Chapter – 1

INTRODUCTION

1.1. FORENSIC SCIENCE

The term forensics means application of science methods to the investigation of crime. Various enforcement agencies in the legal/ criminology department make use of forensic science to investigate and solve wildlife crime with the admissible evidence of scientific backing.

The forensic science is the combination of two different Latin words i.e. Forensic and science. The second is science which is derived from Latin word for knowledge and is today closely tied to the scientific method and way of acquiring knowledge. The two words taken together forensic science is the methods and processes in crime solving.

Forensic scientists collect, preserve, and analyze the evidence during the course of an investigation. Scientists, forest guards and other officials within the department travel to the scene of crime for surveillance through which they collect the evidence, amongst certain evidences requiring biological investigation is taken to the laboratory. The forensic scientists and other science personnel, use scientific techniques for analyzing the objects of evidences.

The forensic science include many divisions liken Forensic Accounting / Auditing, Computer or Cyber Forensics, Crime Scene Forensics, Forensic Archaeology, Forensic Dentistry, Forensic Entomology, Forensic Graphology, Forensic Pathology wildlife forensics, Forensic toxicology, Forensic Psychology, Forensic Podiatry, Forensic Optometry Forensic Odontology Forensic Linguistics, Forensic Geology, Forensic, Forensic Entamology, Forensic Engineering, Forensic DNA Analysis, Forensic Botany, Forensic Narcotics, Forensic Anthropology, Forensic Serology, Forensic Ballistics, Digital Forensics, Criminology, Crime Scene Photography, Forensic Chemistry.

It includes seven principles:

1. Law of individuality
2. Principle of exchange
3. Law of progressive change
4. Law of comparison
5. Law of analysis
6. Law of probability
7. Law of circumstantial facts

Dr. Edmond Locard born in the year 13 December 1877-1966. He, the father of forensic science, a French criminologist, the pioneer in forensic science known as the "Sherlock Holmes of France". He formulated the basic principle of forensic science: "Every contact leaves a trace". This became known as Locard's exchange principle. is Dr. Edmond.

1.2. WILDLIFE FORENSICS

WHAT IS WILDLIFE?

The definition of wildlife forensic science is Animals living in their natural habitat and not within the possession or control of humans. Is taken to comprise those species of animals or plants that are typically found occurring naturally in wild, in contradictory to the "cultivated" species that have certain characteristics engineered or fed in through techniques of scientific backing.

A wild animal is by the definition of species that has not been domesticated. For instance, a wolf is a wild animal whereas a pet dog is domesticated. However, the wolf may be in captive or be free living too.

WILDLIFE FORENSICS

Wildlife forensics is the science applied to legal issues involving wildlife crimes. The scientific procedures to investigate wildlife-related crimes involving the exotic pet trade, poaching, other illegal hunting activities. A wildlife forensic specialist is a scientist who uses science-based techniques to investigate wildlife crimes. These scientists analyze animals, animal parts and products, and other evidence collected by wildlife inspectors and other officials that require scientific inspection.

Its goal is to use the specific procedures to examine and compare evidence from crime scenes and to the evidence with a suspect and victim.

Killing wild animals that are protected from hunting by law is also called poaching, that is one of the most serious crimes investigated by wildlife forensic scientists. The international organization that monitors trade in wild animals and plants is the convention on international trade in endangered species of wild flora and fauna (CITES), which is established in 1963 and , includes 183member countries the endangered species act was authorized in the year 1972, which protects the endangered and threatened species. The types of evidence analyzed by a wildlife forensic laboratory include any part of an animal including blood and tissue samples, carcasses, hair, teeth, bone, claws, talons, tusks, hides, fur, feathers, or stomach contents. Wildlife forensic scientists may also investigate materials used to kill or harm an animal such as poisons, pesticides, projectiles and weapons.

WILDLIFE INVESTIGATION TEAM AND ROLL

1. The investigation team leader
2. The evidence collector
3. The scene photographer
4. The scene sketcher
5. Additional team members.

1.3. WILDLIFE PROTECTION ACT(1972)

An act to provide the protection to wild animals, birds and plants and for matters connected therewith or incidental with a view to ensure the ecological and environmental security of the country.

This act can be called as wildlife protection Act, 1972. It extends to whole of India except the state Jammu and Kashmir. It has six schedules which varying degree of protection. Schedule I and part II of Schedule II provide absolute protection - offences under these are prescribed the highest penalties. Species listed in Schedule III and Schedule IV is also protected, but the penalties are much lower. Schedule V includes the animals which may be hunted. The specified endemic plants in Schedule VI are prohibited from cultivation and planting. The hunting to the Enforcement authorities has the power to compound offences under this Schedule.

The legislatures of the state of Andhra Pradesh, Bihar, Gujarat, Haryana, Himachal Pradesh, Madhya Pradesh, Manipur, Punjab, and west Bengal passed resolution empowering parliament to pass the necessary legislation on the subject. According to the wildlife protection act bill was introduced in the parliament.

1.4. IMPORTANCE OF SCIENTIFIC ANALYSIS IN WILDLIFE FORENSICS

Forensic science enables scientists to identify an animal by sample of DNA in the form of blood or tissue cells. Molecular studies involving reptiles often overlook shed skins as a source for high-quality DNA. In most cases, tissues or blood samples are preferred by researchers, but the process of sampling for these tissue types can be harmful or otherwise adversely affect the animals involved. While reptile breeders or zoological institutions are a potential source of specimens, most will likely decline requests for samples if the sampling will harm their prized animals. Generally, breeders and curators would be much more amenable to part with a shed skin—which is something they usually discard anyway.

Genetic data can provide a powerful tool for those interested in ecology and management of wildlife, especially when it is combined with behavioral, demographic, or spatial information. The applications of genetic analyses are becoming feasible and cost effectively. Genetic data can provide a powerful tool for those interested in the ecology and management of wildlife, especially when it is combined with behavioral, demographic, or spatial information. Genetic data can be used to assess mating systems, hybridization, gene flow, effective population size, and population viability. Genetic can also be used to define management units, identify individuals, sex, and species, and to provide insights into demographic patterns associated with the reduction and expansion of population. All of these factors are either related to species ecology or provide information of management and conservation. As the genetic methods become progressively more accessible and adaptable to an ever-widening array of questions, it is expected that they play an increasingly important role in the ecology and management of wildlife.

Wildlife forensics contains the molecular biology including identification by DNA profiling and sequencing. Illegal hunting of wildlife is serious worldwide concern for wildlife controlling the genes located on mitochondrial DNA are the source of most effective molecular marker used in wildlife forensics.

There are a developing range of modern DNA approaches that can be used in wildlife crime investigations. DNA profiling is one of the most effective protocols so far in dealing wildlife crime scenes, which is the DNA basis for the DNA wildlife forensics.

As such the Examination is done by using the Molecular and Morphological metods such as DNA Extraction, Quantification, Gel Electrophoresis, PCR by using the samples such as Skin, Tissue, Blood, Bone, Scat, Hair etc,.

As like all animals in the web of life, Reptiles play an important role in our ecosystem by maintaining a balance to the food web. Humans hunt the reptiles for the food since history. Present humans mostly hunt the animal skin for use of ornamental use like shoes, coat, and medicines.

Chapter – 2

AIM & OBJECTIVES

2.1. AIM: Quantification of DNA from snakeskin exuviates by Molecular Analysis.

2.2. OBJECTIVES:

1. DNA Isolation from shed snake skin.
2. Analysis and Quantification of isolated gDNA (genomic DNA).
3. Polymerase chain reaction using 12S and Cytb Mitochondrial markers.

Chapter - 3

LITERATURE REVIEW

The term Forensics means application of science methods to the investigation of crime. Various enforcement agencies in the legal/ criminology department make use of forensic science to investigate and solve wildlife crime with the admissible evidence of scientific backing.

The forensic science is the combination of two different Latin words i.e. Forensic and science. The second is science which is derived from Latin word for knowledge and is today closely tied to the scientific method and way of acquiring knowledge. The two words taken together forensic science is the methods and processes in crime solving.

Wildlife forensics is the science applied to legal issues involving wildlife crimes. The scientific procedures to investigate wildlife-related crimes involving the exotic pet trade, poaching, other illegal hunting activities. A wildlife forensic specialist is a scientist who uses science-based techniques to investigate wildlife crimes. These scientists analyze animals, animal parts and products, and other evidence collected by wildlife inspectors and other officials that require scientific inspection.

The world is dealing with illegal wildlife trade, threatening to overturn decades of conservation gains. Wildlife trade is a big business run by dangerous international network and animal parts are trafficked like illegal drugs and arms. It is impossible to obtain reliable figures for value of illegal wildlife trade. Experts at TRAFFIC (trade record analysis of flora and fauna) the wildlife trade monitoring network.

Illegal wildlife trade is well known such as poaching of Elephant for Ivory and Tigers for their Skin and bones and shells of pangolin. Products demanded by trade includes Exotic pets, food, traditional medicine, clothing, jewelry made from animals such as Tusk, Fins, Skin, Shells, Horns, Teeth, Nails, Hair, Rhinoceros Horns and other internal organs and Various other plants.

As such the Examination is done by using the Molecular and Morphological methods such as DNA Extraction, Quantification, Gel Electrophoresis, PCR by using the samples such as Skin, Tissue, Blood, Bone, Scat, Hair etc.,

3.1. A Method to Predict the Percutaneous Permeability of Various Compounds: Shed Snake Skin as a Model Membrane

Penetration of various compounds through shed snake skin was measured *in vitro* to examine the effect of lipophilicity and molecular size of a compound on permeability through this model membrane. The permeabilities were found to be controlled by the lipophilicity and the molecular size of the permeant. (Tomoo Itoh et al…1990)The smaller and the more lipophilic the compound, the greater the permeability. Equations have been developed to predict the permeability from the molecular weight and the distribution coefficient of a compound. Further, the lipophilicity of shed snake skin is similar to that of human skin and the response of shed snake skin to the molecular size of a permeant is more similar to human skin than to hairless mouse skin. Considering the similarities between shed snake skin and human stratum coraeum in terms of structure, composition, and permeability characteristics, the same considerations may apply to permeability through human stratum corneum.

3.2. Molecular Identification of Three Indian Snake Species Using Simple PCR–RFLP Method

Three endangered Indian snake species, *Python molurus, Naja naja*, and *Xenochrophis piscator* are known to be significantly involved in illegal trade. Effective authentication of species is required to curb this illegal trade. In the absence of morphological features, molecular identification techniques hold promise to address the issue of species identification. They presented an effective PCR–restriction fragment length polymorphism method for easy identification of the three endangered snakes species. A 431- bp amplicon from cytochrome *b* gene was amplified using novel snake- specific primers following restriction digestion with enzymes *Mbo* II and *Fok* I (Bhawna Dubey et al…2010) . The species- specific reference fragment patterns were obtained for the target species, which enabled successful identification of even highly degraded shed skin sample confirming the utility of the technique in case of poor- quality DNA. The assay could be effectively used for forensic authentication of three Indian snake species and would help strengthen conservation efforts.

3.3. Shed skin as a source of DNA for genotyping seals

They evaluate the ability to genotype ringed seals using a novel source of DNA, skin cells shed by the seal as it moults on sea ice and found that shed skin samples yielded a lower quantity and purity of DNA compared to tissue samples. The shed skin cells were a viable source of DNA for microsatellite analysis. They found no significant difference in allelic diversity or heterozygosities between tissue samples and shed skin cells.(B. J. swanson et al…2006) This source of DNA should allow the rapid collection of a large number of noninvasively collected DNA samples in ice- breeding phocids and has proven difficult and has limited the ability to use molecular genetics on these species.

3.4. Trace evidence scrapings: a valuable source of DNA

Collection and analysis of trace evidence (e.g., hairs and fibers) from evidentiary materials may indicate an association with a suspect, a victim, or both to the evidence. The collected trace evidence debris may also contain sufficient cellular material removed from an item to permit identification of the wearer of that item through DNA analysis. To test this hypothesis, T-shirts and hosiery were worn by FBI Laboratory personnel for a period of time and then scraped for trace evidence. The pillboxes used to collect the scrapings were swabbed with applicators moistened with sterile water and processed alongside a friction swab of the item. The amount of DNA obtained from trace evidence scrapings was compared to the amount of DNA obtained from a friction swab of an item.(Stacy L. Stouder et al…2001) Samples were then amplified by the PCR using the AmpFlSTR® Profiler Plus™ Amplification Kit (PE Applied Biosystems 1998), and the results were compared. This study demonstrates that trace evidence debris can provide a sufficient quantity and quality of DNA to potentially identify the wearer of an item.

3.5. Purification and characterization of a neurotoxic phospholipase A_2 from Indian cobra (*Naja naja naja*) venom

Snake venoms contain multimolecular forms of phospholipase A_2 which are diverse with respect to their pharmacological properties(manoj kumar bhat et al…1991). A neurotoxic PLA_2 from *Naja naja naja* venom has been purified in two steps.

(1) The whole venom was fractionated on CM-Sephadex C-25 column; 4.6% of the total PLA_2 activity recovered was found in the NN-V fraction.
(2) The NN-Vb-PLA_2 fraction was purified to homogeneity by gel filtration of fraction NN-V on Sephadex G-50.
It is a basic protein with a mol. wt between 10,500 and 11,000, and is more toxic than other basic PLA_2s purified from *Naja naja naja* venom. The LD_{50} of NN-Vb-PLA_2 is 0.27 mg/kg body wt. It induced neurotoxic symptoms in experimental mice and is devoid of myotoxic, anticoagulant and edema-inducing activities.

3.6. Multiplex PCR assay for rapid identification of three endangered snake species of India

Species identification has been the core issue in all approaches of conservation of endangered wild life. In this regard molecular techniques for species authentication have proved indispensable(Bhawna Dubey et al…pages1861–1864(2009)). A novel multiplex PCR assay for the identification of three Indian snake species *Python morulus*, *Ptyas mucosus*, and *Naja naja* is successfully demonstrated using 16S rRNA gene. Three reverse primers and a common forward primer were designed to generate three different size species-specific PCR fragments. Absence of any PCR amplification in non-target species proves the specificity of the primers. These four primers were combined in a multiplex assay to enable identification of three snake species in a single reaction. The assay described here shows its utility in identifying unknown snake specimen and in case of samples yielding low quality DNA. This multiplex PCR technique using novel primers is an unprecedented approach offered for forensic identification of exhibits

originating from three Indian snake species. It is expected that this endeavor will help strengthening conservation efforts for these species.

3.7. Using blood and non-invasive shed skin samples to identify sex of caenophidian snakes based on multiplex PCR assay

Molecular sexing is routinely used in the fields of Forensic Investigations, population genetics and conservation biology. However, none of the assays used so far allows non-ambiguous, quick, and cheap sex identification in snakes(Alexander Kupfer et al…2017). They designed a new multiplex PCR sexing assay using two homologous loci on sex chromosomes (snake gametologous genes), *CTNNB1Z*/*CTNNB1W* and *WACZ*/*WACW*, and two combined female-specific *CTNNB1W* loci. This method was successfully tested on 81 samples from 17 caenophidian snakes. The casework with blood spots on filter paper and shed skin as a non-invasive sample confirmed the effectiveness of this assay in the forensic perspective. The assay represents a robust, cheap, rapid, and simple method which should also prove useful in unknown bodies of carcass. This efficient assay should benefit wildlife forensic laboratories and registered breeders to conform to wildlife regulations or certification of commercial trade, and boost profits for snake-breeders and conservationist snake-breeding communities.

3.8. Multiplex PCR assay for rapid identification of three endangered snake species of India

Species identification has been the core issue in all approaches of conservation of endangered wild life. In this regard molecular techniques for species authentication have proved indispensable. A novel multiplex PCR assay for the identification of three Indian snake species *Python morulus, Ptyas mucosus,* and *Naja Naja* is successfully demonstrated using 16S rRNA gene. Three reverse primers and a common forward primer were designed to generate three different size species-specific PCR fragments. Absence of any PCR amplification in non-target species proves the specificity of the primers. These four primers were combined in a to identify the three snake species in a single reaction. They described here is its utility in identifying unknown snake specimen and in case of samples yielding low quality DNA. This multiplex PCR technique using novel primers is an unprecedented approach offered for forensic identification of exhibits originating from three Indian snake species. It is expected that this endeavor will help strengthening conservation efforts for these species.

Chapter - 4

METHODOLOGY

4.1. Sample Collection

Shed snake skin samples of, were collected from Arignar Anna Zoological Park, Chennai, Tamil Nadu. Samples which were collected from individual cages of captive snakes, dried for 48-72 hrs. and stored at room temperature in air-tight zip-lock bags (Fetzner et al., 1999) for 6-8 months were used.

4.2.DNA Isolation

Isolation of DNA from shed snake skin samples was carried out cutting approximately 1 sq. inch of shed skin sample into small pieces, to which900 µL of extraction buffer containing 10 mMTris-base (pH 8.0), 10 mM EDTA, 2% SDS and 9 µL of 20 mg/mL proteinase K was added. The mixture was incubated at 56 ^{0} C for 4 hrs. and then cooled to room temperature to add 300 µL of 7.5 M ammonium acetate. The sample was vortexed and placed on ice for 15 mins. Followed by centrifugation at 12000 rpm for 5 mins. to transfer the supernatant to a fresh tube. 900 µL of ice-cold isopropanol was added to the supernatant, mixed gently by inversion and incubated at (-20^{0} C) overnight for precipitation of DNA. After incubation, the sample was centrifuged at 14000 rpm for 5 mins. And the pellet was washed twice with 500 µL of 70% ethanol. The pellet was air dried, subsequently resuspended in nuclease-free water and stored at (-20 ^{0} C).

Fig. 4.1. Isolation of DNA from Shed Skin

4.3. Analysis and Quantification of Isolated DNA

Isolated DNA samples were visualized on 0.8% agarose gel prepared by dissolving 0.24g of 1X TAE buffer and stained using Novel Juice stain (Sigma). The agarose gel picture was documented using BioRad XR+ gel documentation system. The quantity and quality of the DNA was analyzed using Thermo Scientific Nanodrop One spectrophotometer, to determine the 260/280 and 260/230 ratios.

Polymerase Chain Reaction using 12S and Cytb markers

Polymerase Chain Reaction was carried out to amplify partial fragments of 12S and Cytb markers as described by Kocher et al., 1989. The reactions were carried out in 10 µL volume containing 1X KAPA Taq Buffer B, 2.5 µMdNTPs, 2.5 mM $MgCl_2$, 1 µM forward primer, 1 µM reverse primer, 0.5 U KAPA Taq Polymerase and 50-100 ng of template DNA. The PCR reaction was carried out with an initial denaturation at 95^0 C for 5 mins. Followed by 35 cycles of 95^0 C for 1 min., 50^0 C for 1 min. and 72^0 C for 1.5 mins. Final extension step was carried out at 72^0 C for 10 mins and the PCR products were visualized on a 2% agarose gel and stained using novel juice stain. The agarose gel picture was documented using a BioRad XR+ gel documentation system

Fig. 4.2. Preparation of master mix for PCR amplification

POLYMERASE CHAIN REACTION (PCR)

This shows the PCR reaction conditions for Cytb and 12s gene regions

STEP	TEMPERATURE (° C)	TIME (minutes)
Initial Denaturation	94° C	5’
Denaturation	94° C	1’
Annealing	50° C	1’
Extension	74° C	1.5’

x 35 cycles		
Final Extension	74° C	10’

Table: 4.1. Thermal cycle for DNA Amplification

PCR COMPONENT	VOLUME	FINAL CONCENTRATION
10 X KAPA Taq Buffer B (with 1.5 mM $MgCl_2$)	1 µL	1 X
10 mM dNTPs	1 µL	1 mM
Forward Primer (10 µM)	1 µL	1 µM
Reverse Primer (10 µM)	1 µL	1 µM
KAPA Taq Polymerase (5 U/ µL)	0.1 µL	0.5 U
Nuclease-free water	4.5 µL	
DNA template (~ 40 to 100 ng/ µL)	1 µL	
Total volume of reaction	10 µL	

Table: 4.2. PCR Components with respective Concentrations and volume

4.4.Gel purification of PCR amplicon and Preparation of Sample for Sequencing

The PCR products obtained using 12S and Cytb primers were amplified in higher volume and run on 2% agarose gel and visualized using GeneiBlook illumination system to excise the gel piece containing the DNA band corresponding to a size of 300-400 bp. The agarose gel piece containing the band of DNA was transferred to a fresh centrifuge tube and DNA was purified from it using Qiagen Gel Purification kit. The DNA was eluted using elution buffer provided in the kit and its quantity was determined using Thermo Scientific Nanodrop One spectrophotometer. The sample was stored at (-20 ^{0} C) and thus prepared for sequencing.

Fig. 4.3. Loading of samples in Agarose gel

Chapter - 5

RESULTS & DISCUSSION

DNA ISOLATION - QUANTIFICATION

5.1. Nanodrop Spectrophotometry

S.No	Species name	Conc ((ng/µL)	A260/280	A260/230
1	Indian Rock Python (IRP 1)	276.9	1.39	1.62
2	Indian Rock Python (IRP 2)	566.4	1.48	0.59
3	Reticulated Python (RP 1)	319.3	1.77	1.17
4	Reticulated Python (RP 2)	575.6	1.86	1.66
5	Red sand boa (RSB 1)	612.3	1.74	1.66
6	Red sand boa (RSB 2)	638.1	1.70	1.14
7	King cobra (KC 1)	472.4	1.76	1.19
8	King cobra (KC 2)	833.4	1.88	1.53

Table 5.1. Spectrophotometric quantification of samples in microliters using a Nanodrop 2000 Spectrophotometer showing Concentration and Purity ratios

DISCUSSION

The isolated DNA samples from snake shed skin shows good concentration of DNA
The purity ratio A260/280.

5.2. AGAROSE GEL ELECTROPHORESIS – POST DNA ISOLATION

A. Shed skin DNA Isolated samples

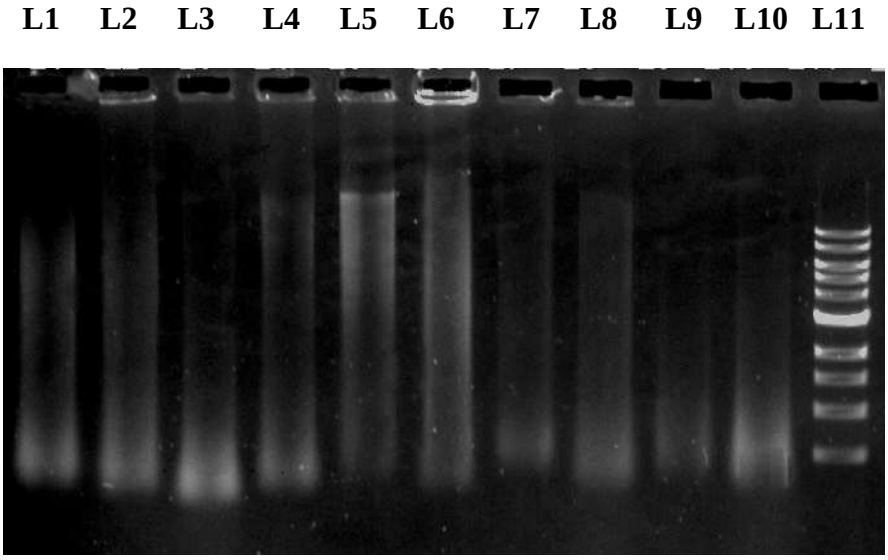

Fig 5.1. : DNA isolated from snake-shed skin of species run on 0.8% agarose gel. Each sample of 3 µL was dyed using 1 µL of Novel juice stain and loaded into the wells

L1 - IRP 1
L2 - IRP 2
L3 – RP 1
L4 – RP 2
L5 – RSB 1
L6 – RSB 2
L7 – KC 1
L8 – KC 2
L9 – IC 1
L10 – IC 2
L11 – 1 Kb DNA ladder

The genomic DNA has been isolated in duplicates using manual method of isolation and run on an agarose gel of 0.8%

All the lanes with DNA samples show a smear with faint band of higher size more than 10Kb

POST PCR AGAROSE GEL ELECTROPHORESIS

1. PCR – 12S

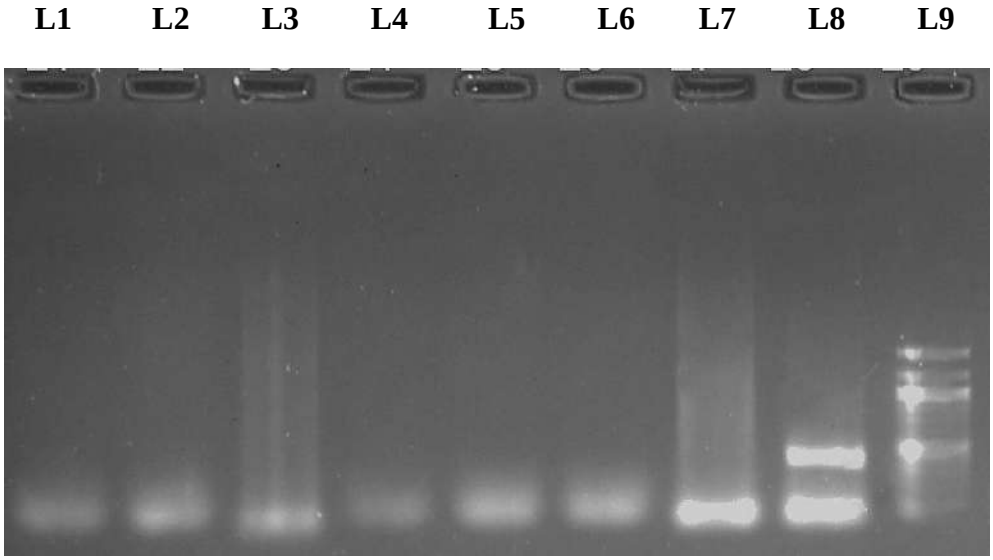

Fig 5.2. : 12S PCR amplification products from snake-shed skin of the following species were run on 2 % agarose gel. Each sample of 3 µL was dyed using 1 µL of Novel juice stain

L1 - Indian Rock Python 50.4 ng
L2 - Indian Rock Python 70 ng
L3 – Rock Python 65 ng
L4 – Rock Python 82.6 ng
L5 – Red Sand Boa 57.8 ng
L6 – Red Sand Boa 75.3 ng
L7 – King Cobra 56.4 ng
L8 – King Cobra 65.7 ng
L9 – 100bp DNA ladder

From the above PCR of 12S gene region, only KC of 65.7 ng/µL appears with a crisp thick band

The other sample will be varied with different template concentrations (40 to 100 ng/ µL) by dilution to obtain PCR amplification

2. PCR – CYTB

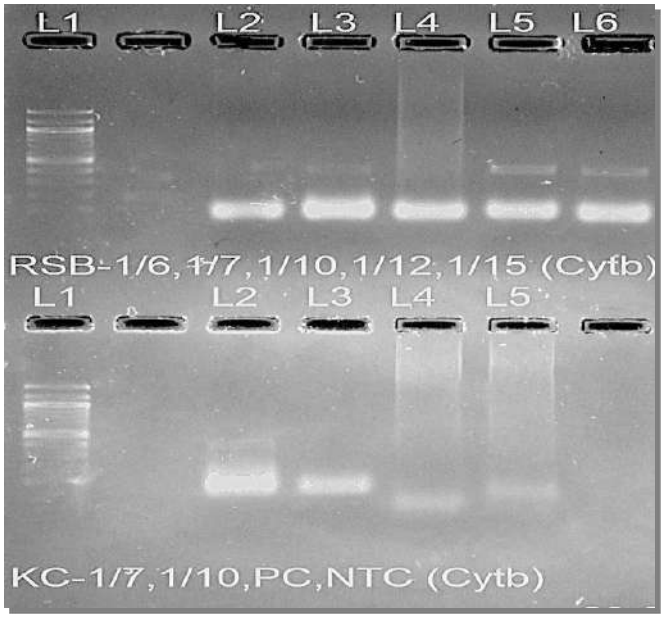

Fig 5.3. : PCR - CYTB amplification products from snake-shed skin

Upper Wells
L1 – 100bp DNA ladder
L2 – Red Sand Boa (1/6)
L3 – Red Sand Boa (1/7)
L4 – 1/10
L5 – 1/12
L6 – 1/15

Lower Wells
L1 – 100bp DNA ladder
L2 – King Cobra (1/7)
L3 – King Cobra (1/10)
L4 – PC (positive control)
L5 – NTC

From the above PCR of 12S gene region, only King Cobra of dilution (1/7) appears with a crisp thick band

The other sample will be varied with different template concentrations (40 to 100 ng/ μL) by dilution to obtain PCR amplification

5.3. PCR – 12S

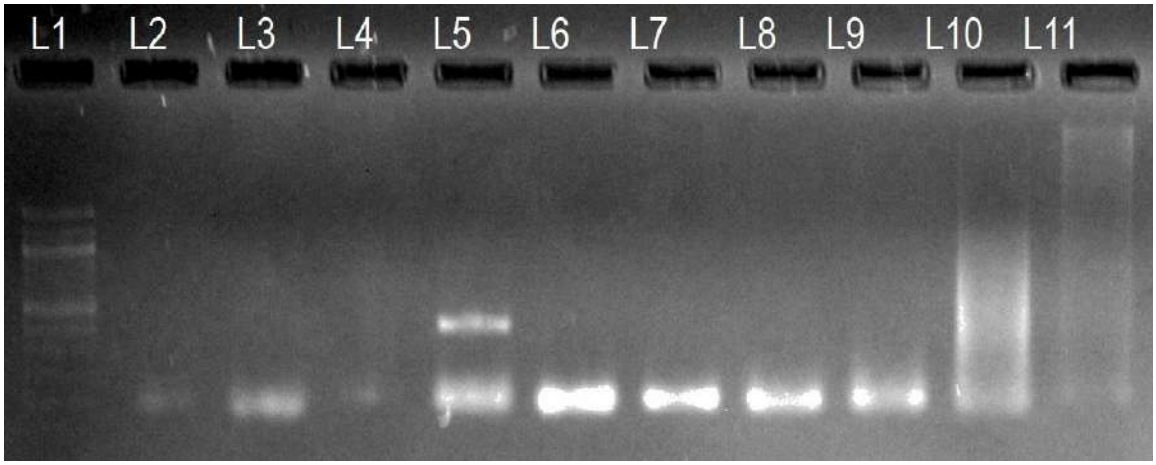

Fig 5.4: gel picture of PCR Amplification using 12s marker of Red Sand Boa and King Cobra

L1 – 100bp DNA ladder
L2 – Red Sand Boa (1/8)
L3 – Red Sand Boa (1/10)
L4 – King Cobra (1/5)
L5 – King Cobra (1/7)
L6 – King Cobra (1/8)
L7 – King Cobra (1/10)
L8 – King Cobra (no dilution)
L9 – PC (positive control)
L10 – NTC (negative control)

From the above PCR of 12S gene region, only King Cobra of dilution (1/7) appears with a crisp thick band.

The other sample will be varied with different template concentrations (40 to 100 ng/ μL) by dilution to obtain PCR amplification

5.4. PCR -12S

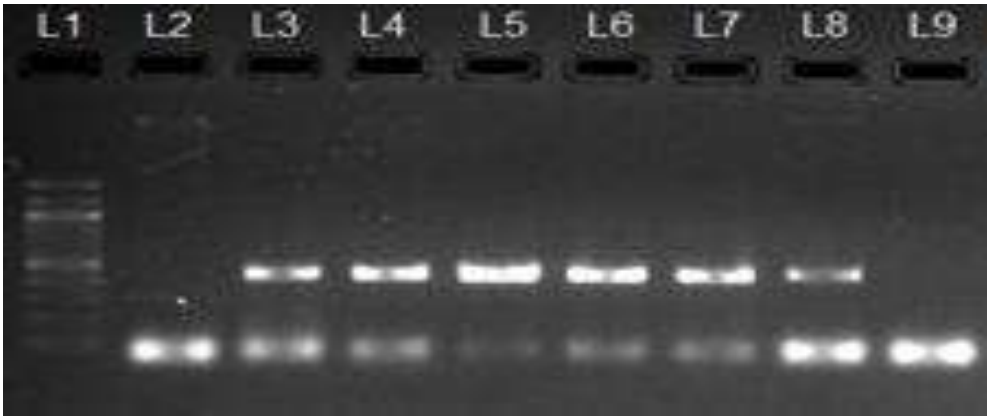

Fig 5.5: gel picture of PCR Amplification using 12s marker for Indian Rock Python and Rock python

L 1- 100bp DNA ladder
L2 – Indian Rock Python (1/6)
L3- Indian Rock Python (1/7)
L4 - Indian Rock Python (1/10)
L5 – Rock Python (1/6)
L6 – Rock Python (1/10)

Chapter - 6

CONCLUSION

I hereby conclude that, I tried DNA isolation and PCR with various forensic samples of snake shed skin of different species of King Cobra, Indian Rock Python, Reticulated Python, Red Sand Boa. And further I need to give the amplicons for sequencing to identify species using bioinformatics tools.

REFERENCE

1. Itoh, T., Magavi, R., Casady, R. L., Nishihata, T., & Rytting, J. H. (1990). A method to predict the percutaneous permeability of various compounds: shed snake skin as a model membrane. *Pharmaceutical research*, *7*(12), 1302-1306.
2. Dubey, B., Meganathan, P. R., & Haque, I. (2010). Molecular identification of three Indian snake species using simple PCR–RFLP method. *Journal of forensic sciences*, *55*(4), 1065-1067.
3. Swanson, B. J., Kelly, B. P., Maddox, C. K., & Moran, J. R. (2006). Shed skin as a source of DNA for genotyping seals. *Molecular Ecology Notes*, *6*(4), 1006-1009.
4. Stouder, S. L., Reubush, K. J., Hobson, D. L., & Smith, J. L. (2001). Trace evidence scrapings: a valuable source of DNA. *Forensic Science Communications*, *4*(4).
5. Bhat, M. K., Prasad, B. N., & Gowda, T. V. (1991). Purification and characterization of a neurotoxic phospholipase A2 from Indian cobra (Naja naja naja) venom. *Toxicon*, *29*(11), 1345-1349.
6. Dubey, B., Meganathan, P. R., & Haque, I. (2009). Multiplex PCR assay for rapid identification of three endangered snake species of India. *Conservation genetics*, *10*(6), 1861-1864.
7. Tawichasri, P., Laopichienpong, N., Chanhome, L., Phatcharakullawarawat, R., Singchat, W., Koomgun, T., ... & Suntrarachun, S. (2017). Using blood and non-invasive shed skin samples to identify sex of caenophidian snakes based on multiplex PCR assay. *Zoologischer Anzeiger*, *271*, 6-14.
8. Zhou, Z., & Jiang, Z. (2005). Identifying snake species threatened by economic exploitation and international trade in China. *Biodiversity & Conservation*, *14*(14), 3525-3536.
9. Daniel JC (1970) "A review of the present status and position of endangered species of Indian reptiles" IUCN Publication No. 18, pp 75–76
10. Fetzner JW (1999) Extracting high quality DNA from shed reptile skin: a simplified method. Biotechniques 26:1052–1054
11. Inskipp T (1981) Indian trade in reptile skins. A report by TRAFFIC International. Cambridge University Press, Cambridge
12. Kumar S, Tamura K, Nei M (2004) MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163. doi:10.1093/bib/5.2.150
13. Parson W, Pegoraro K, Niederstatter H, Foger M, Steinlechner M (2000) Species identification by means of the cytochrome *b* gene. Int J Legal Med 114:23–28. doi:10.1007/s004140000134

14. Zhou, Z. & Jiang, Z. (2005) Identifying snake species threatened by economic exploitation and international trade in China. *Biodiversity and Conservation*, 14, 3525–3536.
15. Fetzner JW (1999) Extracting high quality DNA from shed reptile skin: a simplified method. Biotechniques 26:1052–1054
16. Jerome M, Lemaire C, Verrez-Bagnis V, Etienne M (2003) Direct sequencing method for species identification of canned sardine and sardine type products. J Agric Food Chem 51:7326–7332. doi:10.1021/jf034652t