CHAPTER I
INTRODUCTION

Hair samples are the most probable evidence found in the wildlife crime scene. Thus, they play a vital role in species identification for *Wildlife Forensic Investigation*. In this study, we provide the first in-depth report on a variety of Cuticular and Medullary characteristics of Tiger Guard Hairs (30 hairs per individual from four individuals) of two different morphs. The proposed method could reduce subjectivity of expert opinions on species identification based on hair morphology. Variations in 30 hair morphological characteristics were quantified at three levels: Hair Section, Body Region, and Intra-Species. The results indicate statistically significant variations in most morphological characteristics in all levels. Intra-Species variations of four variables, namely hair length, hair index, scale separation and scale pattern, were low. Therefore, identification of tiger hairs using these multiple features in combination with other characteristics with high inter-species variations (e.g. medulla type) should bring about objective and accurate tiger hair identification. The method used should serve as a guideline and be further applied to other species to establish a wildlife hair morphology database. Statistical models could then be constructed to distinguish species and provide evidential values in terms of likelihood ratios.

1.1 Brief introduction of Mammals

Mammals are distinguished morphologically from other taxa by the possession of body hair and mammary glands in females. Most of the mammals have hairs throughout their life time and some of them like Whales and Dolphins have hairs during their Ontogeny process. Its main function is to protect the body and maintain the body and maintain the body temperature.

The class Mammalia includes about 5416 species were identified and these were grouped into 1229 genera and 153 families and 29 orders there are about 410 species known from India, were about 8.86% of world mammalian species (Wilson & Reeder 2005: Johnsingh & Manjrekar, 2013; Menon, 2014). Many species of Indian animals are facing towards extinction due to several reasons including poaching/ hunting for the illegal trade. Mammals are one of the most target group and their skins are often seized by the different Wildlife enforcement agencies (Chakraborty & De, 2010). The mammalian skins are traded by coating temporary dye for concealing the original characters and are being exported as preserved flat-skins, bags, shawls, brushes, shoes, caps, wallets, etc.Mammals which belongs to the

orders *Artiodactyla* and *Lagomorpha* are poached mainly for the local bushmeat consumption and for their skins. In general, identification of mammal is difficult through morphological characters, if only small part of skin or its derivatives is available, but when a few hairs of skin or derivatives of mammal are available, the species can be identified through hair study i.e., Tricho-Taxonomic study.

1.2 Brief Account on Tiger (*Panthera tigris*)

Tiger (Panthera tigris) are critically endangered; only approximately 3900 of the Tiger population remains in the wild (2019 ZSI). This tiger species include three different morphs viz,. Royal Bengal Tiger, Melanistic Tiger and White Tiger. The decrease in the population is mainly due to extreme poaching of tigers for their skins and body parts. A number of organizations such as the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and the wildlife trade monitoring network (TRAFFIC) have taken an action to enforce tiger trade regulation in order to protect and manage wild tiger populations at a sustainable level. National legislation's are enacted in response to these international controls. Forensic science aids law enforcement by determining if seized materials contain tiger body parts. For species identification, two reliable and court-accepted methods commonly employed by most forensic laboratories are molecular testing and microscopic examination. The advantage of the *molecular approach* is its high *accuracy and sensitivity*. However, it is *time-consuming, expensive and destructive*. Tiger hairs rather than other tissues are commonly found in a crime scene and are sometimes the only evidence found, hence the importance of the non-destructive method. The *traditional hair morphological examination is a viable alternative for tiger species identification*. In an actual wildlife crime scene, it is common to find hairs from many species including domestic animals such as cat and dog. Species identification based on *hair morphology* has been reported in many animal species.

A wide range of hair morphological features has also been reported in these different species. However, most studies cannot be reliably used in the forensic context, as the critical process of validation is lacking. An important aspect of validation is a large enough sample size and a reporting of scientific findings with statistics such as likelihood ratios. Along with this data base and the likelihood ratios the difference in the hair morphometry of two different morphs will also help in the statistical analysis of Tiger population. The conventional hair morphology-based method is subjective, relying heavily on expert opinions.

Comparisons can only be made when reference samples are readily available. Also, variations in hair morphology, which are biologically meaningful, have been overlooked in previous studies. By the present knowledge, trustworthy data on hair morphological features of tiger and quantifiable, statistics-based method for the identification of this species have never been reported. Therefore, in this study is aimed to establish a tiger hair morphological characteristic reference as well as quantify variations due to difference between individuals and also between two different morphs, body regions and hair sections. By using this information, we hope to identify the characteristics that are suitable for identification of two different morphs in the tiger species. This study should serve as a guideline for how *hair morphology in animals should be reported in order to establish a wildlife hair morphology database to assist reporting hair evidence with probability statements?*

1.3 Tricho-taxonomy

Tricho-taxonomy is one of the method for identification of mammals based on their hair characteristics which is relatively significant in the study of the food habits of the carnivores and supportive to control the illegal trade of wildlife and its derivatives.While, the Morpho-taxonomy is unable to provide a fruitful result in case of small part of skin of mammal, the Trico-taxonomy may be used for identification of species on the basis of combination characters. There are many scientists viz, Mayer (1952): Stains (1958); Brunner & Coman (1974); Moore et al. (1974); Koppikar & Sabins (1976); Teerink (1991); Wallis (1993): Chakraborty & De (2010) have well documented the different hair characters of mammals.The Zoological Survey of India (ZSI) is one of the premier institutes using this technique for identification of mammalian species. Presently; Trico-taxonomy is helping different enforcement agencies for identification of confiscated materials of wildlife and its derivatives for implementation of Indian Wildlife (Protection) Act, 1972. Moreover, the analysis of mammal hairs, which has long been useful in the study of prey-predator food habits using scat-hair analysis (Day, 196; Korschgen, 1971; Foran et al, 1997; Perrin & Campbell, 1980; Rajaram & Manon, 1956 Amersinghe & Ekanayake, 1991).

Systematic knowledge of structure of dorsal guard hair is necessary to identify the species and data generated from macroscopic and microscopic characteristics of dorsal guard hair will provide for a preparation of keys for respective species. In India, the Tricho- taxonomic studies have been carried out by many workers on different orders of class Mammalia viz, Koppikar & Sabins (1976); Rajaram & Manon (1986); De (1993); Bahuguna (2008); Chakraborty & De (2010): Sarkar (2012). However, tricho-

taxonomic studies on the species of the order *Artiodactyla* are scanty and yet there are no tricho-taxonomic studies on the Indian species of the order *Lagomorpha*. Hence, the present study was undertaken to make detailed study of dorsal guard hairs of different species of Indian mammals to the orders *Artiodactyla* and *Lagomorphav* with help of optical light microscope.

1.4 Brief Account on Hair Characteristics of Mammals

Hair is regarded as Exo-Skeletal structure of mammals. The length of a hair extends from the root embedded in the follicle, continues into a shaft, and ends at the tip. A hair is composed of three layers such as cuticle, cortex and medulla.

Types of hair

A body of mammal is composed of various types of hair, the main components of coat are guard hair (over hair) and the under hair

Over hair - The guard hair or over hair is the long and stiff hair with thickening in the distal part is called the shield and a thinner proximal part is called shaft

Under hair - The under hair or woolly hair is much thinner and less stiff and has an undulating appearance and these fine hairs cover the bodies of all mammals.

Vibrissae - These are the whiskers of many animals. They are normally tactile and sensitive.

Bristle - This is the coarse bristle that provides an animal with a protective coat.

Spines or quills - The greatly enlarged and modified hairs present in the hedgehogs and porcupines.

Dorsal guard hair

The elastic, horny, large and shiny outer coat fibers from the mid-dorsal region of the back, which give mammals their characteristic appearance. These coarse outer hairs, are differentiated, in one way, from the finer, shorter, vellum-like hairs by usually having medulla.

1.5 Macroscopic Characteristics of Hair

1.5.1 Hair regions

A full length of hair may be divided into four major regions such as basal, shield, sub-shield and tip.

Basal- The area of shaft containing the root end, usually comprising about the basal one-fifth of the shaft.

Shield- A widened, flattened area of shaft. If the widest area is not flattened the hair is considered as unshielded. The banding pattern of the hair is usually predominant on the shield region.

Sub-shield -The area of immediately below the shield region between the basal and shield.

Tip- The end opposite of the root or basal area, is pointed on the full hair. Sometimes it blunt or cut off.

1.5.2 Colour of hair

The effect of light on pigment and structure of hair which is permitted gives the hair its characteristic colour. Pigments may be present in the cortex or medulla or both regions of a hair, and some occasionally on cuticular scales may. Accordingly, the hairs colours may be divided into unicoloured and bicoloured.

Unicolour or Unbanded- The hair is uniformly pigmented without any bands. Usually many hairs are unbanded

Bicoloured or multi-coloured hair - Two or more distinct colours; one blending into the next Banded hairs is distinguished from bicoloured hairs by the sharpness of the colour charge in the banded type.

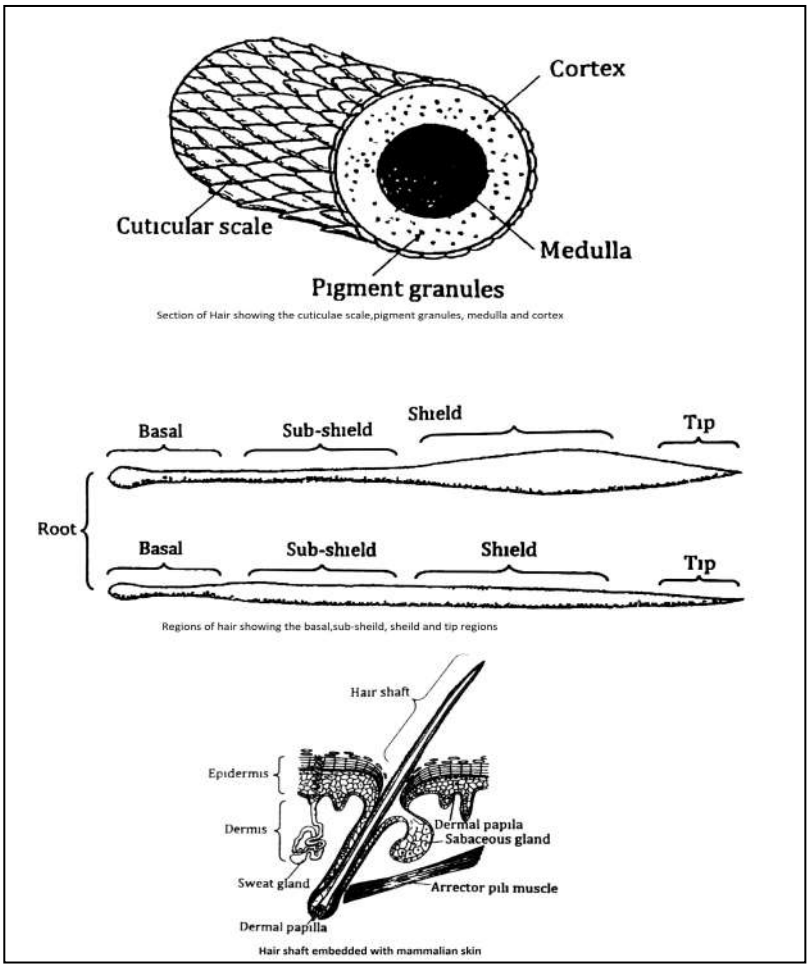


Fig.1.1 showing section of hair, regions of hair and hair shaft

1.6 Microscopic characteristics of hair

1.6.1 Cuticle characteristics

The cuticle is non-nucleated, translucent- outer layer, formed by overlapping scales of Keratin that always point toward the tip of the hair. The size and shape of the scales vary relative to the position on the hair. At the tip, the first scales to appear are small. On the widest part of shield, the scales are much larger and lie transversely.

1.6.2 Scale position

The arrangement of scales along the length of the hair is known as scale position n general, the scales are formed in three different positions in relation to the longitudinal axis of the hair such as transversal, longitudinal and intermediate.The transversal scales lie at right angles and their width is greater than their length scales lie parallel and their length is greater than the width, the transversal scales the longitudinal the intermediates.

1.6.3 Scale patterns

The overlapping scale are formed in a definite pattern along with the hair shaft and has the following patterns

Broad petal-The overlapping scales arranged in wider

Diamond petal-Overlapping scales giving the appearance of a diamond pattern

Elongate petal-The overlapping scales are rather short

Mosaic-Non-overlapping scales that are non-uniform in size and shape.

Regular wave-Non-overlapping, wavy in appearance and usually continuous, with wavy in appearance and usually continuous with the length of the waves on the same and different scales.

Irregular wave-Non-overlapping scales wavy in appearance and usually continuous, but length of the waves on the same and different scales unequal.

Streaked-Non-overlapping, wavy scales similar Irregular wave, but the waves are interrupted at regular intervals longitudinally-running columns of scales having steeply inched margins.

1.7 **Structure of scales margins**

The external edge of scale is called margins and have two different distinct patterns, they are:

Smooth-The margins of the scales have no interruptions, irregularities or indentations.

Rippled-The margins of the scales have a saw-toothed appearing edge with deeper indentations. Scales may have more than one type of margin.

1.8 Distance between scale margins

The distance consecutive scales may vary significantly, based on comparisons of scale casts viewed at the same magnification. They are categorized as *close, distant* and *near.*

Close-The margins of the scales are quite near or close to each other **.**

Distant-The margins of the two consecutive scales lie away from each other**.**

Near-The margins of the two consecutive scales are either too close or too away from each other.

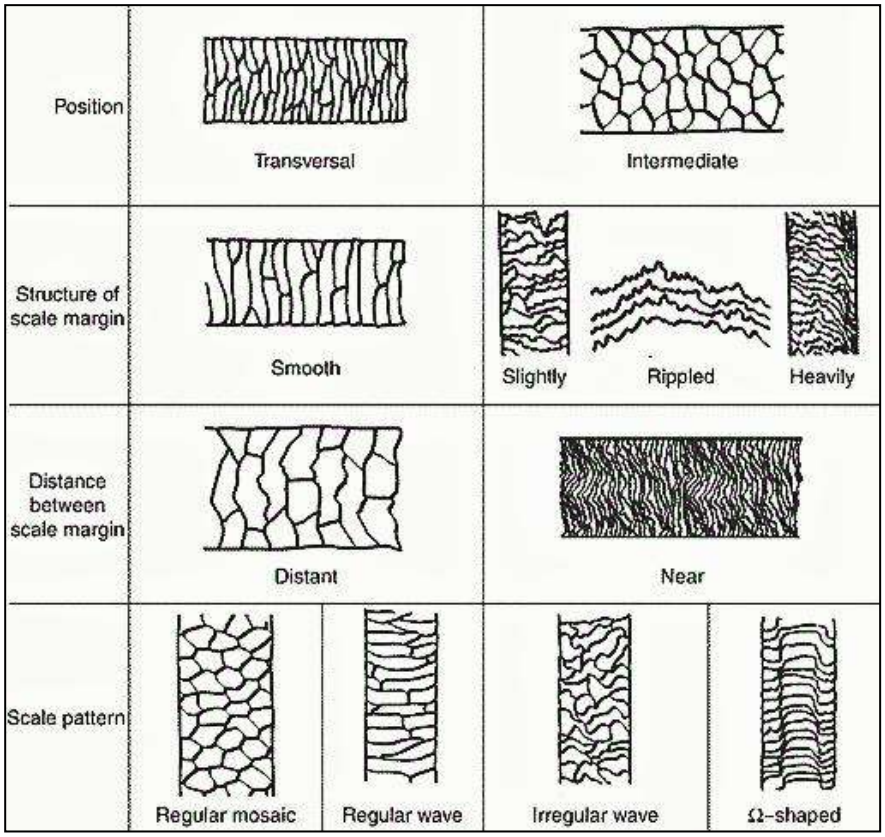


Fig. 1.2 Showing the Position of scales, Structure of Scale Margin, distance between Scale margin and Scale patterns of Mammalian hair.

1.9 Cortex

The cortex is the part of the hair that contains most of the pigment granules (Melanin) that give the hair its color lies between the cuticle and the medulla. It is mainly composed of elongated and fusiform (spindle-shaped) cortical fusy, pigment granules and shrunken cells.

1.10 Medullary characteristics

The medulla is a collection of cells which appear as a canal running through the center of the hair. The medulla is composed by closely packed cortex and shrunken dead cell with inter-cellular spaces filled with air, giving it a characteristic appearance

1.10.1 Composition of medulla

The medulla is composed of single or more layers, they are

Unicellular -The medulla is composed of a continuous single column of discrete cells formed by transversal position. The pattern may be regular or irregular.

Multicellular- The medulla is composed of continuous two or more columns of cells. The pattern may be regular or irregular.

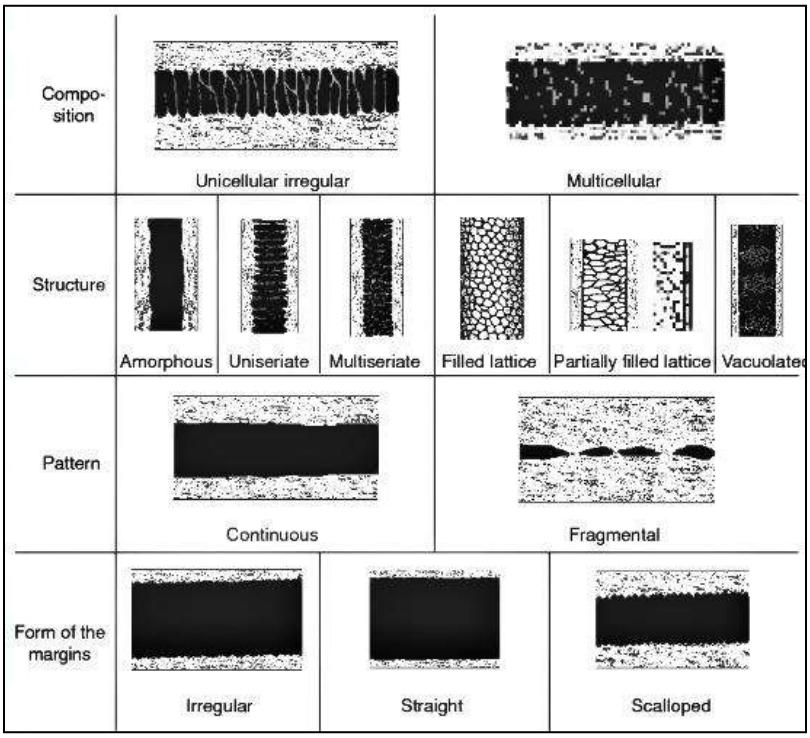


Fig.1.3 Showing composition, Structure pattern and form of Margins of medulla of mammalian hair

1.10.2 Structures of medulla

The structure of medulla is the arrangement of the cells forming the medulla by the following forms

Ladder- A continuous single column of cells interrupted with cortical matter and looks like a septa This pattern is usually restricted to the shaft.

Filed- The medullar cells fill the entire width of the hair and the cortex do not rows center each.

Intermediate - A ladder pattern is sometimes so indistinct that a sort of 'wreath' pattern called intermediate arises. This pattern also occurs usually on the shaft.

Isolated- The dark cells are occasionally continuous, but separated to a variable degree and easily recognized. The shape is circular to oblong.

Crescent- The dark cells form a pattern imposed by their shape. The cells are rather long and slightly curved, tapering at the ends. Many of them touch and overlap each other. The spaces between the curved cells have the shape of crescent.

Interrupted-This pattern takes its name from the absence of the medulla at one or several little pieces of the medulla are present is called fragmental.

1.10.3 Margins of the medulla

Straight-The margins of the medulla form a smooth and straight line.

Fringed-Small protrusions extend into the cortex.

Scalloped-series of convex, rounded projections from the margin of medulla

1.10.4 Cross-section

The shape (appearance) or *cross-section* or *transverse section* of hair shows considerable variations such as Oval, Oblong, Biconvex, Concave-Convex, Reniform, Dumb-bell, carved and H-shaped Biconcave, Triconcave.

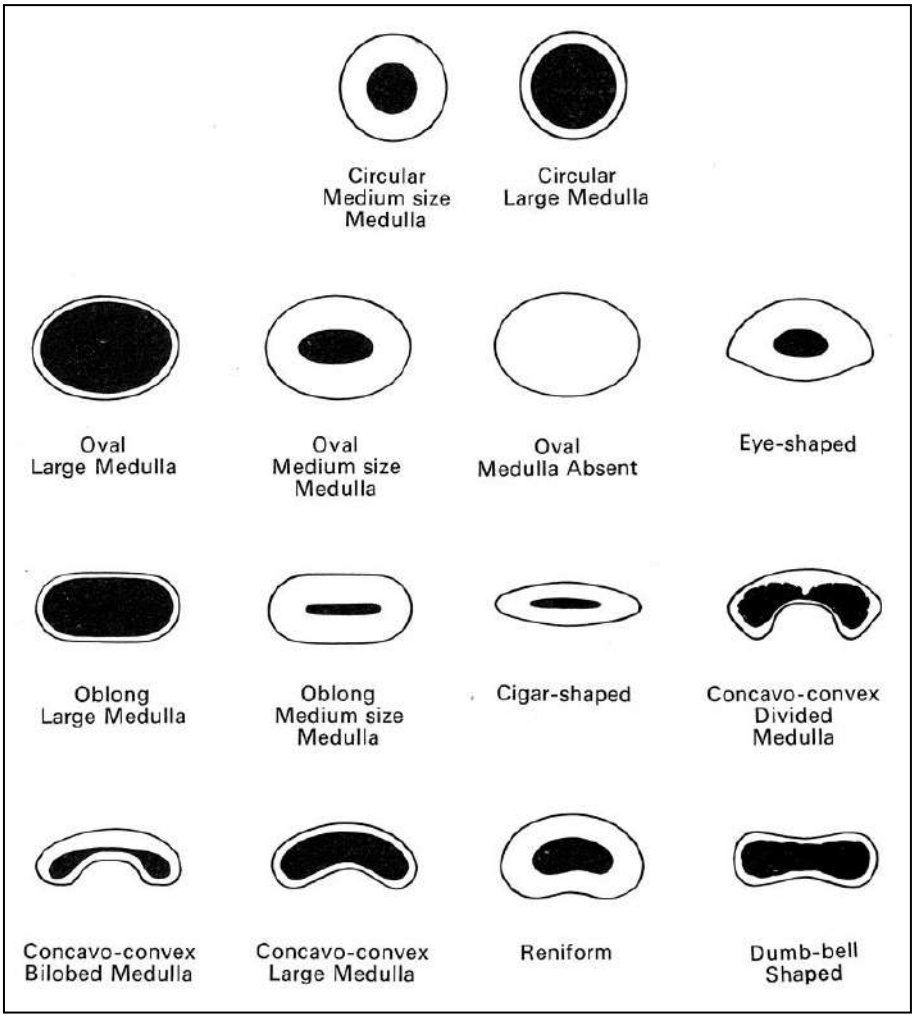


Fig1.4 Image showing different types of appearance of hair during cross section

CHAPTER II
LITERATURE REVIEW

The tricho-taxonomy is one of the practical applications used mainly in the animal forensic and ecological sciences in recent years. The aim of this study is to help in the identification of respective species in wildlife crime investigation in relation to the identification of confiscated materials and prey-predator food habit analysis (Mathiak 1938; Foran et al, 1997; Chakraborty & De, 2010; Sahajibal et al, 2010). The microscopic characters and their combinations show specific or generic trends, and often reveal genetic, phylogenetic and other morphometric information (Kennedy, 1982; Chakraborty et al, 1998; Mcdaniel, 2000; Mowat & Strobeck, 2000). Although genetic analysis have become more common (Foran et al, 1997; Mowat & Strobeck, 2000), hair is usually identified to species using simple method with macroscopic and microscopic characteristics of following published guides and using other reference collections: Mayer (1952); Stains (1958); Brunner & Coman (1974); Moore et al. (1974); Teerink (1991); Wallis (1993).

During the 19th and early 20th centuries hairs were mainly studied in Anthropology. According to Tupinier (1973), studies on mammalian hair were first started in the 19h century by Brewster (1837) and Quekett (1844). Pocock (1914) studied the facial vibrissae of some mammals through the external cutaneous structure however, as a taxonomic character, the hairs of mammals were first studied by Hausman (1920, 1924, 1930, 1932, 1944), he reviewed the morphological hair research of 166 fur bearing mammals, and the descriptions, drawings and the terminology he introduced is widely used. Several investigators have contributed in their own way in tricho-taxonomy. Williams (1934) introduced a simple method for sectioning mammalian hairs for identification. Lochte (1938) published an atlas for hair structure of leopard, puma and gorilla. In the same year, Williams (1938) gave the identification key to mole and shrew hairs with general descriptions. Dearborn (1939), Stoves (1944) and Nason (1948) were studied various kind of hairs of mammals and their descriptions were provided.

Later, Appleyard & Greville (1950) reviewed the cuticle of mammalian hair and stated that the thickness of hair cuticles depends upon the arrangement of scales and the amount of area Tasmanian marsupials and monotremes. Mayer (1952) studied the dorsal guard hairs and provided the key for identification of Californian mammals. Based on various characteristics of hair it overlaps of its neighbour. Lyne & Mc Mohan (1951) examined the hair structure of structures, several keys and hair

identification manuals done by Stains (1958) and Adorjan & Kolenosky, (1969) for North American land mammals.

Growth and morphology of various kind of hair and their descriptions, and terminology of cuticle, medulla and cross-section of hairs were revised by Wildman (1954, 1961). Further, to support this work Appleyard (1960) published a guide to the identification of animal fibres. Dobb et al. (1961) studied the morphology of Cuticle layer in wool fibres and other animal hairs. During 1960s the hair structures on different groups of mammals studied by Day (Mustelidae, 1966), Dreyer (Bovidae, 1966) and Cave (Rhinocerotid ae, 1969).

Identification of hairs through electron microscope was first carried out by Tupinier (1973) on the cuticle of 29 West-European bat species under 10 genera using scanning electron microscope. Later, transmission electron microscopy (Muto et al., 1981; Slepecky et al, 1981; Weedon & Strutton, 1981; Maxwell et al, 1982) and scanning electron microscopy (Anderson & Lipson, 1970; Spiers, 1973; Brunner& Coman, 1974; Short, 1978; Riggott et al, 1981; Hess et al, 1985; Sun et al, 2003), and combinations of both (Hino et al, 1982; Raphael et al, 1982) used for the ultra- structure of hairs.

The comprehensive atlas on the identification key of hair along with drawings and images are well documented by the following authors: **'Identification of mammalian hair'** by Brunner & Coman (1974), describes the important information on medulla, cuticular characteristics and cross-section of hairs with detailed descriptions and terminology for hair identification. **'Mammals of Wyoming'** by Moore et al. (1974) gives detailed description and drawings of medulla and cuticular structure of various groups of mammals**. 'Atlas and identification key for hair of west European mammals'** by Teerink (1991) provides the detailed keys with many drawings and comprehensive pictures. The present tricho-taxonomic studies on mammals rely heavily on these publications.

A pioneer work has been carried out on hairs of Indian mammale researchers. Koppikar & Sabins (1976, 1980, and 1981) studied the different group mammals using Camera Lucida drawings. An extensive tricho-taxonomic has been carried out on 3 orders of class Mammalia such as Primates, Carnivors and Rodentia by the scientists of the Zoological Survey of India [Primates by De (19921 Sarkar (2012) and Sarkar et al. (2010, 2011); Carnivora by Venkataraman (1994), Chakraborty & De (1995, 2001, 2002, 2005), Chakraborty et al. (199 Bahuguna (2010a), De & Chakraborty (1995, 2002, 2006) and De et al. (199811 Later, Chakraborty & De (2010) published

an atlas of dorsal guard hairs of 54 species of Indian Carnivores with the detailed structure and description of hair such as physical characters which includes cuticular, medullary and cross-sectional. Similarly, identification of dorsal guard hairs on the order Rodentia has been well documented by Bahuguna (family Sciuridae, 2007, 2008, 2010b) and Sarkar (2012). Some works on hairs of few Artiodactyls of India were carried out by Gopal et al. (hard ground barasingha *Rucervus duvaucelii branderi*, 1993); Bahuguna & Mukherjee (Tibetan antelope *Pantholops hodgsonii*, 2000); Chatterjee et al. (yak *Bos mutus*, 2005); Bahuguna et al. (selected Carnivores & Artiodactyls, 2010); Sahajpal et al (Tibetan antelope *Pantholops hodgsonii* & Capra sp, 2010); Joshi et al. (4 species of deer, 2012);: Dharaiya & Soni (cross-section of ungulates, 2012); De & Chakraborty (9 species of Bovidae, 2013).

However, the above studies have only touched upon the preliminary investigations and no quantitative data is available in this regard and based on the literature survey none of tricho-taxonomic study is carried out on Identification of hair characters of two different morphs in India. Hence, the present study was initiated to provide detailed macroscopic and microscopic characters of hairs for the identification of the two different Morphs of Tiger species was studied and the comparative characters are noted.

CHAPTER III
AIM & OBJECTIVES

AIM:

To prepare the standards of the Tiger hair to identify the two different morphs of tigers by using Tricho-taxonomic studies.

OBJECTIVES:

1. Formulation of identification keys using microscopic characteristics viz., cuticular and medullary characteristics, of dorsal guard hairs of tiger species *Panthera tigers.*
2. Observation of presence of change in hair characteristics of different morphs (Melanistic Tiger, Royal Bengal Tiger) of tigers in AAZP (Arignar Anna Zoological Park), Vandalur, Chennai, by using *cuticular* and *medullary observations.*

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials Required:

1. Hand gloves
2. Forceps
3. Zip-lock covers
4. Distilled water and 70% ethanol (Cleaning process)
5. Gelatin (Cuticle observation)
6. Mthylene blue (dye)
7. Xylene (Medullary observation)
8. Acetone
9. Paraffin wax (Cross section)
10. Drinking straw

4.2 METHODOLOGY

4.2.1 Sample Collection and Hair Specimen Preparation

Hair samples were obtained from AAZP –Arignar Anna Zoological Park, Vandalur, Chennai., were from four mature, healthy individuals of *Panthera tigris*. For each individual, guard hairs were taken from tiger of two different morphs i.e. Melanistic Tiger and Royal Bengal Tiger (n = 30 for each individual). These hairs were de-greased using an ethanol (70%) and then dried prior to further analysis. From each hair, three types of specimen, i.e., Gelatin slide Casting, Xylene treatment, Paraffin wax molding technique was used for examination under a light microscope.

4.2 Methods for Examination of Tiger Hair

4.2.1 Cuticular structure Examination

For cuticular examination, a cast of hair is made in a solution of Gelatin (0.2 gms of gelatin crystals in 20 ml of water followed by heating up to 30 min under 100ºC at 250 rpm by using magnetic stirrer) in water, with a small amount of Methylene Blue added to the solution to obtain a better contrast. Then a thin layer of gelatin solution is applied on a microscope slide with the help of glass rod, tilt the glass plate such that a uniform thin layer of gelatin is formed on it. Hairs are then delicately placed on the glass slide before it dried, so that the impression of hair is formed on the Gelatin surface. Once the Gelatin is dried, the hairs are removed from the slide and kept for the microscopic observation, hence the

cuticular impression left on the glass slide was observed under the microscope, software and the clear photographs should be taken for the further reference.

4.2.2 Medullary Structure Examination

For the view of medullary structure, it is necessary to remove the air from the medulla of the hair. For obtaing the best quality of slides, hair should be washed firstly with distilled water and then with 1-2% of detergent water followed by treating with 70% ethanol. This process lasts for 6-8 hrs. Then the hair was treated with acetone over night and then hair was cut into pieces and kept it in Xylene for 12 hrs. As Xylene percolates into the medulla, the air gets removed, then the slide and kept for the microscopic observation so that the structure becomes visible under light microscope. Then the thickness of the medulla and cortex were measured by using software and the clear photographs should be taken for the further reference.

4.2.3 Cross section of hair Examination

For the cross section of the hair, few hairs are taken and placed in a drinking straw, the hair should be kept straight in such a way that all the hairs comes at middle of the straw, in order to get a clear mould. Paraffin Wax (wax which is prepared by melting the paraffin at the melting for few minutes such that it becomes semi solid material) is sucked or can also be poured in to the straw, and then the straw is bent to one side so as to prevent the wax from flowing out of it. The wax allowed to solidify, (and can also kept in freezer for getting a good mould) then the straw is removed with the help of a longitudinal cut along its side .The paraffin wax block was horizontally cut into thin sections manually with the help of blade. The cut sections are de-waxed using Xylene and placed on a slide for subsequent observation under a microscope and the clear photographs should be taken for the further reference.

CHAPTER V

OBSERVATIONS AND DISCUSSIONS

5.1 OBSERVATIONS

5.1.1 Hair coloration

Pigmentation or coloration's of hair of both the morphs (Royal Bengal Tiger and Melanistic Tiger) were physically examined and observed that, tiger hairs are present in four colors: White, Black, Yellow and Brown. The proximal and distal hair parts are dominantly Pale Orange and black, whereas white and yellow are almost equally found in the middle part or medial part.

Table 5.1: Showing Physical Characteristic observations of Melanistic Tiger.

	Physical Characters			
Sl.no	**Color**	**Shape**	**Texture**	**Length (cm)**
1.	Pale Orange to Brown	Short and Straight	Rough	4.0
2.	Orange to Black	Long and Straight	Rough	6.0
3.	Dark orange to Dark Brown	Short and Straight	Rough	5.7
4.	Orange to Black	Short and Straight	Rough	4.8
5.	Dark orange to Dark Brown	Short and Straight	Rough	5.7
6.	Orange to Black	Short and Straight	Rough	5.6
7.	Pale Orange to Brown	Short and Straight	Rough	3.3
8.	Orange to Black	Short and Straight	Rough	3.1
9.	Orange to Black	Short and Straight	Rough	4.3
10.	Orange to Black	Short and Curved	Rough	3.5
11.	Pale Orange to Brown	Short and Straight	Rough	3.0
12.	Pale Orange to Brown	Short and Straight	Rough	5.3
13.	Dark orange to Dark Brown	Short and Straight	Rough	5.5
14.	Dark orange to Dark Brown	Short and Straight	Rough	3.0
15.	Pale Orange to Brown	Short and Straight	Rough	4.4

16.	Orange to Black	Short and Straight	Rough	4.1
17.	Dark orange to Dark Brown	Short and Straight	Rough	3.1
18.	Pale Orange to Brown	Short and Curved	Rough	2.8
19.	Orange to Black	Short and Straight	Rough	3.5
20.	Orange to Black	Short and Straight	Rough	2.3
21.	Pale Orange to Brown	Long and Straight	Rough	6.1
22.	Dark orange to Dark Brown	Short and Straight	Rough	4.8
23.	Pale Orange to Brown	Short and Straight	Rough	5.7
24.	Orange to Black	Short and Straight	Rough	5.9
25.	Pale Orange to Brown	Short and Straight	Rough	4.3
26.	Orange to Brown	Short and Straight	Rough	5.5
27.	Orange to Black	Short and Straight	Rough	4.5
28.	Dark orange to Dark Brown	Short and Straight	Rough	5.5
29.	Pale Orange to Black	Short and Straight	Rough	5.1
30.	Pale Orange to Brown	Long and Curved	Rough	6.1

Table 5.2: Shows Physical Characteristic observations of Royal Bengal Tiger

	Physical characters			
Sl.no	**Color**	**Shape**	**Texture**	**Length (cm)**
1.	Pale Orange to Black	Short and Straight	Rough	3.4
2.	Pale Orange to Brown	Short and Straight	Rough	4.1
3.	Pale Orange to Black	Short and Straight	Rough	3.7
4.	Orange to Black	Short and Straight	Rough	4.8
5.	Pale Orange to Black	Long and Straight	Rough	7.8
6.	Orange to Black	Short and Straight	Rough	3.7
7.	Orange to Black	Short and Straight	Rough	5.6
8.	Pale Orange to Black	Short and Straight	Rough	3.0
9.	Orange to Brown	Short and Straight	Rough	4.5
10.	Yellow to Black	Short and Straight	Rough	4.4
11.	Orange to Black	Short and Straight	Rough	4.9
12.	Yellow to Black	Short and Straight	Rough	4.8
13.	Yellow to Black	Short and Straight	Rough	3.5
14.	Pale Orange to Black	Short and Straight	Rough	4.0
15.	Orange to Black	Short and Straight	Rough	3.0
16.	White to Black	Short and Straight	Rough	4.9
17.	Orange to Black	Short and Straight	Rough	4.1
18.	Orange to Black	Short and Straight	Rough	4.9
19.	White to Black	Short and Straight	Rough	4.7
20.	Pale Orange to Brown	Short and Straight	Rough	3.6
21.	Orange to Black	Short and Straight	Rough	5.1
22.	Pale Orange to Black	Short and Straight	Rough	5.0
23.	Orange to Black	Short and Straight	Rough	3.2
24.	Pale Orange to Black	Short and Straight	Rough	4.4
25.	Orange to Black	Short and Straight	Rough	4.5
26.	Pale Orange to Black	Short and Straight	Rough	3.0
27.	Orange to Dark Brown	Short and Straight	Rough	4.1
28.	Pale Orange to Black	Short and Straight	Rough	5.0
29.	Orange to Black	Short and Straight	Rough	3.7
30.	Pale Orange to Black	Short and Straight	Rough	4.5

5.2 Cuticle characteristics

5.2.1 Scale Patterns

Mainly three types of Cuticular patterns i.e., Regular wave, Irregular wave and Single Chevron type of patterns are found in the hair of both the morphs. (Table1.3) & Table.4).

Table 5.3: Shows different Scale Patterns of Melanistic Tiger.

	Scale Pattern		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Regular wave	Irregular wave	Irregular wave
2.	Regular wave	Irregular wave	Irregular wave
3.	Regular wave	Irregular wave	Irregular wave
4.	Regular wave	Irregular wave	Irregular wave
5.	Regular wave	Irregular wave	Irregular wave
6.	Regular wave	Irregular wave	Irregular wave
7.	Regular wave	Irregular wave	Irregular wave
8.	Regular wave	Irregular wave	Irregular wave
9.	Regular wave	Irregular wave	Irregular wave
10.	Regular wave	Irregular wave	Irregular wave
11.	Regular wave	Irregular wave	Irregular wave
12.	Regular wave	Irregular wave	Irregular wave
13.	Regular wave	Irregular wave	Irregular wave
14.	Regular wave	Irregular wave	Irregular wave
15.	Regular wave	Irregular wave	Irregular wave
16.	Regular wave	Irregular wave	Irregular wave
17.	Regular wave	Irregular wave	Irregular wave
18.	Regular wave	Irregular wave	Irregular wave
19.	Regular wave	Irregular wave	Irregular wave
20.	Regular wave	Irregular wave	Irregular wave
21.	Regular wave	Irregular wave	Irregular wave
22.	Regular wave	Irregular wave	Irregular wave
23.	Regular wave	Irregular wave	Irregular wave
24.	Regular wave	Irregular wave	Irregular wave
25.	Regular wave	Irregular wave	Irregular wave
26.	Regular wave	Irregular wave	Irregular wave
27.	Regular wave	Irregular wave	Irregular wave
28.	Regular wave	Irregular wave	Irregular wave
29.	Regular wave	Irregular wave	Irregular wave
30.	Regular wave	Irregular wave	Irregular wave

Table 5.4: Shows Different Scale Patterns of Royal Bengal Tiger.

	Scale Pattern		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Regular wave	Irregular wave	Irregular wave
2.	Regular wave	Irregular wave	Irregular wave
3.	Regular wave	Irregular wave	Irregular wave
4.	Regular wave	Irregular wave	Irregular wave
5.	Regular wave	Irregular wave	Irregular wave
6.	Regular wave	Irregular wave	Irregular wave
7.	Regular wave	Irregular wave	Irregular wave
8.	Regular wave	Irregular wave	Irregular wave
9.	Regular wave	Irregular wave	Irregular wave
10.	Regular wave	Irregular wave	Irregular wave
11.	Regular wave	Irregular wave	Irregular wave
12.	Regular wave	Irregular wave	Irregular wave
13.	Regular wave	Irregular wave	Irregular wave
14.	Regular wave	Irregular wave	Irregular wave
15.	Regular wave	Irregular wave	Irregular wave
16.	Regular wave	Irregular wave	Irregular wave
17.	Regular wave	Irregular wave	Irregular wave
18.	Regular wave	Irregular wave	Irregular wave
19.	Regular wave	Irregular wave	Irregular wave
20.	Regular wave	Irregular wave	Irregular wave
21.	Regular wave	Irregular wave	Irregular wave
22.	Regular wave	Irregular wave	Irregular wave
23.	Regular wave	Irregular wave	Irregular wave
24.	Regular wave	Irregular wave	Irregular wave
25.	Regular wave	Irregular wave	Irregular wave
26.	Regular wave	Irregular wave	Irregular wave
27.	Regular wave	Irregular wave	Irregular wave
28.	Regular wave	Irregular wave	Irregular wave
29.	Regular wave	Irregular wave	Irregular wave
30.	Regular wave	Irregular wave	Irregular wave

Table 5.5 Images of different Patterns at various regions of Melanistic Tiger Hair

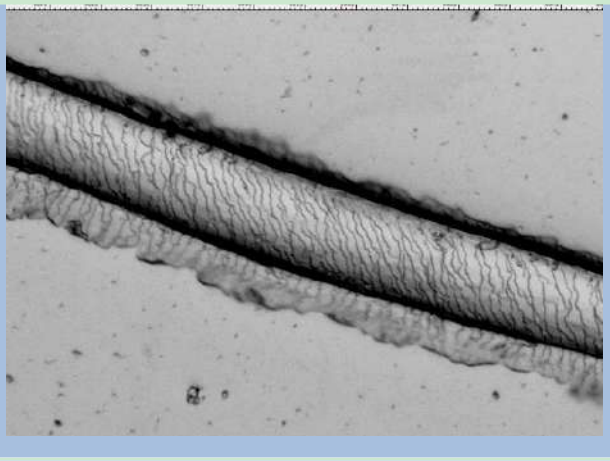
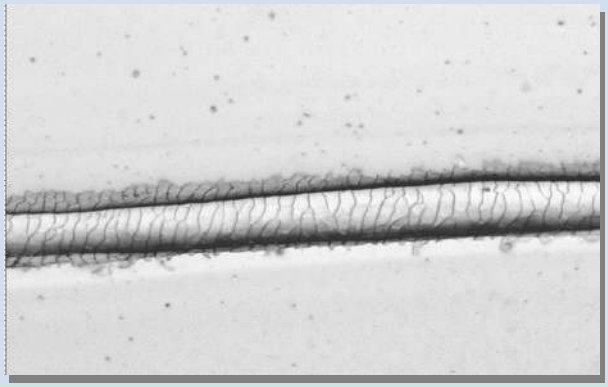
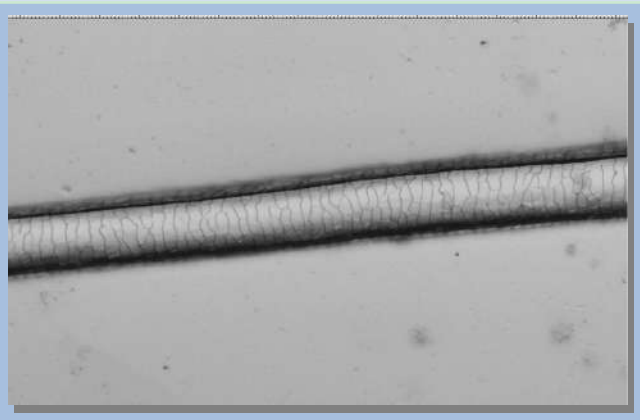
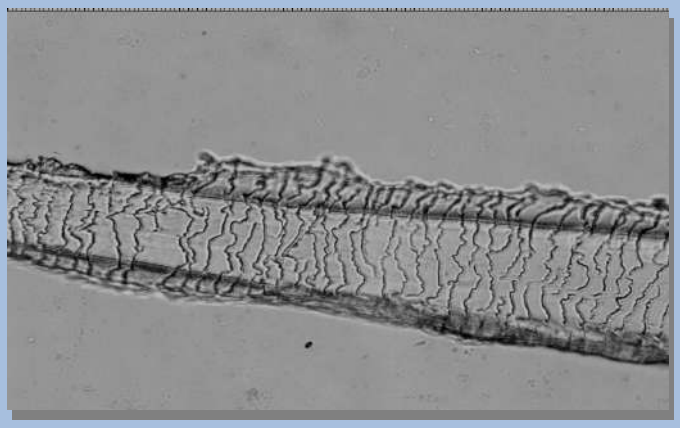
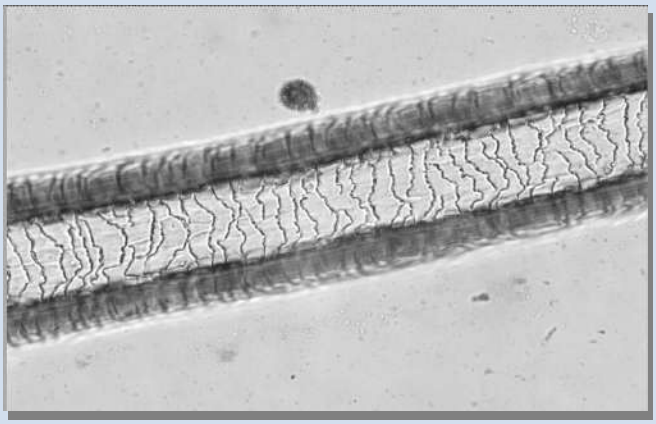
Sl.no	Image	Region of hair
Image 1		Apical region
Image 2		Medial region
Image 3		Basal region

Table 5.6 Images of different Patterns at various regions of Hair of Royal Bengal Tiger.

Sl.no	Image	Region of hair
Image 1		Apical region
Image 2		Medial region
Image 3		Basal region

5.2.2 Scales Margins

Mainly three different distinct scale margins in both the morphs of the Tiger are observed during cuticular examination, they are tabulated below.

Table 5.7: Shows the Scale Margins of Melanistic Tiger.

	Scale Margin		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Smooth &Slant	Rippled	Crenate
2.	Smooth &Slant	Rippled	Crenate
3.	Smooth&Slant	Rippled	Crenate
4.	Smooth &Slant	Rippled	Crenate
5.	Smooth &Slant	Rippled	Crenate
6.	Smooth &Slant	Rippled	Crenate
7.	Smooth &Slant	Rippled	Crenate
8.	Smooth &Slant	Rippled	Crenate
9.	Smooth &Slant	Rippled	Crenate
10.	Smooth &Slant	Rippled	Crenate
11.	Smooth &Slant	Rippled	Crenate
12.	Smooth &Slant	Rippled	Crenate
13.	Smooth &Slant	Rippled	Crenate
14.	Smooth &Slant	Rippled	Crenate
15.	Smooth &Slant	Rippled	Crenate
16.	Smooth &Slant	Rippled	Crenate
17.	Smooth &Slant	Rippled	Crenate
18.	Smooth &Slant	Rippled	Crenate
19.	Smooth &Slant	Rippled	Crenate
20.	Smooth &Slant	Rippled	Crenate
21.	Smooth &Slant	Rippled	Crenate
22.	Smooth &Slant	Rippled	Crenate
23.	Smooth &Slant	Rippled	Crenate
24.	Smooth &Slant	Rippled	Crenate
25.	Smooth &Slant	Rippled	Crenate
26.	Smooth &Slant	Rippled	Crenate
27.	Smooth &Slant	Rippled	Crenate
28.	Smooth &Slant	Rippled	Crenate
29.	Smooth &Slant	Rippled	Crenate
30.	Smooth &Slant	Rippled	Crenate

Table 5.8: Shows the Scale Margins of Royal Bengal Tiger

	Scale Margin		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Smooth	Rippled	Crenate
2.	Smooth	Rippled	Crenate
3.	Smooth	Rippled	Crenate
4.	Smooth	Rippled	Crenate
5.	Smooth	Rippled	Crenate
6.	Smooth	Rippled	Crenate
7.	Smooth	Rippled	Crenate
8.	Smooth	Rippled	Crenate
9.	Smooth	Rippled	Crenate
10.	Smooth	Rippled	Crenate
11.	Smooth	Rippled	Crenate
12.	Smooth	Rippled	Crenate
13.	Smooth	Rippled	Crenate
14.	Smooth	Rippled	Crenate
15.	Smooth	Rippled	Crenate
16.	Smooth	Rippled	Crenate
17.	Smooth	Rippled	Crenate
18.	Smooth	Rippled	Crenate
19.	Smooth	Rippled	Crenate
20.	Smooth	Rippled	Crenate
21.	Smooth	Rippled	Crenate
22.	Smooth	Rippled	Crenate
23.	Smooth	Rippled	Crenate
24.	Smooth	Rippled	Crenate
25.	Smooth	Rippled	Crenate
26.	Smooth	Rippled	Crenate
27.	Smooth	Rippled	Crenate
28.	Smooth	Rippled	Crenate
29.	Smooth	Rippled	Crenate
30.	Smooth	Rippled	Crenate

5.2.3 Distance between scale margins

Mainly three types of scale margins i.e., *close, distant* and *near* are observed during examination of hair of the both morphs i.e., Royal Bengal Tiger and Melanistic Tiger.

Table 5.9: Shows the scale margins of Melanistic Tiger hair.

	Scale Distance		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Near	Near	Close
2.	Near	Near	Close
3.	Near	Near	Close
4.	Near	Near	Close
5.	Near	Near	Close
6.	Near	Near	Close
7.	Near	Near	Close
8.	Near	Near	Close
9.	Near	Near	Close
10.	Near	Near	Close
11.	Near	Near	Close
12.	Near	Near	Close
13.	Near	Near	Close
14.	Near	Near	Close
15.	Near	Near	Close
16.	Near	Near	Close
17.	Near	Near	Close
18.	Near	Near	Close
19.	Near	Near	Close
20.	Near	Near	Close
21.	Near	Near	Close
22.	Near	Near	Close
23.	Near	Near	Close
24.	Near	Near	Close
25.	Near	Near	Close
26.	Near	Near	Close
27.	Near	Near	Close
28.	Near	Near	Close
29.	Near	Near	Close
30.	Near	Near	Close

Table 5.10: Shows the scale margins of Royal Bengal Tiger hair.

	Scale Distance		
Sl.no	**Proximal**	**Medial**	**Distal**
1.	Near	Near	Close
2.	Near	Near	Close
3.	Near	Near	Close
4.	Near	Near	Close
5.	Near	Near	Close
6.	Near	Near	Close
7.	Near	Near	Close
8.	Near	Near	Close
9.	Near	Near	Close
10.	Near	Near	Close
11.	Near	Near	Close
12.	Near	Near	Close
13.	Near	Near	Close
14.	Near	Near	Close
15.	Near	Near	Close
16.	Near	Near	Close
17.	Near	Near	Close
18.	Near	Near	Close
19.	Near	Near	Close
20.	Near	Near	Close
21.	Near	Near	Close
22.	Near	Near	Close
23.	Near	Near	Close
24.	Near	Near	Close
25.	Near	Near	Close
26.	Near	Near	Close
27.	Near	Near	Close
28.	Near	Near	Close
29.	Near	Near	Close
30.	Near	Near	Close

5.3 Cortex

The cortex is the part of the hair that contains most of the pigment granules that give the hair and its color lies between the cuticule and the medulla. It is mainly composed of elongated and fusiform (spindle-shaped), pigment granules and shrunken cells inside of it.

5.4 Medullary characteristics

5.4.1 Structures of medulla

The medulla of tiger hairs consists of three types, viz. simple, uniserial ladder and a mixture of both. The medulla type of each hair will not change along the length of the hair but the width becomes narrow towards the distal end. In the Royal Bengal tiger the simple narrow type of medulla was seen and when it comes to Melanistic tiger the Simple and Wider than the expected was seen.This characteristic is quite unique for tiger and can be used to distinguish it from other species for example mongoose (wide medulla with cortical intrusions and vacuoles).

(i) (ii)

Figs5.4.1 i and ii showing the length of medulla of hair of Both morphs., viz Melanistic Tiger and Royal Bengal tiger.

(i) Medulla of Royal Bengal Tiger (L1=69.02um and L2=25.52um), (ii) Medulla of Melanistic Tiger (L1=71.83 and L2=33.08)

(Magus - Stereo microscope 40X zoom)

5.4.2 Medullary Percentage

Tiger hair medulla is narrow, resulting in a small medulla index (35.2-11.3) when compared with mongoose (59.6-0.4 to 79.2-0.5). On the other hand, tiger hair medulla index is larger than that of four Indian bear species (4-0.3 to 36-0.9).it is been almost same in 30 samples of each morphs of the tigers that are been observed.

Table 5.4.1: Shows the Medullary percentages of Melanistic Tiger hairs.

	Medullary Characters			
Sl.no	**Type**	**Thickness (µm)** L1 Cortex	**Thickness (µm)** L2 Medulla	**Percentage in hair**
1.	Simple and Narrow	62.38	27.76	44.50144277
2.	Simple and Narrow	310.1	175.35	56.5462754
3.	Simple and Narrow	231.22	95.21	41.1772338
4.	Simple and Narrow	231.5	92.68	40.03455724
5.	Simple and Narrow	59.15	22.15	37.44716822
6.	Simple and Narrow	91.71	41.81	45.58935776
7.	Simple and Narrow	69.02	25.52	36.97478992
8.	Simple and Narrow	61.49	20.28	32.98097252
9.	Simple and Narrow	69.85	29.37	42.04724409
10.	Simple and Narrow	71.83	33.08	46.05318112
11.	Simple and Narrow	60.74	28.19	46.41093184
12.	Simple and Narrow	103.39	47.74	46.1746784
13.	Simple and Narrow	63.06	27.05	42.89565493
14.	Simple and Narrow	64.03	26.01	40.62158363
15.	Simple and Narrow	73.51	36.62	49.81635152
16.	Simple and Narrow	52.13	20.54	39.40149626
17.	Simple and Narrow	64.6	23	35.60371517
18.	Simple and Narrow	50.87	18.3	35.9740515
19.	Simple and Narrow	55.98	20	35.72704537
20.	Simple and Narrow	70.89	40.63	57.31414868

Average medullary percentage of Melanistic Tiger is *42.66%*

Table 5.4.2: Shows the Medullary percentages of Royal Bengal Tiger hairs.

		Medullary characters		
Sl.no	**Type**	**Thickness (µm)** L1 Cortex	**Thickness (µm)** L2 Medulla	**Percentage of hair**
1.	Simple and Narrow	68.84	32.85	47.71934922
2.	Simple and Narrow	79.74	35.89	45.00877853
3.	Simple and Narrow	57.05	25.64	44.94303243
4.	Simple and Narrow	59.49	26.36	44.30996806
5.	Simple and Narrow	60.38	27.49	45.52832064
6.	Simple and Narrow	65.87	28.12	42.69014726
7.	Simple and Narrow	77.95	36.32	*46.5939705*
8.	Simple and Narrow	80.56	36.26	45.00993049
9.	Simple and Narrow	82.27	39.66	48.20712289
10.	Simple and Narrow	64.86	28.65	44.1720629
11.	Simple and Narrow	71.47	33.11	46.32713026
12.	Simple and Narrow	65.78	29	44.08634843
13.	Simple and Narrow	69.26	32.69	47.19896044
14.	Simple and Narrow	72.77	33.93	46.62635702
15.	Simple and Narrow	67.36	31	46.02137767
16.	Simple and Narrow	67.03	31.29	46.68059078
17.	Simple and Narrow	67.27	31.85	47.34651405
18.	Simple and Narrow	45.98	20.02	43.54066986
19.	Simple and Narrow	52.28	24.53	46.92042846
20.	Simple and Narrow	77.4	36.11	46.65374677

Average medullary percentage of Royal Bengal Tiger is *45.77%*

5.5 Cross-section

The cross-section shape of tiger hairs is found in two forms mostly: Circular and Oval, with mostly medium-sized medulla. While in general the cross-section shapes are similar to Asiatic lion.

Table 5.5.1 : Shows the type of cross section of Melanistic Tiger hairs.

Sl.no	Species	Cross section Type
1.	Melanistic Tiger	Mostly Circular
2.	Melanistic Tiger	Circular
3.	Melanistic Tiger	Circular
4.	Melanistic Tiger	Mostly Circular
5.	Melanistic Tiger	Circular
6.	Melanistic Tiger	Circular
7.	Melanistic Tiger	Circular
8.	Melanistic Tiger	Oval
9.	Melanistic Tiger	Circular
10.	Melanistic Tiger	Circular

Table 5.5.2: Shows the type of cross section of Royal Bengal Tiger hairs.

Sl.no	Species	Cross section Type
1.	Royal Bengal Tiger	Oval
2.	Royal Bengal Tiger	Circular
3.	Royal Bengal Tiger	Oval
4.	Royal Bengal Tiger	Circular
5.	Royal Bengal Tiger	Oval
6.	Royal Bengal Tiger	Circular
7.	Royal Bengal Tiger	Circular
8.	Royal Bengal Tiger	Oval
9.	Royal Bengal Tiger	Oval
10.	Royal Bengal Tiger	Circular

Table5.5.3: Shows the type of cross section of Melanistic and Royal Bengal Tiger hairs.

Sl.no	Species	Cross section Type
1.	Melanistic Tiger	
2.	Royal Bengal Tiger	

5.6 DISCUSSIONS

In this study, the two Tiger hairs which are morphologically different, were observed and examined by few important examinations and are quantified and the key features useful for Tiger Hair identification were noted. It is expected that the output reported in this study will serve as a guideline for tiger hair identification and raise caution about using data from studies that overlook internal variations of hair morphology.

Observations shown that there are some variations due to hair section, hair region and individual animal in most tiger hair characteristics. A considerable amount of variations was found in studied hair parameters between species as well as sometimes within species between different regions of the body. Traits like Color & Cuticular pattern showed a good variation within & between the species but Medullary pattern & Cortex varied very less.

CHAPTER VI

RESULT AND CONCLUSION

6.1 RESULTS

In the present study, pigmentation or coloration's of hair of both the morphs (Royal Bengal Tiger and Melanistic Tiger) were physically examined and observed that, tiger hairs are present in four colors: White, Black, Yellow and Brown. The P*roximal and Distal hair* parts are dominantly Pale Orange and Black, whereas White and Yellow are almost equally found in the M*iddle part or Medial part.*

Mainly three types of Cuticular patterns i.e., Regular wave, Irregular wave and Single Chevron type of patterns are found in the hair of both the morphs.Mainly three different distinct scale margins in both the morphs of the Tiger are observed during Cuticular examination. Those are Smooth & Slant, Rippled and Crenate in Melanistic Tiger and Smooth, Rippled and Crenate at Royal Bengal Tiger at Proximal, Medial and Distal regions respectively. During hair examination of both morphs i.e., Royal Bengal Tiger and Melanistic Tiger mainly three types of scale margins are observed.i.e., *close, distant* and *near* which shows the same scale margins compared to the standards given by WII.

Medullary structure : In the Royal Bengal tiger, the simple narrow type of medulla was seen and in Melanistic tiger the simple and wider medulla was seen than the expected .The Medullary percentage of the Melanistic tiger is either be positive (greater) or negative (less than) of the exact standard. Medullary percentage (46%) provided by *WII (Wildlife Institute Of India),* where as Royal Bengal Tiger is having of almost same medullary percentage as of the standards of *WII.*

6.2 CONCLUSION

These findings indicate that it is very hard to differentiate between related species based on any one trait of hair, instead all the characters must be considered and also this identification was been continued with the samples of another morph of Tiger 'White Tiger'. It is expected to perform similar study in same or other species to construct a database of hair morphological characteristics in different morphs of one species.

With this information, it will be possible to use statistical methods to achieve classifications based on hair characteristics of Tigers which are morphologically different and also help to assign probabilities using likelihood ratios in reporting of Species and Morphs identification.

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