereunder; “person” includes a firm;

[(24A) “protected area” means a National Park, a sanctuary, a conservation reserve or a community reserve notified under sections 18, 35, 36A and 36C of the Act; “prescribed” means prescribed by rules made under this Act;

(25A) “recognised zoo” means a zoo recognised under section 38H; (25B) “reserve forest” means the forest declared to be reserved by the State Government under section 20 of the Indian Forest Act, 1927 (16 of 1927), or declared as such under any other State Act;

“sanctuary” means an area declared as a sanctuary by notification under the provisions of Chapter IV of this Act and shall also include a deemed sanctuary under sub-section (4) of section 66; “specified plant” means any plant specified in Schedule VI;

“State Government”, in relation to a Union territory, means the Administrator of that Union territory appointed by the President under article 239 of the Constitution;

“taxidermy”, with its grammatical variations and cognate expressions, means the curing, preparation or preservation or mounting of trophies;

(30A) “territorial waters” shall have the same meaning as in section 3 of the Territorial Waters, Continental Shelf, Exclusive Economic Zone and other Maritime Zones Act, 1976 (80 of 1976);

“trophy” means the whole or any part of any captive animal or wild animal, other than vermin, which has been kept or preserved by any means, whether artificial or natural, and includes—rugs, skins and specimens of such animal mounted in whole

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or in part through a process of taxidermy, and antler, bone, carapace, shell, horn, rhinoceros horn, hair, feather, nail, tooth, tusk, musk, eggs, nests and honeycomb;

“uncured trophy” means the whole or any part of any captive animal or wild animal, other than vermin, which has not undergone a process of taxidermy, and includes a [freshly killed wild animal, ambergris, musk and other animal products];

“vehicle” means any conveyance used for movement on land, water or air and includes buffalo, bull, bullock, camel, donkey, elephant, horse and mule; “vermin” means any wild animal specified in Schedule V;

“weapon” includes ammunition, bows and arrows, explosives, firearms, hooks’ knives, nets poison, snares and traps and any instrument or apparatus capable of anaesthetizing, decoying, destroying, injuring or killing an animal; “wild animal” means any animal specified in Schedules I to IV and found wild in nature;

“wild life” includes any animal, aquatic or land vegetation which forms part of any habitat; “Wild Life Warden” means the person appointed as such under clause (b) of sub-section (1) of section 4;

“zoo” means an establishment, whether stationary or mobile, where captive animals are kept for exhibition to the public [and includes a circus and rescue centres but does not include an establishment] of a licensed dealer in captive animals.

Aim and Scope of the Work

Wildlife resources establish a vital link in the existence of the human species and have been a subject of much interest and research all over the world. Today, a large number of species of wild fauna have become endangered. India is one of the richest biodiversity nations of the world having mixture of Palaearctic, Oriental and Afro-tropical fauna. India has 400 mammal species out of which 129 species are protected under Wildlife Protection Act and is an arbitrator to Convention on International Trade in Endangered Species (CITES). Illegal trade of the wildlife products is the key risk to conservation and is responsible for regional obliteration to these species.

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Wildlife Forensics

Wildlife forensics is a new emerging field of criminal investigation. It deals with the application of scientific procedures to examine, identify and compare evidence obtained from the crime scenes and to link the evidences with a suspect and a victim, which is specifically an animal. Killing wild animals that are protected from hunting by laws, also called poaching, is one of the most serious crimes investigated by wildlife forensic scientists. Other crimes against wildlife include buying and selling protected animals and buying and selling products made from protected animals.

The types of evidence examined by a wildlife forensics lab include any part of an animal including blood and tissue samples, hair, carcasses, bones, feathers, claws, tusks, teeth, talons, hides, fur, or stomach contents etc. Wildlife forensic scientists may also investigate the actual cause of death that is materials used to kill or harm animals, such as pesticides, poisons, weapons and projectiles. Products that are made from animals are also investigated which may include leather goods and medicines, especially those from Asia. One of the most critical problems faced by the wildlife forensic scientists is identifying a particular species from crime scene evidence. For example, wildlife forensic scientists need to distinguish if a piece of leather on a watchband is made from a protected animal, like a zebra or an elephant, or if it comes from a non-protected animal, like a horse or a cow. The scientist must be able to differentiate whether the medicinal powder contains the pulverized remains of a protected animal or not.

Wildlife crime includes an illegal trade, possession of wildlife animal, plants and their products which comes under the action against national and international law (Cooper 2008, Lawton 2009). Currently trade of illegal wildlife flora and fauna is taking place all over the country as per WWF (World Wildlife Fund) of India in 2014. The major fears are hunting for meat, skins, and traditional remedies (International Union for Conservation of Nature (IUCN) 2016). The illegal wildlife trade involves thousands of wild animals and associated products which are shipped daily around the globe as medicines, food, pets, trophies, clothing, and religious amulets (Wyler 2013, Wilson-Wilde 2010) as shown in Figure 1. Under the wildlife protection Act (WPA) 1972, trade of wild animals, plants and their derivatives is illegal. After narcotics and firearms, wildlife products are third in the row of illegal trade (Sahajpal 2009, Mayer 1952). The wildlife crimes are increasing day by day and area covered under this is indefinite. The major cause of misuse of wildlife natural resources is illegal trade of wildlife animal skin, sport,

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food and clothing, elephant ivory and its medicinal needs for anti-aging and growth medicines (Sreepada 2002). In India to protect wild animal's project are launched for



showing the image of Tiger and Leopard trophy and articles

many species such as Tiger, Sea turtle, Hangul (Red Deer), Crocodile, Elephant and one Horned rhino.

A variety of scientific techniques are used now a days which allows wildlife forensic scientists to distinguish various species from the animal products which they obtained from crime scene or illegal trade. Various analytical markers for species identification in wildlife forensic involves morphological study of the species, microscopic examinations of the body parts, footprints analysis at the crime sites, anatomical studies and serological methodology. These studies are performed on the basis of characteristics of long bones, hair, species-specific tooth morphology and other associated traits (Bell 2011). Recently, DNA markers are also being used for accurate identification of species. Identification of species based on DNA microsatellites was developed in 1999 which is being utilized in identification of species from several samples (Verma 2014). Identification of species based on Mitochondrial DNA (mt-DNA) is also applied. National Center for Biotechnology Information (NCBI) is the source data base of DNA sequences from different species which helps in comparison after PCR amplification and sequences produced with genetic analyser from wildlife evidences. But the

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repository of DNA sequences and markers are not available for a majority of wildlife flora and fauna. The illegal trade of wildlife flora-fauna and their products can be reduced with the help of wildlife forensics. But despite advances in wildlife detection technology and general descriptive work on the illegal trade (Cowdrey 2002, Smith et al. 2009, Rosen 2010, Oswell 2010, South 2011, Pires 2012, Milliken 2012, Ayling 2013, Underwood 2013, Karesh 2007, Goss 2013), current prevention and control approaches are failing (World Wildlife Fund Dalberg 2012, World Bank 2008). More quantitative research is still required on the techniques which can help in easy identification of species without the much destruction of samples and can provide results with high accuracy (Vira 2014, Schneider 2008, Toledo 2012).

Current Status of Royal Bengal Tigers in India

India has over half the world's tiger population. Throughout India, every two to four years, a comprehensive tiger census is conducted. In 1972, the first census was conducted, 1827 tigers were recorded. Further establishment of Project Tiger in 1973 led to an increase in the tiger population. In 1989 census 4344 tigers were recorded, while in 1993 census only 3750 tigers were recorded thus showing a decline from four years earlier. Figure 2 shows the graph of Asian big cats illegal trading from 2010 to 2016. In the early 1990s, the tiger's conservation status shown shattering news with the discovery

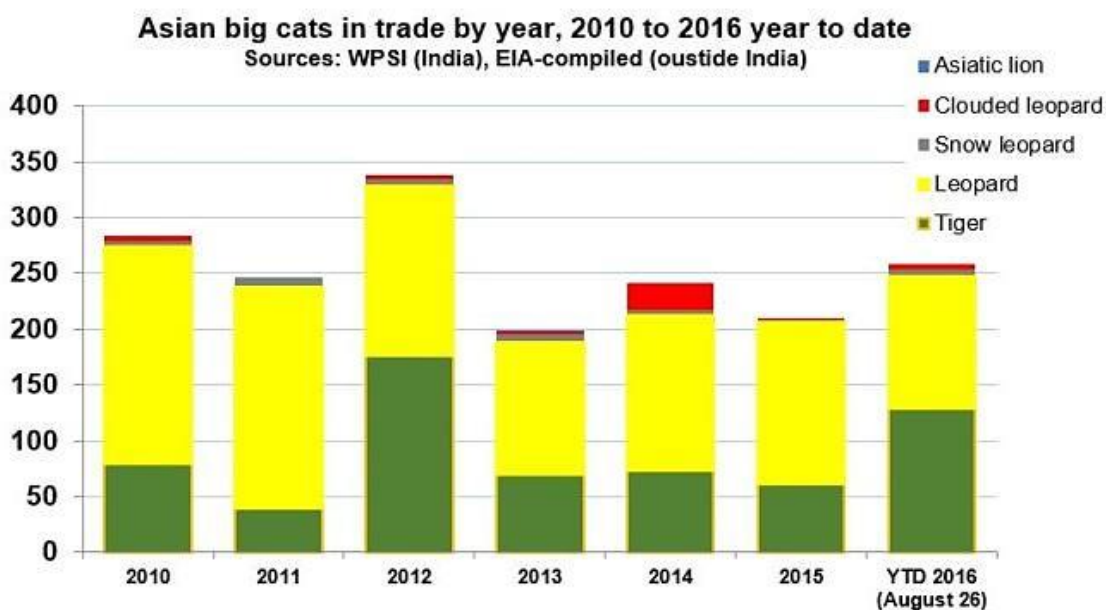
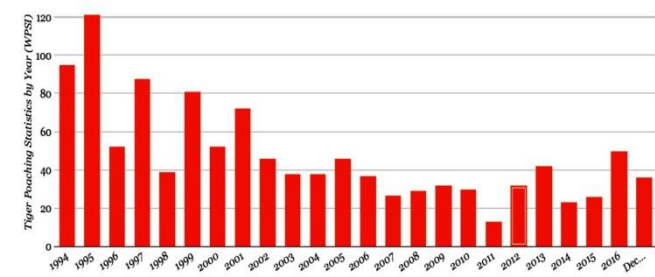


Figure 2 shows the graph of Asian big cats illegal trading from 2010 to 2016 Source: <https://encrypted->

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of large scale poaching and trafficking for the illegal international trade of tiger parts. The huge demand for tiger bones for the use in Oriental traditional medicine outside of India and as a macho supplement, was an additional threat to India's tiger population. Practically every part of the tiger, from its whiskers to its tail are utilized for the traditional Chinese medicine. Figure 3 shows the historical tiger poaching statistics from 1994 to Dec 2017.



shows the historical tiger poaching statistics from 1994 to Dec 2017.

Poaching is done by the mafia gangs and is a part of the flourishing trade. In 1993–94, 36 tiger skins and 667 kg of tiger bones were seized in north India. Poachers usually kill wild tigers via poison, steel traps, or firearms. The estimated cost for the poachers for each tiger killed is \$1 for poisoning, \$9 for trapping, and \$15 for shooting which is further distributed among four poachers. If charged and proven guilty, the maximum punishment is of three years in jail and/or Rs.25,000 in fines also there is no minimum sentence. The Wildlife Protection Society of India has made intensive effort to gather correct information and document cases of tiger poaching and unnatural deaths of tigers throughout the India. As per government sources 596 tigers are known to have been killed from 1994 to 2002; however, a non-government organization puts that number much higher as mention in Table 1. Under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) the international trade in tiger products has been banned and only 8 out of the 14 countries within the tiger's range comply with it. Out of the original nine subspecies of tigers, three have become extinct in the last 80 years; an average of one every 20 years. It has been predicted that all the tigers may become extinct in the wild within the next decade. The total number of all the wild populations of the six remaining subspecies of tigers that is Bengal, Indochinese, Malayan, Siberian, South China,

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and Sumatran is estimated to be between 3,000 – 3,600 tigers. Figure 4 shows the tiger mortality from 2008 to 2017.

TIGER MORTALITY BETWEEN 2008-2017			
YEAR	Poaching & Seizure	Other Mortalities	Total
2008	28	23	51
2009	32	53	85
2010	30	28	58
2011	13	48	61
2012	32	57	89
2013	43	38	81
2014	23	58	81
2015	26	65	91
2016	50	82	132
2017	22	50	72

shows the tiger mortality from 2008 to 2017

Current Status of Asiatic Lion

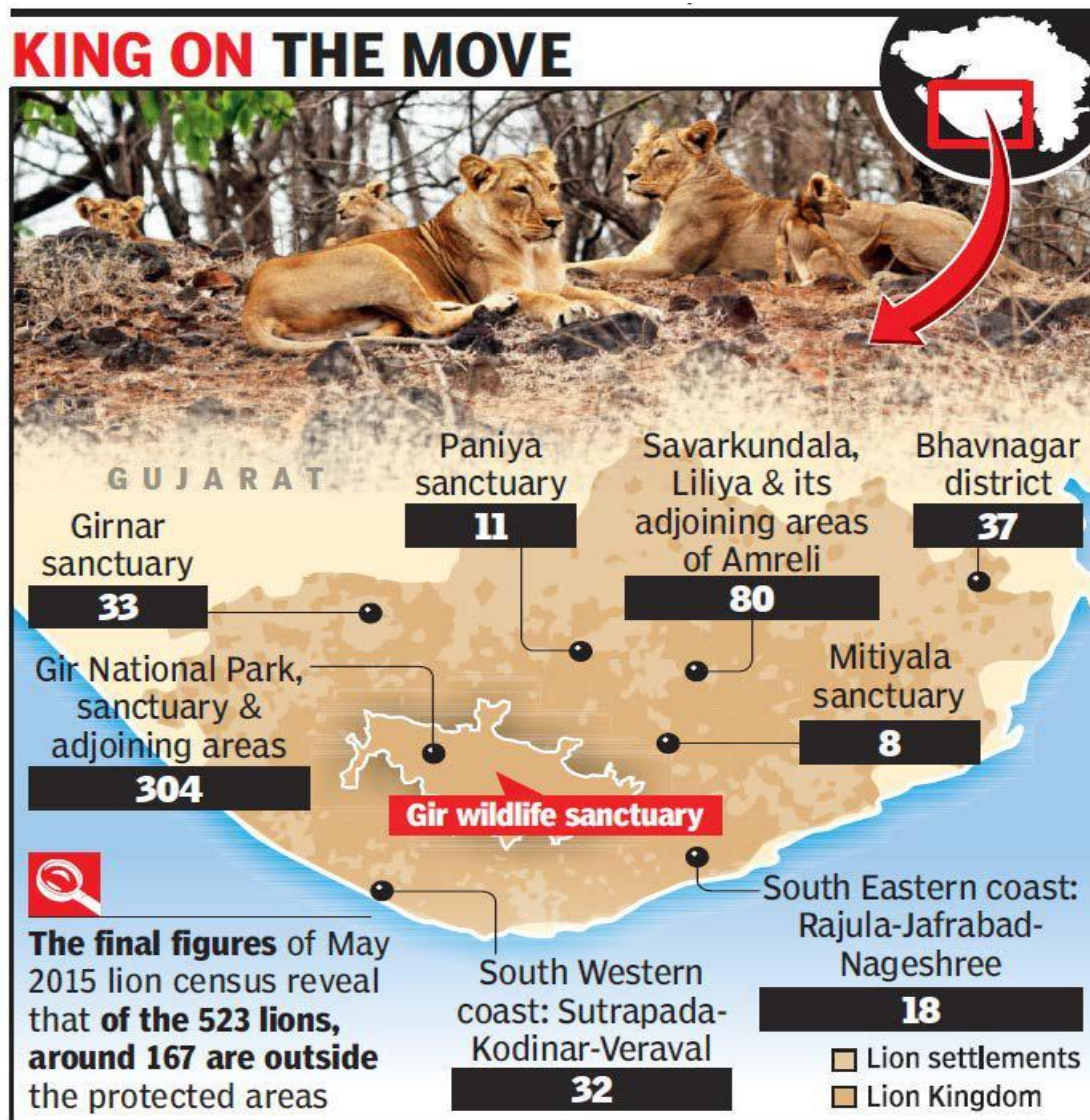
Slowly and gradually the Asiatic Lion species are becoming extinct from all the countries of the world, except India. Hunting of animal on a large scale led to their total extinction from most of the countries. The species became extinct in Turkey around late 1800s and in Iran and Iraq, Asiatic Lions were last sighted in the year 1942 and 1918 respectively. At present the population of the majestic animal has also dropped to alarming proportions in India also. The population of Asiatic Lions in India fell to only 13 lions in 1907. Due to this scenario, the Nawab of Junagadh gave lion's complete protection and banned their hunting within his province. The Gir National Park of India is the only place in the world where Asiatic lions are found. As per the Indian Government first census based on identification of individual pugmarks of lions in Gir, in the year 1936 the count was 234 animals. The next census was conducted between 1968 and 1979 based on animal counts at live baits and the population of lion was found to be around 100. As per the census of 1990, there were 221 adults lions existing in the Gir Lion Reserve and around 30-40 lions were found to be inhabiting in the adjacent agricultural areas. The current status of the Asiatic Lions is based on the 2006 census revealed

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a population of around 359 lions in the Gir National Park. Further as per the census in 2015 there are currently 523 lions are left. The entire population is living within an area of 1,412 sq km inside the sanctuary. Though the population of Asiatic lions in India has increased considerably since 1907, much still needs to be done.

The current status of Asiatic lions has improved from 1907, when there were only 13 lions in India. But still, it is less than what it should have been. Various factors contributed to this. Though Gir National Park provide a complete protection for the species but still one can find incidences of poaching here. Another major reason is poisoning by the nearby residents as well as natural epidemics like floods, fires, etc. Also the wells dug by farmers for irrigation purposes causes their death as they get trap in there. Other instances like the fence made by farmers with crude and illegal electrical fences to protect their crops from Nilgai also causes death of animal. The Maldhari

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Figure 5 shows the Gir wildlife sanctuary distribution of lion population Source:

community of Gir also threatens the existence of lions. All these factors together results in the large decline of the Lion population. Figure 5 shows the Gir wildlife sanctuary distribution of lion population.

Current Status of Panther

The cat family Felidae (order Carnivora) originated in the late Miocene (Johnson 2006) and has evolved into one of the world's most successful carnivores. The leopard, *Panthera pardus* is the most widely distributed and adaptable large cat in this family. The geographical range of leopards covered all of sub-Saharan and North Africa, the Middle East and Asia Minor, South and Southeast Asia, and is extended up to the Amur Valley in the Russian Far East (Seidensticker 1991, Nowell & Jackson 1996). Occupying diverse habitats across most of Africa and Asia (Nowell & Jackson, 1996) the leopard *Panthera pardus* is one of the most widespread species of large cat (Perez 2006). Leopards are widely distributed across Africa and Asia, but their populations have reduced, isolated and now destroy completely from large portions of their historic range. Leopard populations have been intensely reduced due to continued persecution with habitat fragmentation (UN 2014), increased illegal wildlife trade (Datta et al. 2008), increased human populations (Thorn et al. 2013, Selvan et al. 2014), excessive harvesting for ceremonial use of skins, poorly managed trophy hunting (Balme et al. 2009) and prey base deteriorations (Hatton et al. 2001, du Toit 2004, Fusari and Carpaneto 2006, Datta et al. 2008, Lindsey et al. 2014, Selvan et al. 2014). Also factors which affect leopards reduction includes increased in Human populations as well as deforestation. These factors have a significant impact on suitable leopard range. Leopards have suffered marked reductions and regional destruction due to poaching for illegal wildlife trade, fragmentation, habitat loss and prey loss, throughout East and South-east Asia, Middle East, North, East and West Africa. A regional survey found that leopards in India are poached at a rate of four individuals per week for the illegal wildlife trade (Raza et al. 2012b). According to Nowell and Pervushina (2014) the illegal trade of leopard parts are comparable to that of Tigers present in Asian range States and derivative seizures with an average of 3.5 leopard's seizure cases per month in India since 2000. Also the illegal trade of leopard skins for cultural purpose is widespread in southern Africa. Figure 6 shows the articles of leopard been poached. It is

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proposed that 4,500-7,000 leopards' area is harvested annually to fuel the demand for leopard's skins by followers of the Nazareth Baptist (Shembe) Church only (Balme unpub. data).



Figure 6 shows the articles of leopard been poached

Leopards have declined substantially (>30%) since the earlier assessment as determined by extensive population surveys, expert input and indirect measures (criteria A2cd). Leopards are also killed for their skins in traditional ceremonies and their other parts are used for medicinal use in eastern cultures.

Various researches are reported on various endangered species for their identification purpose. Determination keys for getting accurate identification are based on microscopic characteristics such as size, shape, profile and colour of the nails, scale pattern etc. Researchers have done molecular based studies, hair examination, genetic analysis, application of captured images etc for the identification of species. However, the identification is problematical by the occurrence of significant discrepancy caused by differences of species, breed and gender of the animals, by environmental conditions (climate, habitat, nutrition) and various other factors. Now a days, the crime related to animals of high economic impact (Scheduled under Wildlife Protection

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Acts) has gained specific focus, where nail can be used for identification of the animals in issues related to species identification.

Nail plays a vital role as the key evidence found in cases related to wildlife crimes of animals scheduled under Wildlife Protection Act. These animals are mostly poached for their meat, skin, bones, claws, nails, ivory etc. where nail can be used to identify the species. The present study shows how claw varies between species and also the development of a database of claw samples of different species that can be used by wild life forensic scientists in investigating cases of illegal trafficking of the world's most endangered animals. The study focusses on the keratin which is a structural proteins found abundantly in the outer layer of human skin, in hair, and in nails. Keratin is rich in the amino acid cysteine. It get self-assemble into bundles of fibers and each of these fiber bundles contains individual strands crosslinked through S-S (sulfur-sulfur) bonds involving the cysteine side chains. Thus keratin forms tough, insoluble structures that are among the strongest non-mineralized tissues found in nature. The ongoing research on microscopic characteristics of claw can be used to offer more information for wildlife forensics and provide vital information for the identification of prohibited transfer of animals, poaching or wildlife crime, fraud in textile and fur industry, and recognition of their victims etc. Further, the application of newly developed non-destructive techniques such as Fourier transform infrared spectroscopy - attenuated total reflection (FTIR-ATR) and video spectral comparator (VSC) can prove to be of great significance as they do not require any kind of sample pre-treatment or any hazardous chemical which can disrupt the integrity of an evidence, as in forensic case work the evidential value has to be maintained. Application of non-destructive techniques also preserve the samples for longer duration for any further analysis. Here, the aim of the present study is to explore the applicability of various non-destructive techniques for the identification of species of various endangered species which can prove to be useful in forensic investigation and can also provide an identification library using different advanced tools, which will help government as well as forensic scientist in relation to any wildlife crime.

CONCLUSIONS

Increased taxon sampling of Trigonostominae, including four new species, in a comprehensive molecular phylogeny inferred from 18S and 28S rRNA sequences allowed us to demonstrate inter- and intrageneric relationships within this group. This phylogenetic context enabled us to

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reassess homology statements on some key morphological traits associated with the stylet and the afferent system. Our results show that (1) species of Ceratopera and Messoplana cannot be clearly separated based on morphological and molecular data leading to the suppression of the latter genus in favour of Ceratopera; (2) traditional species groups within Trigonostomum are partly supported by molecular data, while traditional species groups within Proxenetes are not; (3) the presence of a mantle accompanying the stylet proper reflects the plesiomorphic condition of the Trigonostominae; (4) the spermatic duct and seminal receptacle in Ptychopera is inferred to be homologous with (parts of) the bursal appendage and the insemination duct in other genera; and (5) the ancestral bursal appendage resembled a simple, slightly sclerotized tube. Further taxon sampling and additional molecular markers will be necessary to more fully resolve the phylogeny of Trigonostominae and refine our inferences about the evolution of their highly complex reproduction structures.

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