



Tamil Nadu Forest Department
ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(Research, Training & Education)
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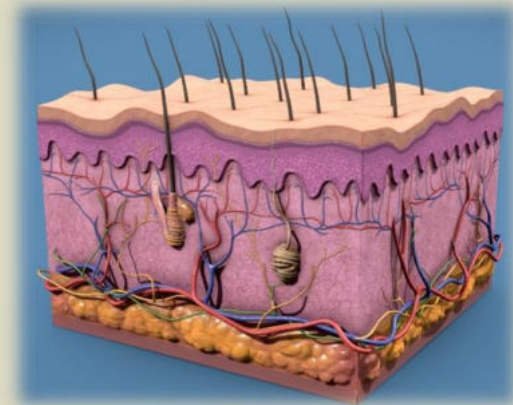
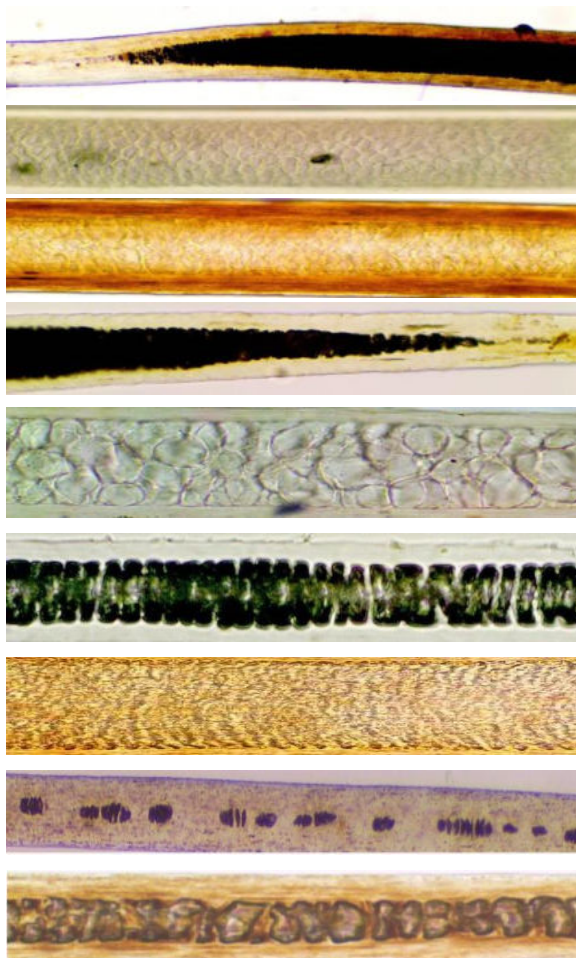


Project Completion Report

Research Theme 1: Application of forensic science in wildlife crime investigations and in enhancement of species conservation

Morphometric Approach

‘Development of Mammalian Hair Reference Repository for Species Identification and Tricho-taxonomic studies’



**Annual Plan of Operations
(APO)
2020-21**

Submitted by

Dr. Nittu George
Project Scientist



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On

‘Development of Mammalian Hair Reference Repository for Species Identification and Tricho-taxonomic studies’

**Annual Plan of Operations (APO) Project
(2020-21)**



August 2023

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INTRODUCTION

Taxonomic studies play a vital role in species identification in wildlife forensics, often used in food habitat studies, forensic sciences, taxonomy, archaeological studies, etc. (Raval *et al.*, 2018). It is significant in the study of the food habits of carnivores and is supportive of controlling the illegal trade of wildlife and its derivatives. While the morpho-taxonomy is unable to provide a fruitful result in the case of a small part of the skin of a mammal, the tricho-taxonomy may be used for the identification of species on the basis of combination characters.

A Concise Overview of Mammals

Mammals are distinguished morphologically from other taxa by possessing body hair and mammary glands in females. Most mammals have hairs throughout their lifetime, and some, like Whales and Dolphins, have hairs during their Ontogeny process. Its main function is to protect the body and maintain the body temperature. The class Mammalia includes about 5,416 identified species grouped into 1,229 genera, 153 families, and 29 orders. In India, 410 mammalian species have been recorded, which is 8.86% of the world's total mammalian species (Wilson & Reeder, 2005).

Many Indian animals are on the verge of extinction due to several reasons, including poaching/ hunting for the illegal trade. Mammals are one of the most targeted groups in India. Their skins are often seized by different Wildlife enforcement agencies (Chakraborty & De, 2010). The mammalian skins are traded by concealing their original characters by coating temporary dyes. They are exported as preserved flat skins, bags, shawls, brushes, shoes, caps, wallets, etc. The two mammalian orders, Artiodactyla and Lagomorpha, are poached mainly for the local bushmeat consumption and their skins.

Identification of mammal species by using a small part of the skin or its derivatives is difficult. But mammalian skin or skin derivatives with hairs are

very useful in species identification through the morphometric study of hairs, i.e., Tricho-taxonomy.

Tricho-taxonomy

Tricho-taxonomy is one of the reliable methods of identification of mammalian species. It is based on the study of hair characteristics. Tricho-taxonomic study is significant in studying the food habits of carnivorous animals that predate on other mammal species. Tricho-taxonomy is helpful in controlling the illegal trade of wildlife and its derivatives. Many investigators already studied and documented mammalian hairs' macroscopic and microscopic features (Stains, 1958; Brunner & Coman, 1974; Teerink, 1991; Wallis, 1993).

The tricho-taxonomic study is helping different enforcement agencies identify confiscated materials of wildlife and its derivatives for implementation of the Indian Wildlife (Protection) Act, 1972. Moreover, the tricho-taxonomic analysis of mammal hairs in scat samples is useful in studying predatory animals' food habits (Perrin & Campbell, 1980). Systematic knowledge of the structure of dorsal guard hair is necessary to identify the species. Data generated from macroscopic and microscopic characteristics of dorsal guard hair aid in preparing identification keys for respective species. In India, Tricho-taxonomic studies have been carried out by many workers on different orders of class Mammalia (Bahuguna, 2010; Chakraborty & De, 2010; Sarkar, 2012).

The present study attempted to create an in-house reference repository of guard hairs of animals involved in trade to provide supportive data for solving wildlife crime.

Brief account of hair characteristics of Mammals

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

Types of hair

The body of a mammal is composed of various types of hair, the main components of the 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 called the shield and a thinner proximal part called the shaft.

Underhair: The underhair 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 coarse bristle provides an animal with a protective coat.

Spines or quills: The greatly enlarged and modified hairs in hedgehogs and porcupines.

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

Macroscopic Characteristics of Hair regions

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

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

Shield- A widened, flattened area of the shaft. If the widest area is not flattened

the hair is considered as unshielded. The banding pattern of the hair is usually predominant in the shield region.

Sub-shield -The area immediately below the shield region between the basal

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

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, medulla, or both regions of a hair, and some occasionally on cuticular scales. Accordingly, the hair colours may be divided into unicoloured and bicoloured.

Unicolour or Unbanded hair: 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 are distinguished from bicoloured hairs by the sharpness of the colour change in the banded type.

Medullary characteristics

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

Composition of medulla

The medulla is composed of single or more layers; they are:

Unicellular -The medulla comprises a continuous single column of discrete

cells formed by transversal position. The pattern may be regular or irregular.

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

Structures of medulla

The structure of the 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 hair width, and the cortex does not row centre each.

Intermediate - A ladder pattern is sometimes so indistinct that a '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 a 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.

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 the medulla.

Brunner and Coman (1974) identified four major structural groups of hair medullae. These could be further subdivided into a number of medulla types. Classification of the medullary pattern described by Brunner and Coman (1974) is given below (Fig. 1).

- a) Wide lattice
- b) Narrow lattice
- c) Wide aeriform lattice
- d) Narrow aeriform lattice
- e) Multi-serial ladder
- f) Uniserial ladder
- g) Wide simple
- h) Narrow simple
- i) Beaded interrupted
- j) Chain like

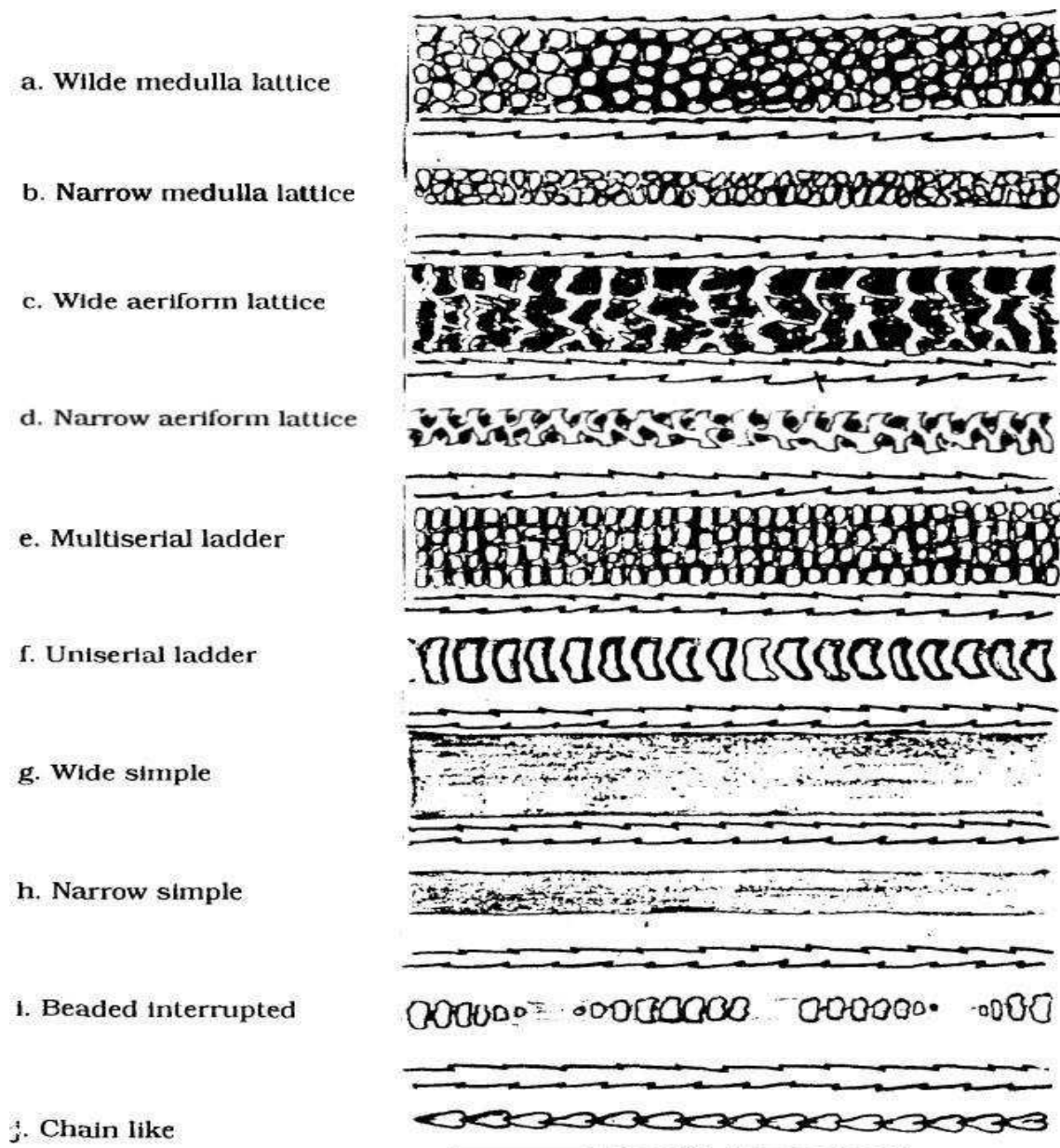


Figure 1. Types of medullary structure

The Present study focused on the medullary characteristics of proximal, medial and distal regions of guard hairs of different species of mammals. This guard hair reference database will serve as supportive data to identify a species involved in wildlife crime.

OBJECTIVES OF THE STUDY

- To establish standards of guard hair parameters for species identification that provide supportive data for solving wildlife crime.
- To Develop a reference repository of guard hairs of various wild animals involved in trade.

MATERIALS AND METHODS

Sample collection

Most of the hair of wild animals used in the present reference repository was collected from Arignar Anna Zoological Park (AAZP), Vandalur, Tamil Nadu, and some from domestic animals. Hair samples were collected from different regions of the animal body to prepare reference slides. Hair samples were collected from the dorsal, ventral and head portions in general. However, samples were collected from all possible parts as far as possible. While collecting, care was taken to get the maximum accessible length of each hair. Especially, guard hairs, the primary ones, exhibit the most diagnostically useful features and hence are paramount in hair identification (Brunner & Coman. 1974). Hence, guard hairs were selected for the present study.

Slide preparation

The hair was washed in distilled water, cleaned in 70% alcohol and mounted in DPX (Dibutylphthalate Polystyrene Xylene). Some hairs, especially thick hairs, were treated with xylene for 4-5 days for better results. A total of 28 species were selected for the study and hairs were observed under a microscope for studying the characteristic features of the cortex and medulla. Three to four hairs from the same species were selected for the study to confirm the characteristics. Several such samples were examined in each species to select uniform characteristics and record variations based on the general shape and arrangement of air spaces and medullary material.

The present reference repository targeted the three parts of the medulla, proximal, medial and distal (Fig.2) portions of the hair. Species can be identified based on the variations of the medullary structure of these three regions. The slides prepared were used as reference slides at the AIWC

morphology laboratory.

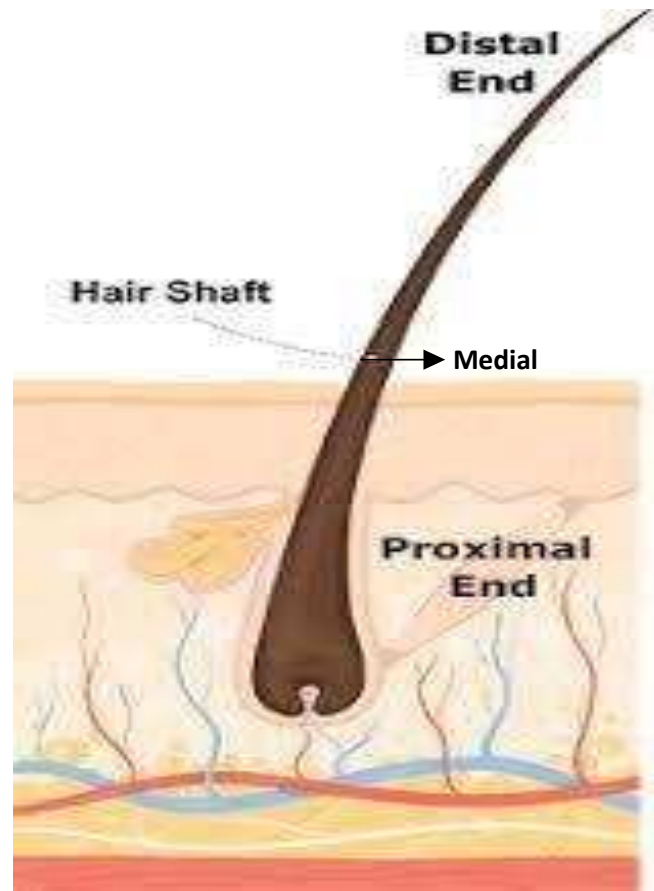


Figure 2. The structure of hair shows three different parts (Proximal, medial and distal)


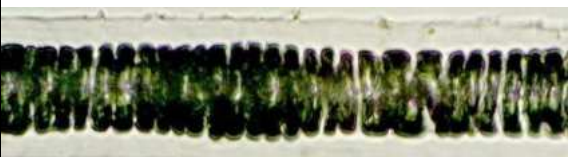
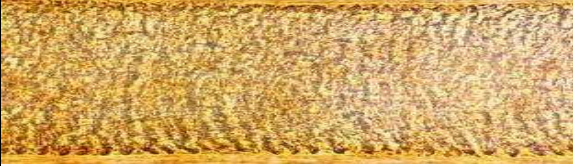
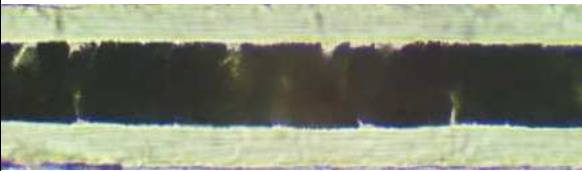

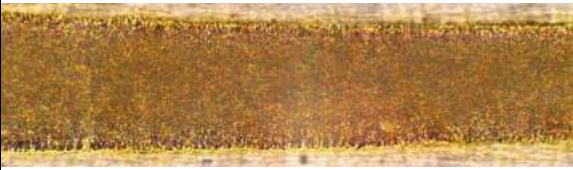
RESULTS AND DISCUSSION

The list of 28 animal species selected for the reference repository is presented in Table 1. Species identification through hair morphology was done by examining the cortex and medulla of the hairs under a microscope. The proximal, medial and distal parts of hair shaft were also studied (Table 2). The microscopic examinations of hairs provided information on the structure of the medial part of the medulla and images were recorded. Table 3 gives hair identification keys based on different hair characteristics as described by Easa (1995), which helps identify or confirm the mammalian species. Comparing photographs of three portions of the medullary structure of the hair provides greater clarity in species identification.

Table 1. List of 28 species selected for the reference repository is given below (Species are classified based on their taxonomic order and family)

Sl. No.	SPECIES	Sl. No.	SPECIES
Family: CERVIDAE		Family: CANIDAE	
1.	Spotted deer (<i>Axis axis</i>)	17	Indian wolf (<i>Canis lupus pallipes</i>)
2.	Sambar deer (<i>Rusa unicolor</i>)	18	Domestic dog (<i>Canis lupus</i>)
3.	Mouse deer (<i>Moschiola memmina</i>)	19	Dhole (<i>Cuon alpinus</i>)
Family: BOVIDAE		Family: HYAENIDAE	
4.	Barasingha (<i>Rucervus duvaucelii</i>)	20	Striped Hyena (<i>Hyaena hyaena</i>)
5.	Barking deer (<i>Muntiacus muntjack</i>)	Family: URSIDAE	
Family: FELIDAE		21	Sloth bear (<i>Melursus ursinus</i>)
6.	Blackbuck (<i>Antilope cervicapra</i>)	Family: HERPESTIDAE	
7.	Nilgiri Tahr (<i>Nilgiritragus hylocrius</i>)	22	Indian grey mongoose (<i>Herpestes edwardsii</i>)
8.	Domestic cattle (<i>Bos taurus</i>)	Family: VIVERRIDAE	
9.	Indian Gaur (<i>Bos gaurus</i>)	23	Asian Palm civet (<i>Paradoxurus hermaphroditus</i>)
Family: CANIDAE		Family: SCIURIDAE	
10.	Tiger (<i>Panthera tigris</i>)	24	Malabar giant squirrel (<i>Ratufa indica</i>)
11.	Leopard (<i>Panthera pardus</i>)	25	Indian palm squirrel (<i>Funambulus palmarum</i>)
12.	Domestic cat (<i>Felis catus</i>)	Order: PRIMATES	
13.	Jungle cat (<i>Felis chaus</i>)	Family: CERCOPITHECIDAE	
14.	Leopard cat (<i>Prionailurus bengalensis</i>)	26	Nilgiri Langur (<i>Semnopithecus johnii</i>)
Family: CANIDAE		27	Bonnet macaque (<i>Macaca radiata</i>)
15.	Indian fox (<i>Vulpes bengalensis</i>)	Family: LORISIDAE	
16.	Golden Jackal (<i>Canis aureus</i>)	28	Slender loris (<i>Loris</i>)

Table 2. Structure of medial part of medulla and possibility of species similarity table.

Sl. No.	Medulla-medial	Species possibility
1.		Deer species; Nilgiri tahr; Indian fox
2.		Jungle cat; domestic cat; leopard cat
3.		Mongoose
4.		Leopard; Indian wolf; Hyena
		Leopard
5.		Tiger


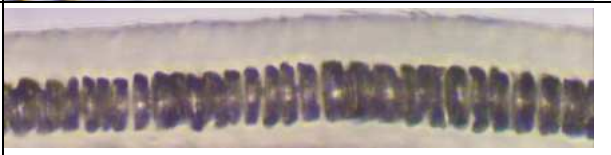
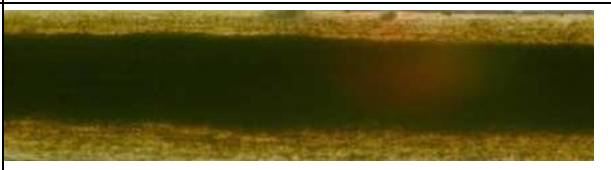
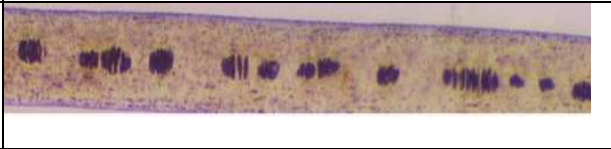

6.		Indian palm squirrel
7.		Indian palm squirrel; Loris
8.		Domestic cattle; Gaur; Wild dog
9.		Macaque
10		Langur

Table 3. Hair identification keys of some mammals

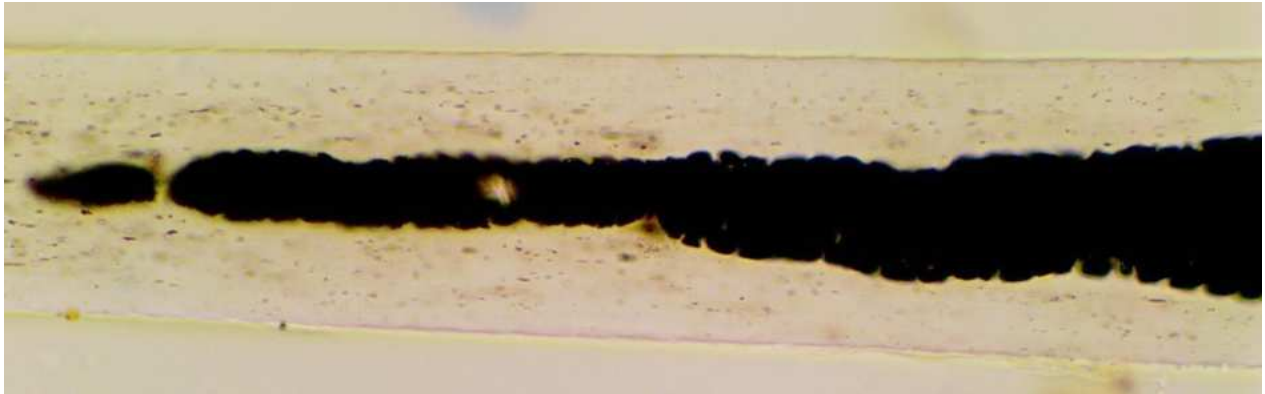
Sl. No.	Hair characteristics	
1.	• Medulla lattice type.....	2
	• Medulla simple, ladder or aeriform.....	10
2.	• Medulla with narrow lattice.....	3
	• Medulla with wide lattice.....	4
3.	• Medulla starts away from the base.....	
	Mongoose	
4.	• With polygonal cells.....	5
	• Without polygonal cells.....	8
5.	• Long thick hair.....	Sambar Deer
	• Hair not so long and thick.....	6
6.	• Lattice scaly, cream-coloured portion just behind the Up.....	Mouse Deer
	• Lattice not scaly, no distinct colour pattern at the Tip.....	7
7.	• Proximal region of the medulla with segregated pattern.....	Spotted Deer
	• Proximal region of the medulla without segregated pattern.....	Mouse Deer
8.	• Cortex highly serrated in the proximal region.....	9
9.	• Medulla starts in a conical fashion.....	Leopard
	Cat	
10.	• Medulla simple in the middle Medulla.....	11
	• Ladder or aeriform lattice in the middle.....	15
11.	• Medulla simple with globular white patches at regular intervals.....	Leopard
	• Medulla simple without globular white patches.....	12
12.	• Cortical width less than that of Medulla.....	Nilgiri Tahr
	• Cortical width greater than that of medulla.....	13
13.	• Medulla uniserial ladder in the proximal region.....	
	Jackal	
	• Medulla simple in the proximal region.....	20
14.	• Hair black throughout and the blackness increases towards the tip.....	Gaur
	• Hair yellow white or with alternate cream and black.....	Tiger
15.	• Medulla aeriform.....	22
	• Medulla uniserial ladder.....	16

16.	• Medulla beaded in the middle portion.....	17
	• Medulla spindle-shaped cells.....	20
17.	• Medulla beaded chain-like hair very narrow.....	Slender Loris
	• Medulla not chain like hair not narrow.....	18
18.	• A yellow portion before the tip of the hair.....	Bonnet macaque
	• No yellow portion before the tip of the hair.....	19
19.	• Hair throughout black or white.....	Nilgiri Langur
20.	• Cortex feebly serrated.....	Civet
	• Cortex highly serrated.....	21
21.	• White patches between the spindle cells.....	Wild dog
	• No white patches between the spindle cell.....	Jungle cat
22.	• Medulla with alternate narrow and wide aeriform lattice in the middle.....	Palm squirrel

Order: ARTIODACTYLA

Family: CERVIDAE

1. Spotted Deer (*Axis axis*)



Proximal



Medial

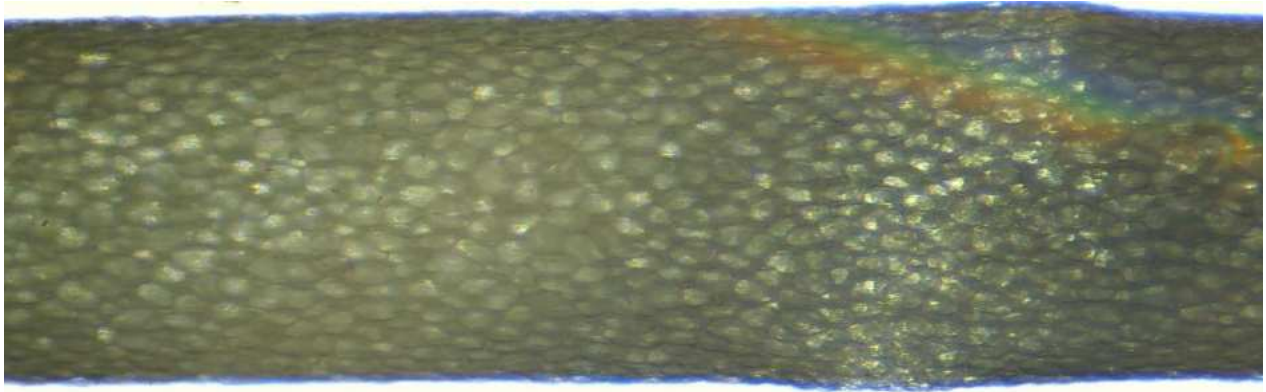


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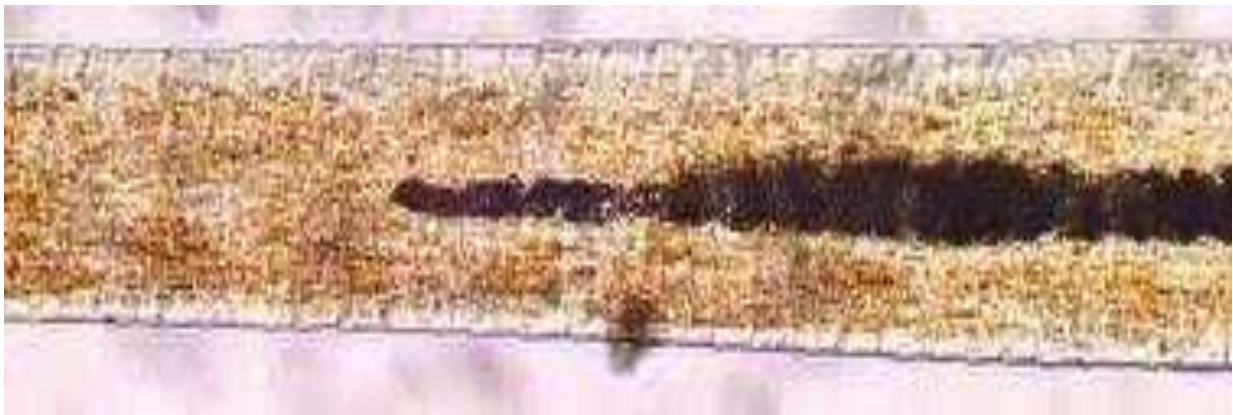
2. Sambar Deer (*Rusa unicolor*)



Proximal



Medial

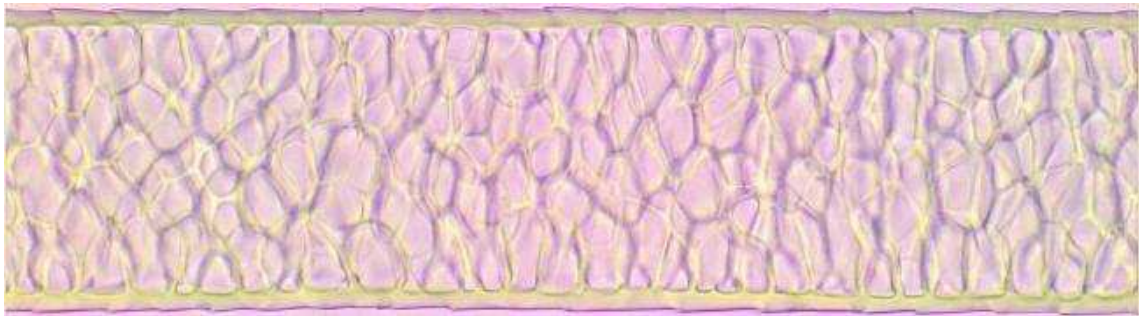


Distal

3. Mouse Deer (*Moschiola memmina*)



Proximal

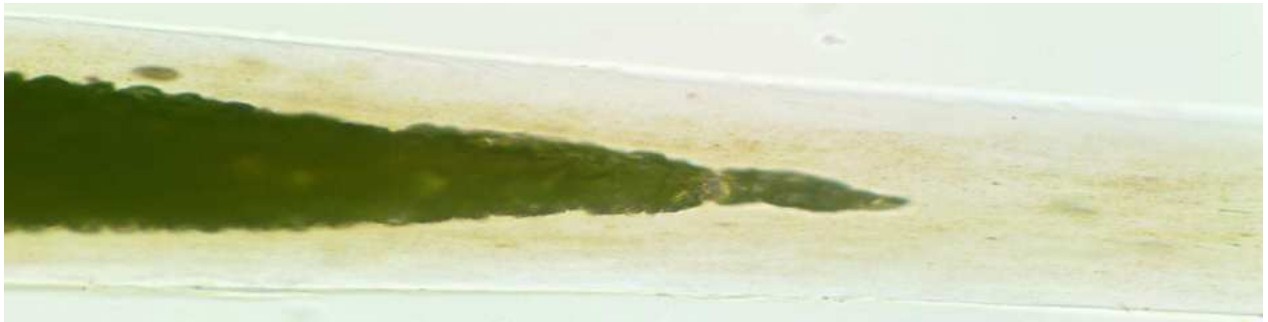


Medial

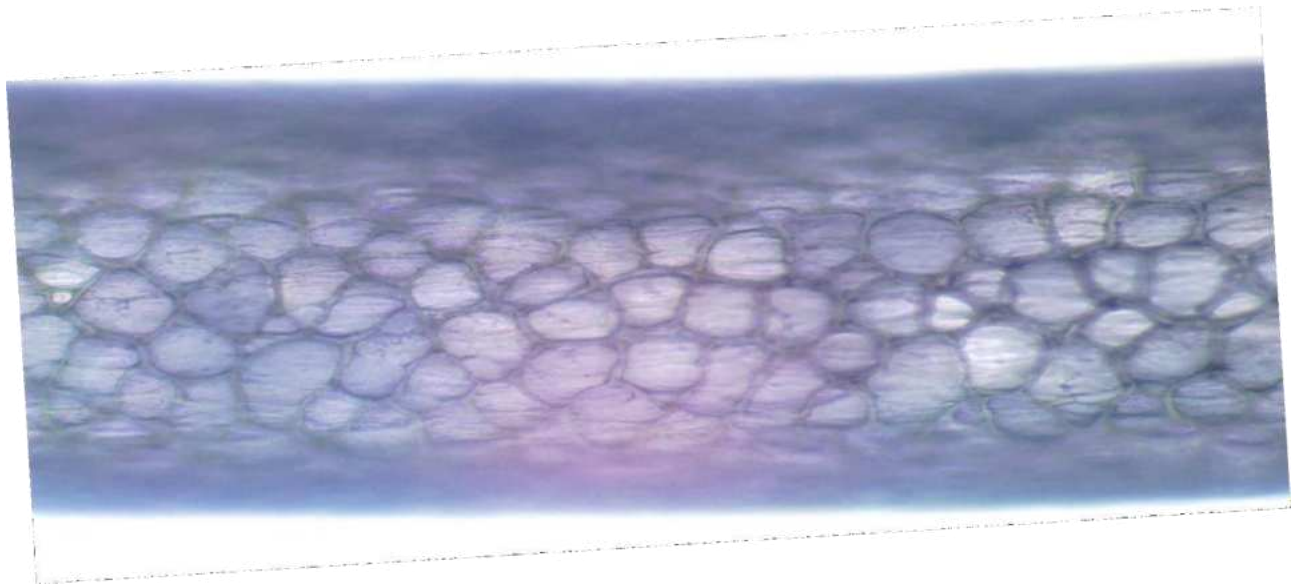


Distal

4. Barasingha (*Rucervus duvaucelii*)



Proximal

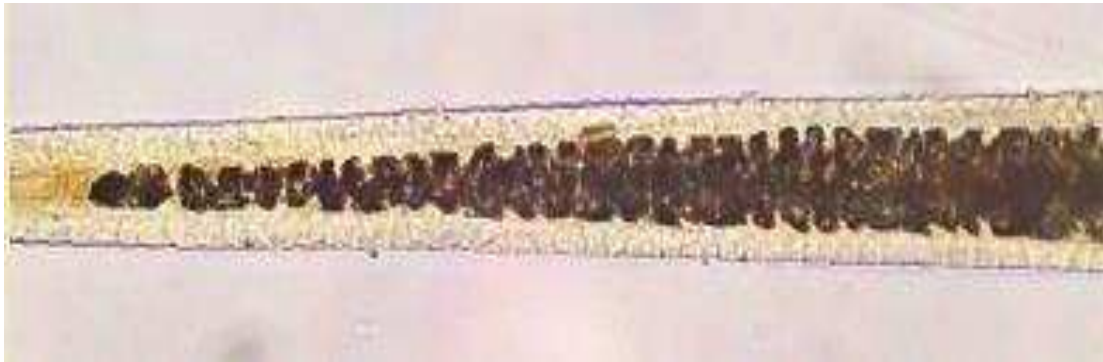


Medial

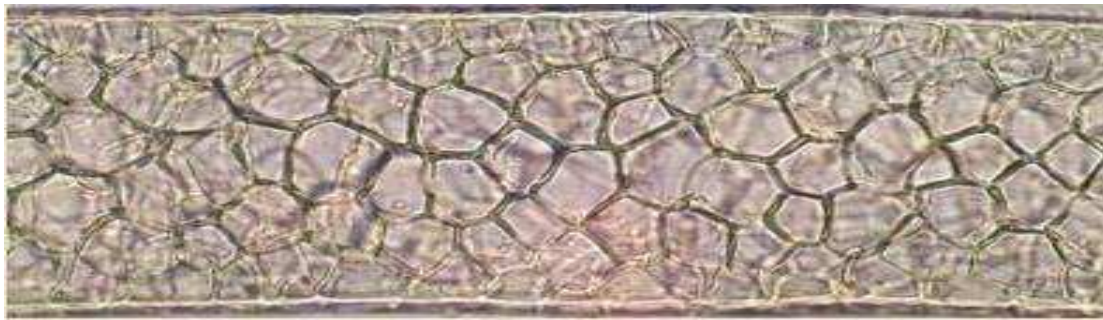


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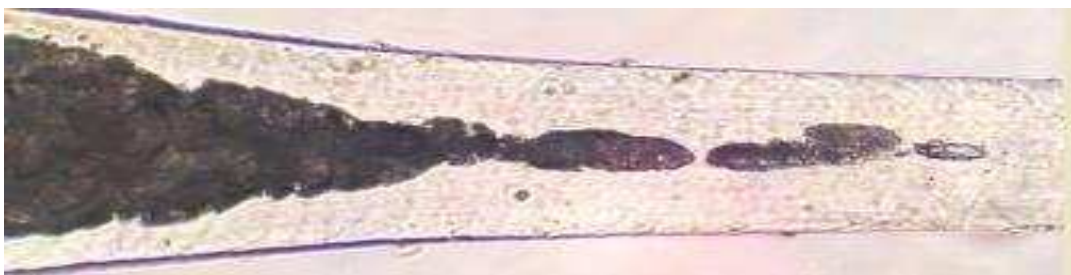
5. Barking Deer (*Muntiacus muntjack*)



Proximal



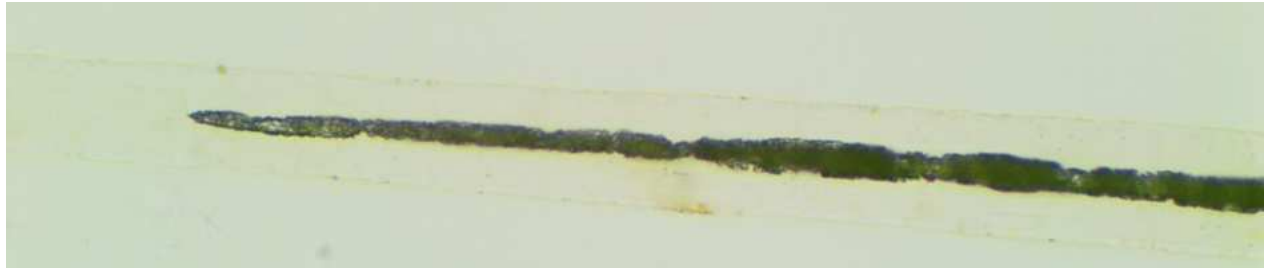
Medial



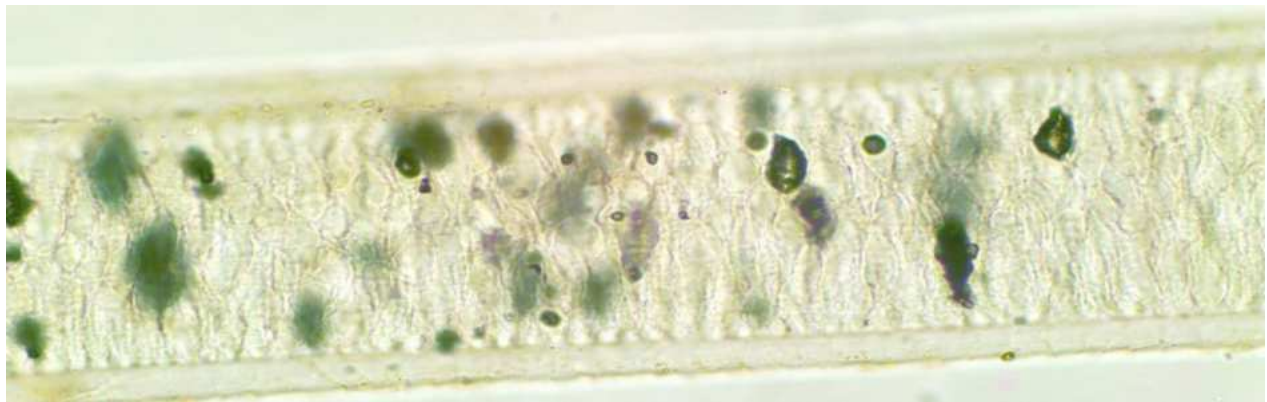
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Family: BOVIDAE

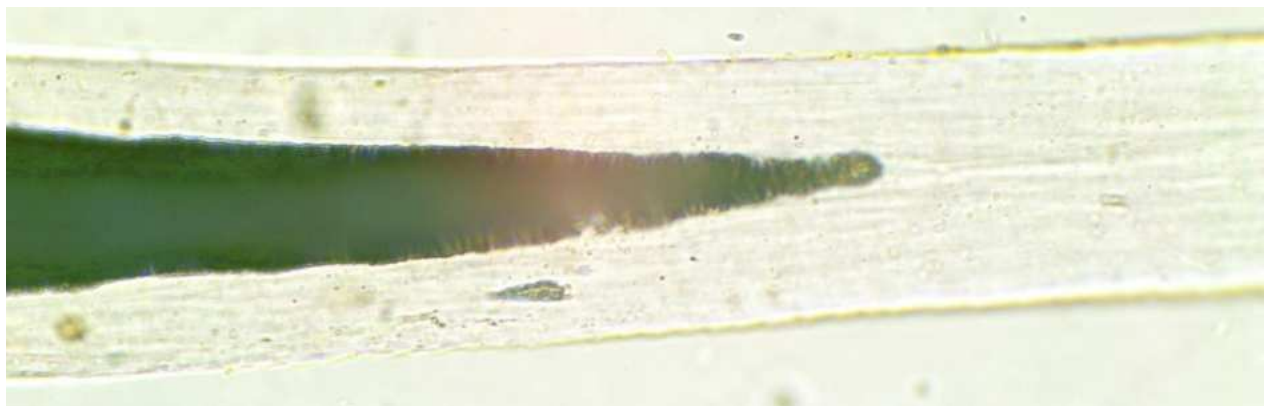
1. Blackbuck (*Antilope cervicapra*)



Proximal



Medial

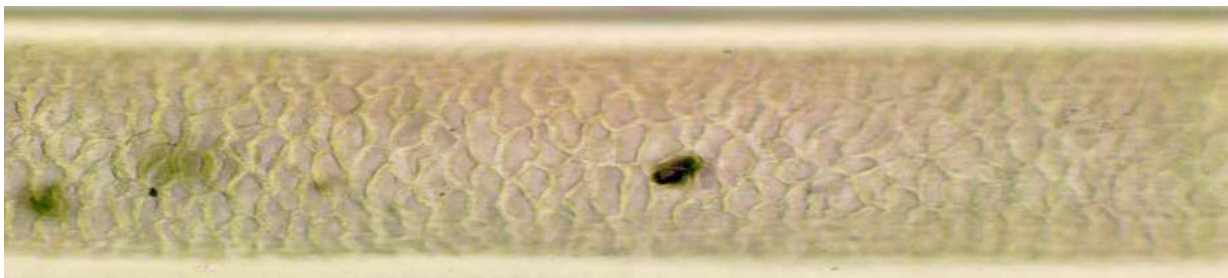


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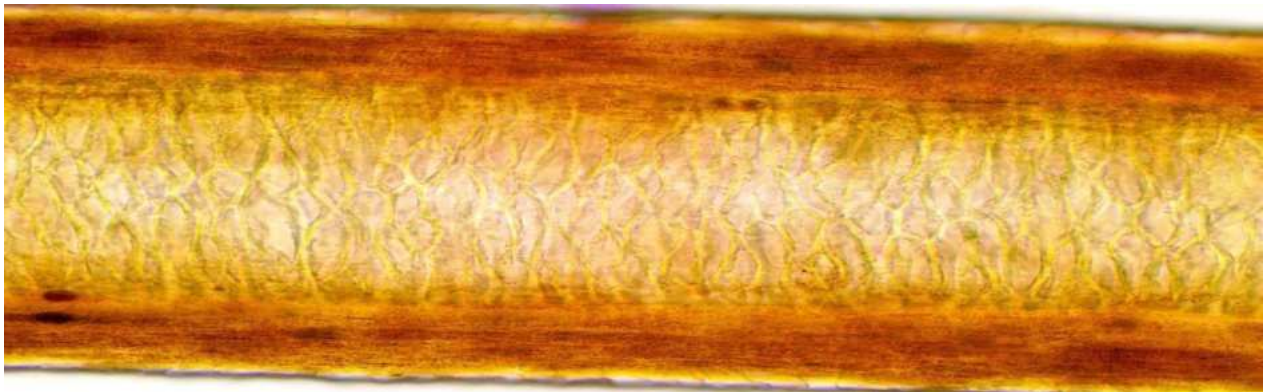
2. Nilgiri Tahr (*Nilgiritragus hylocrius*)



Proximal

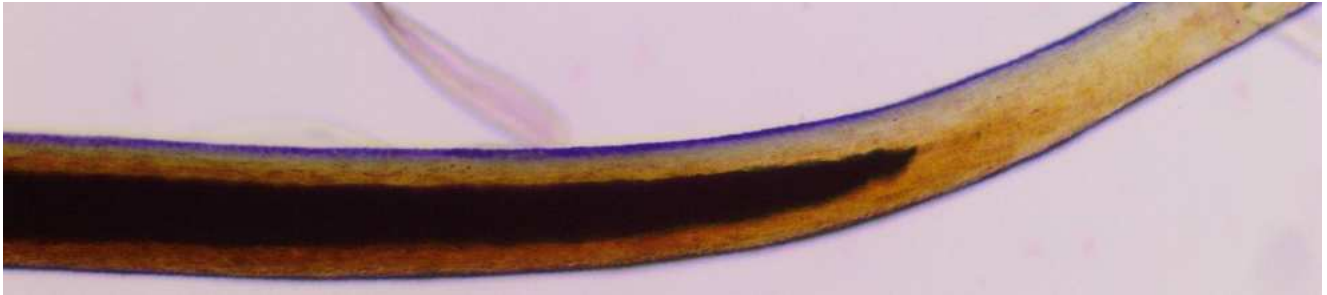


Medial



Distal

3. Domestic cattle (*Bos taurus*)



Proximal

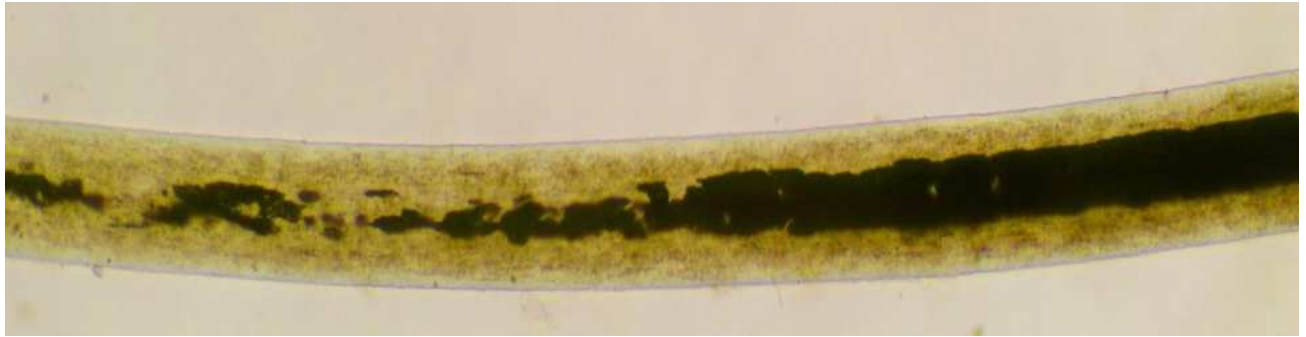


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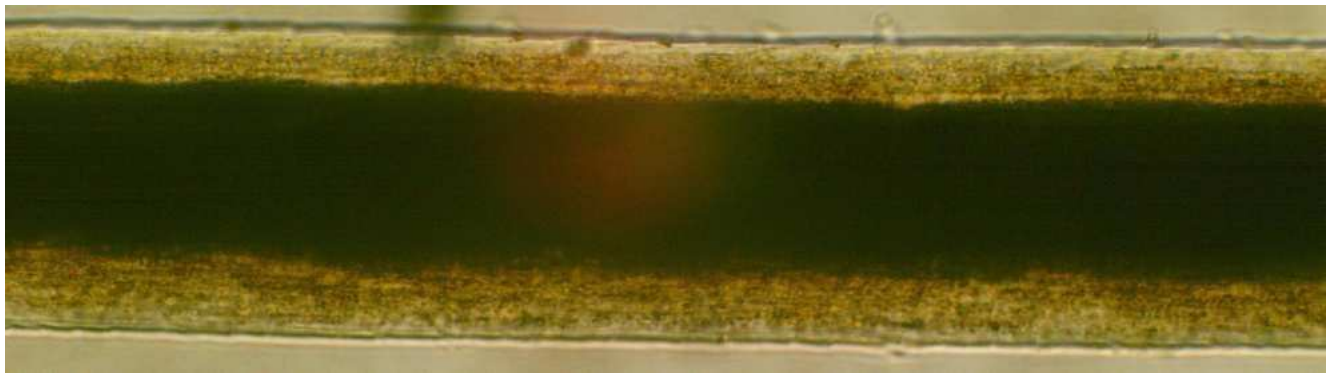


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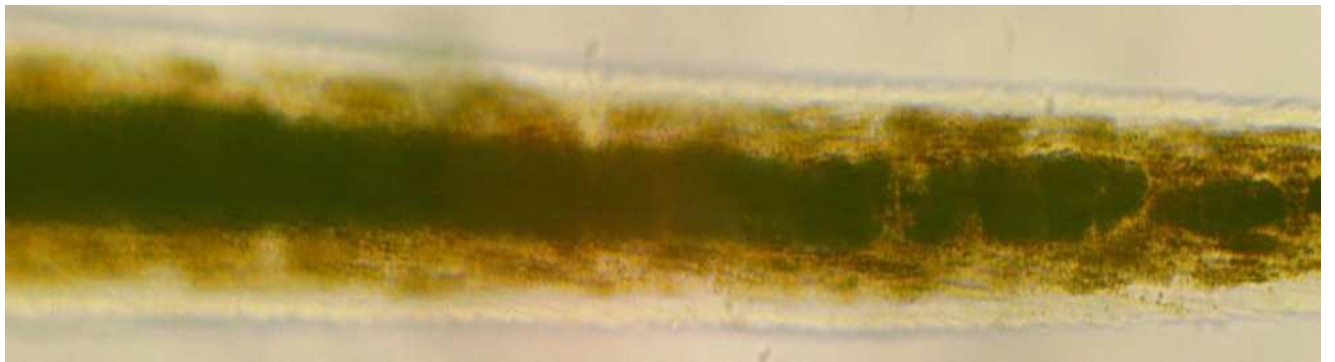
4. Indian guar (*Bos gaurus*)



Proximal



Medial

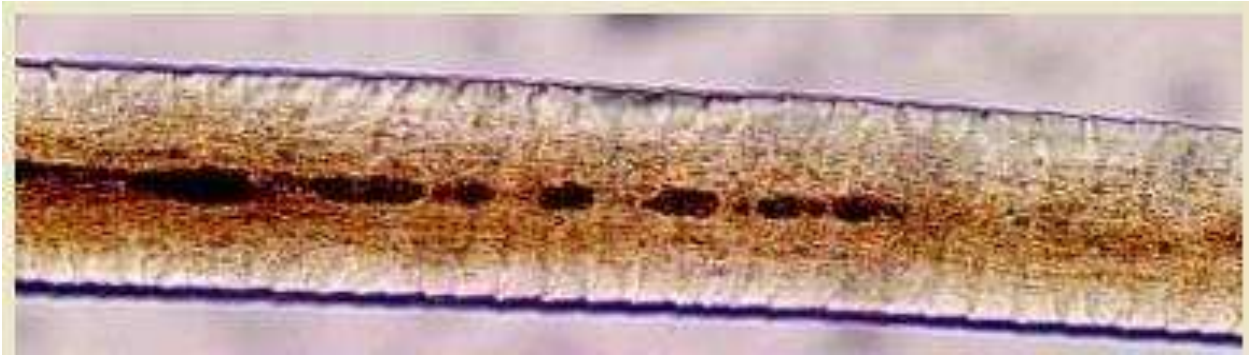


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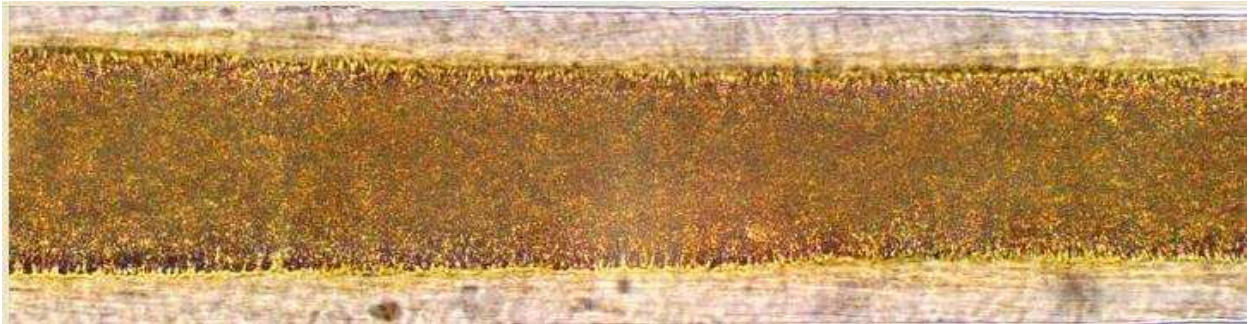
Order: CARNIVORA

Family: FELIDAE

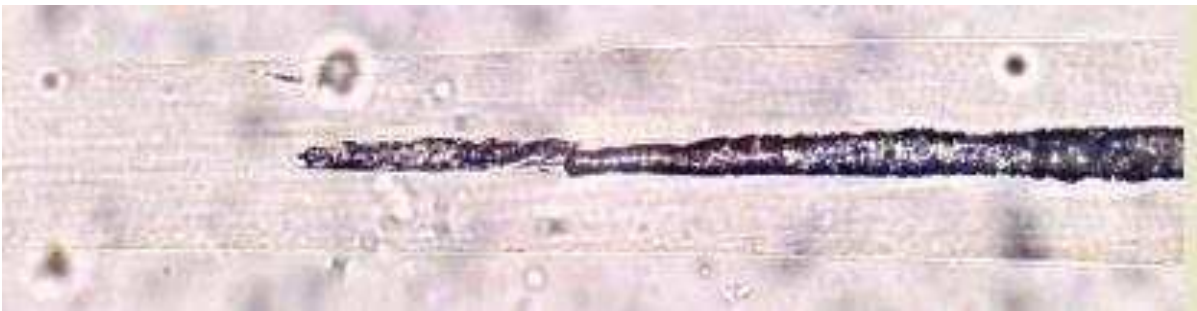
1. Tiger (*Panthera tigris*)



Proximal

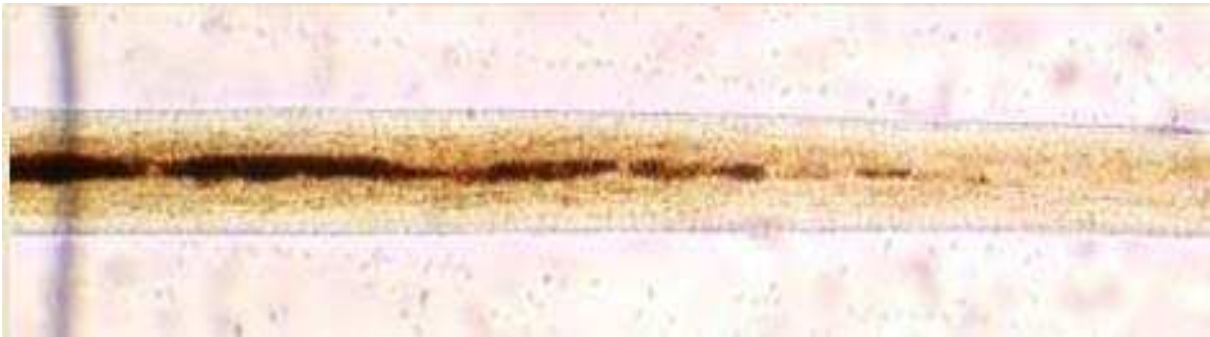


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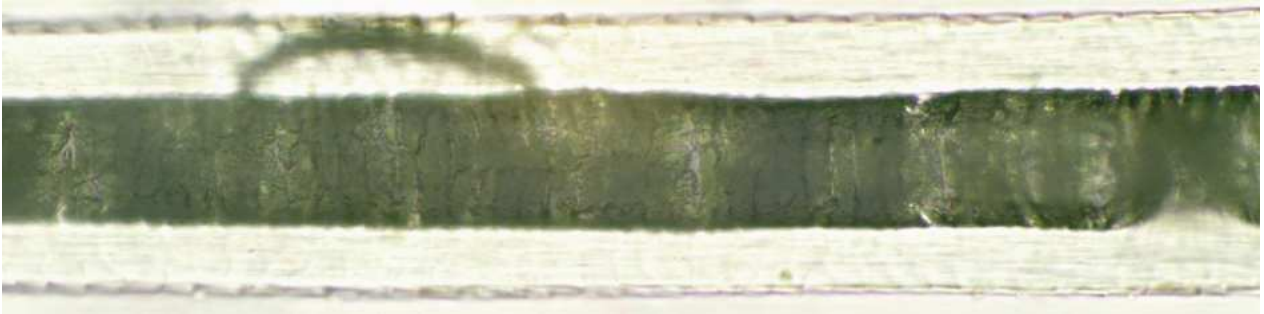
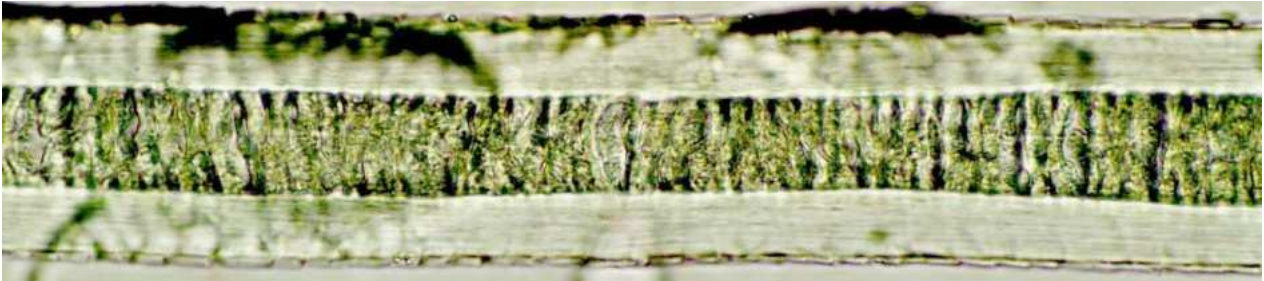


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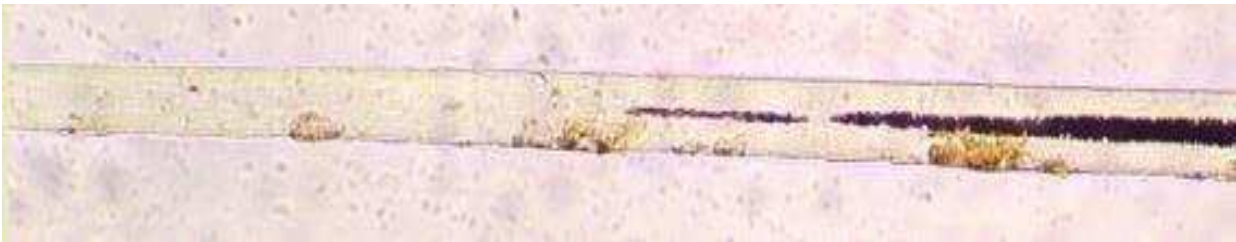
2. Leopard (*Panthera pardus*)



Proximal



Medial

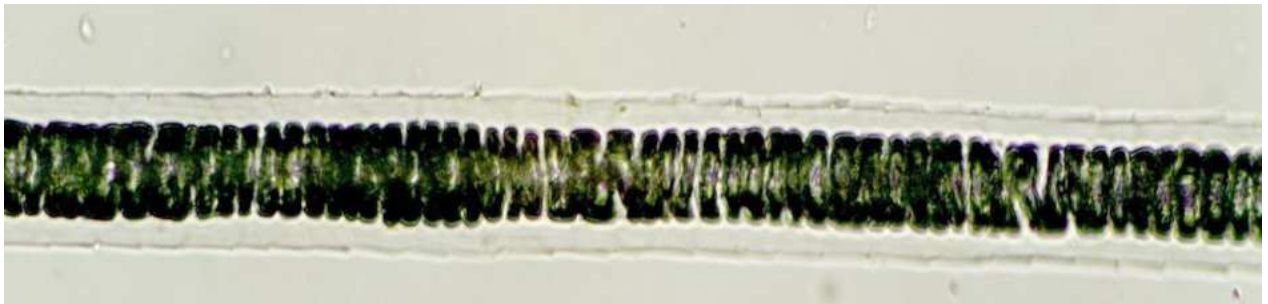


Distal

3. Domestic cat (*Felis catus*)



Proximal



Medial

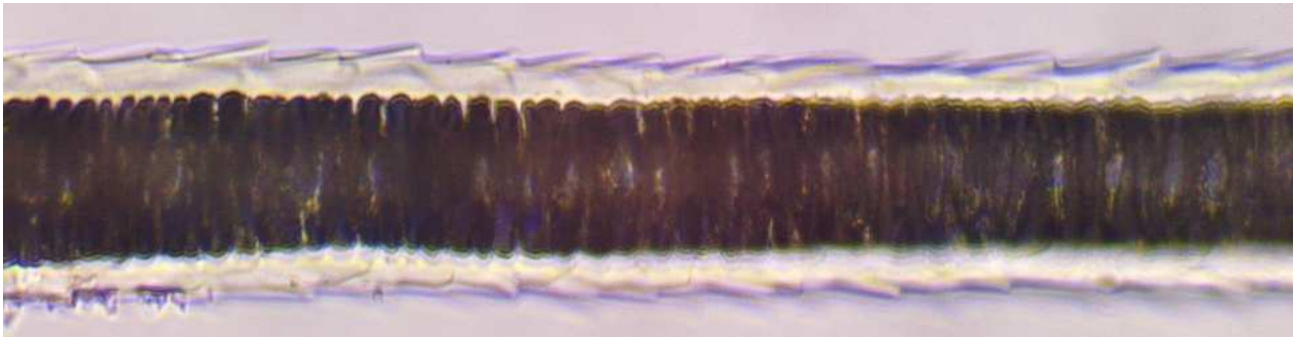


Distal

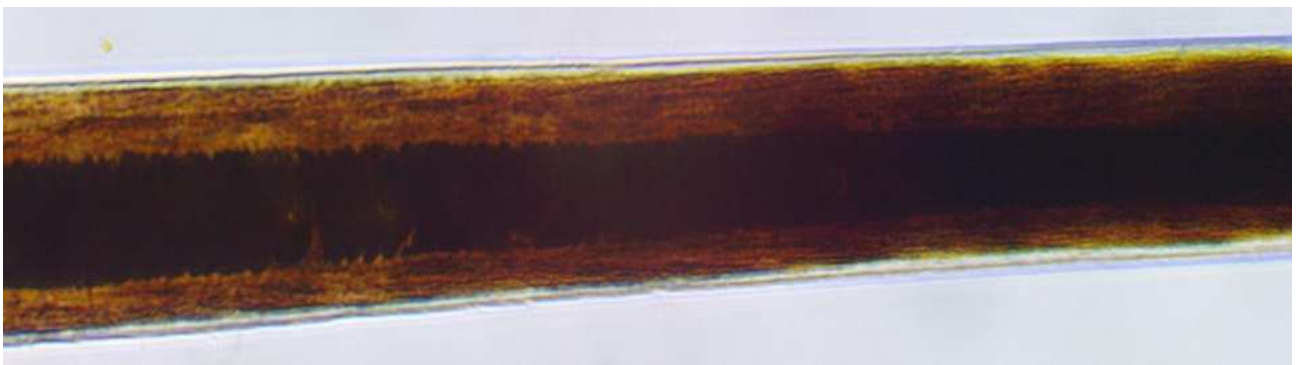
4. Jungle cat (*Felis chaus*)



Proximal



Medial



Distal

5. Leopard cat (*Prionailurus bengalensis*)



Proximal



Medial



Distal

Family: VIVERRIDAE

1. Asian Palm civet (*Paradoxurus hermaphroditus*)



Proximal



Medial



Distal

Family: CANIDAE

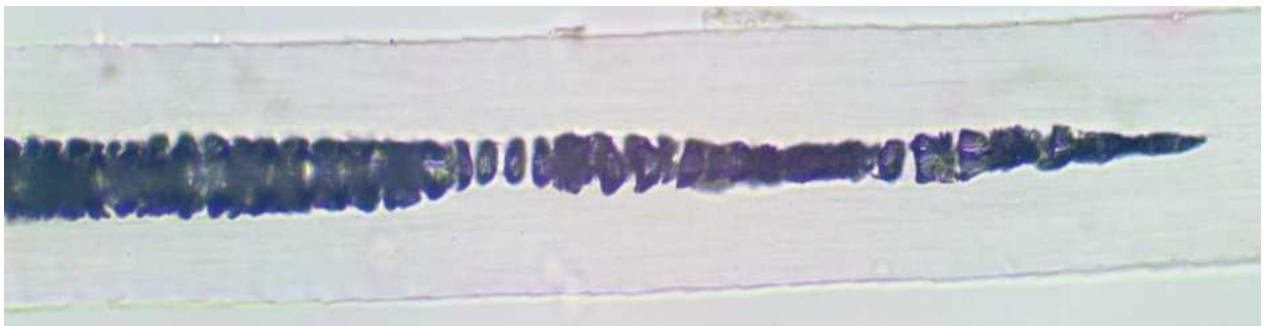
2. Indian fox (*Vulpes bengalensis*)



Proximal

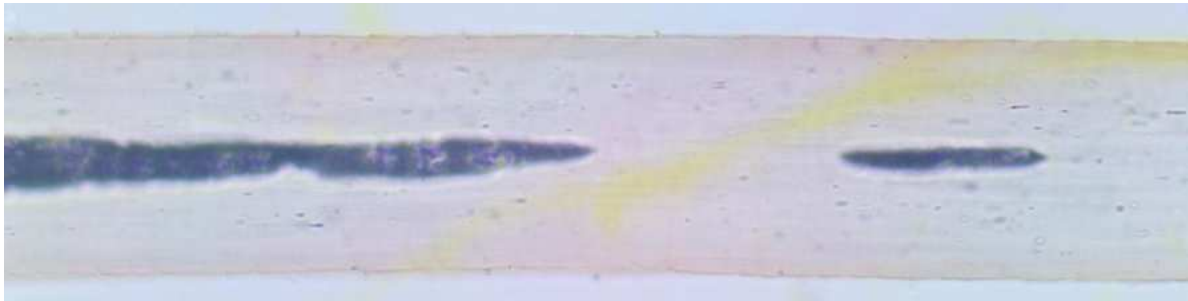


Medial

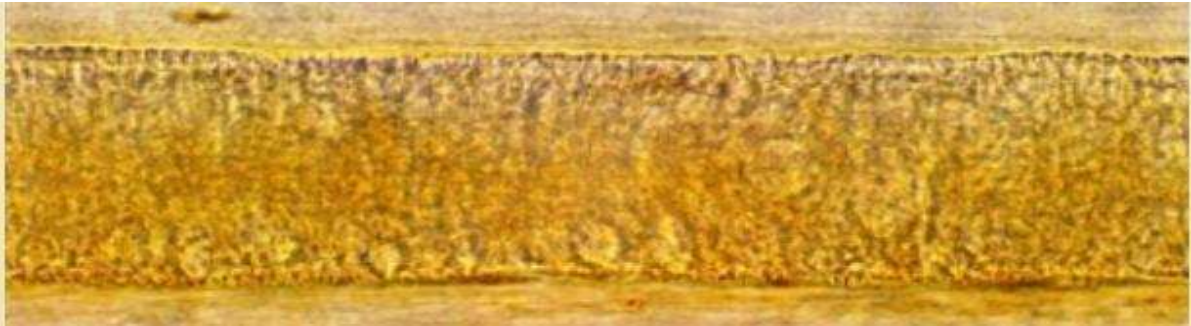


Distal

3. Golden Jackal (*Canis aureus*)



Proximal

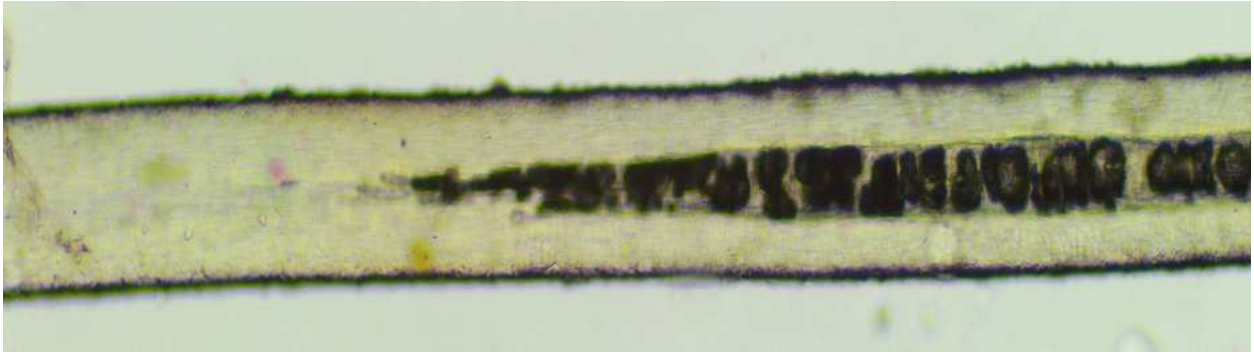


Medial

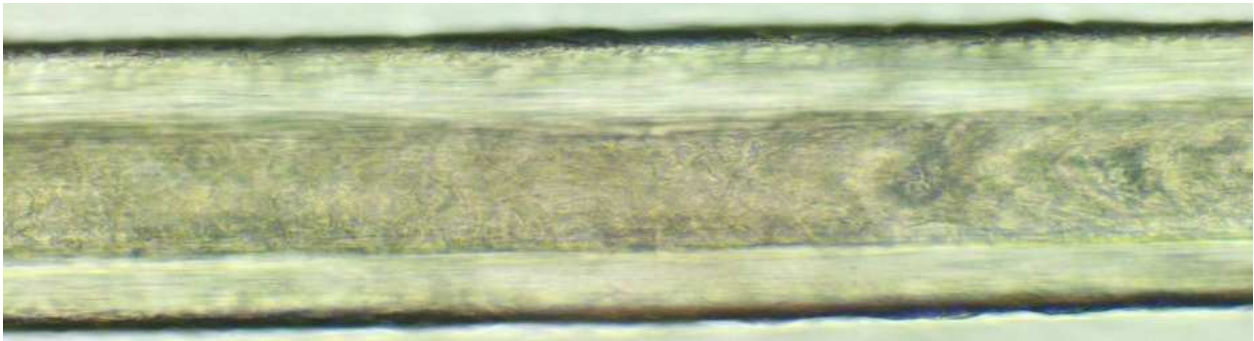
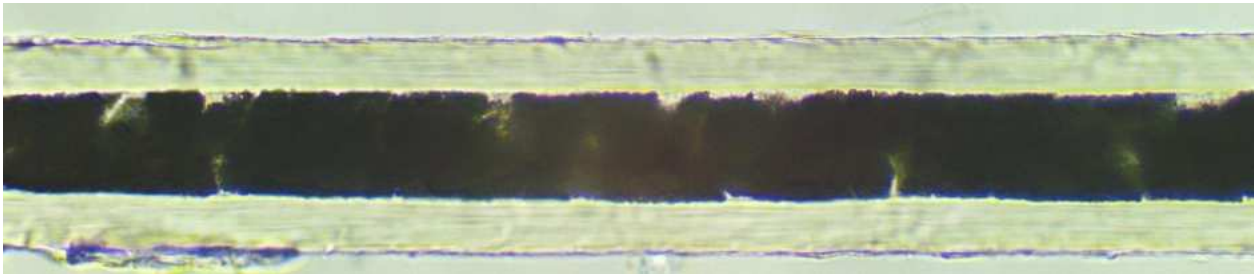


Distal

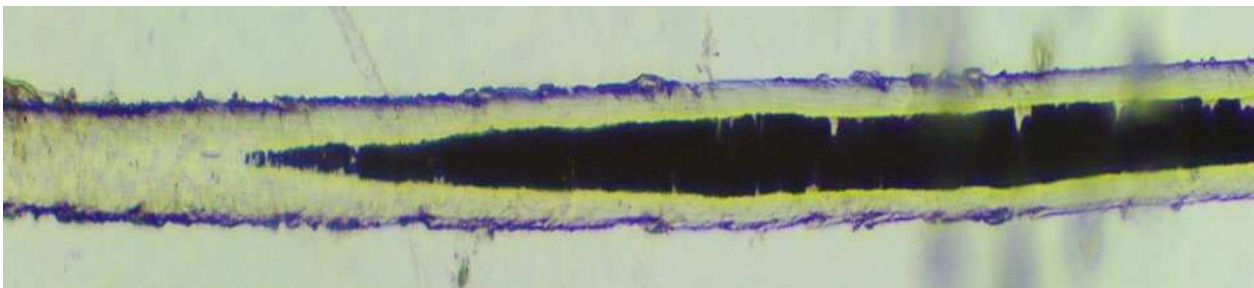
4. Indian wolf (*Canis lupus pallipes*)



Proximal

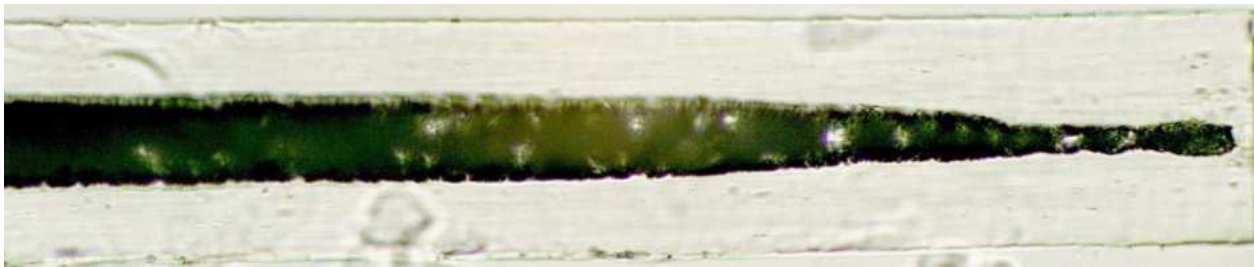
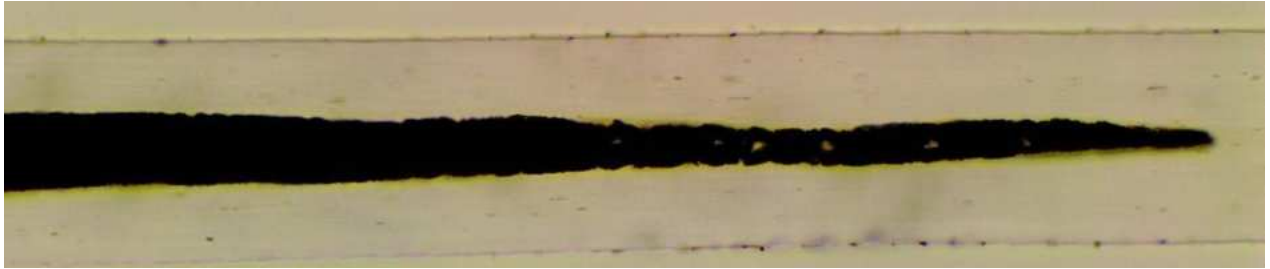


Medial



Distal

5. Domestic dog (*Canis lupus*)

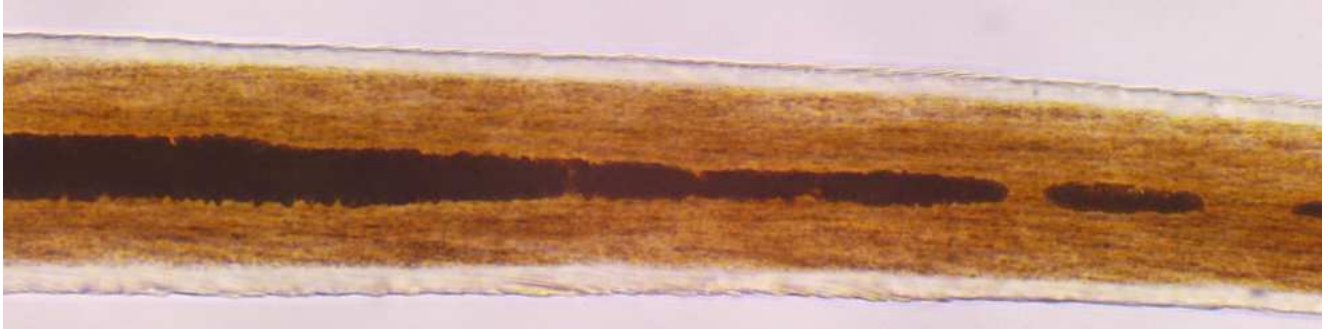


Proximal

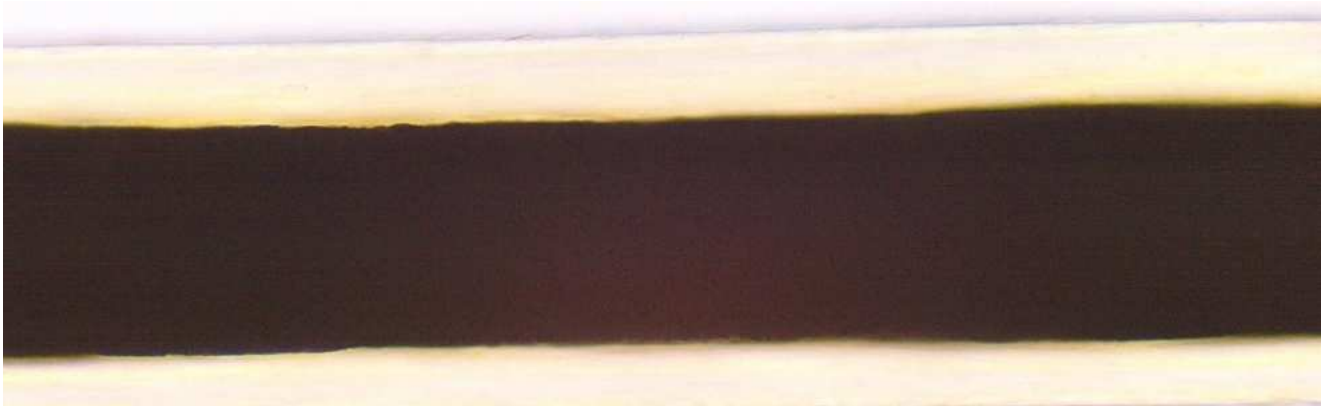


Medial

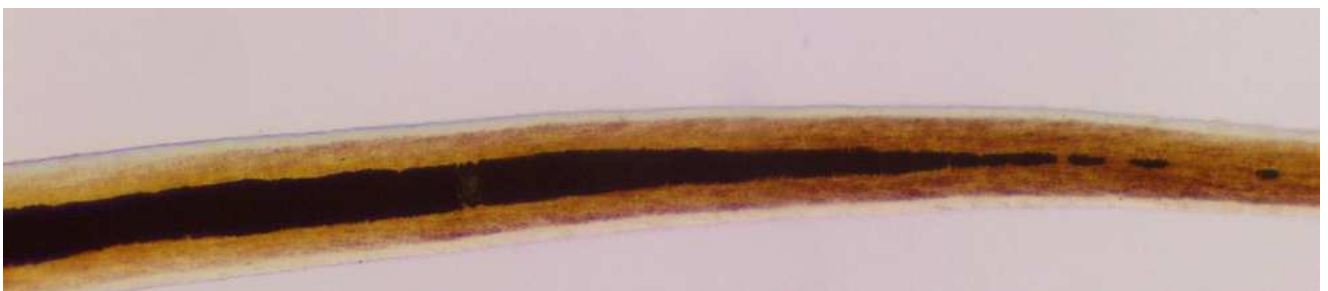
6. Dhole (*Cuon alpinus*)



Proximal



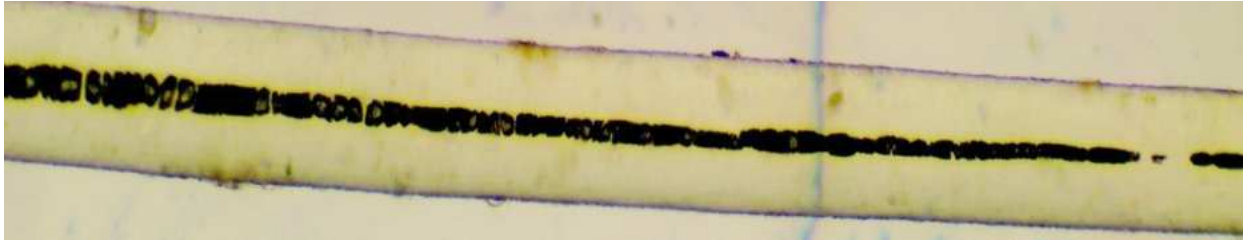
Medial



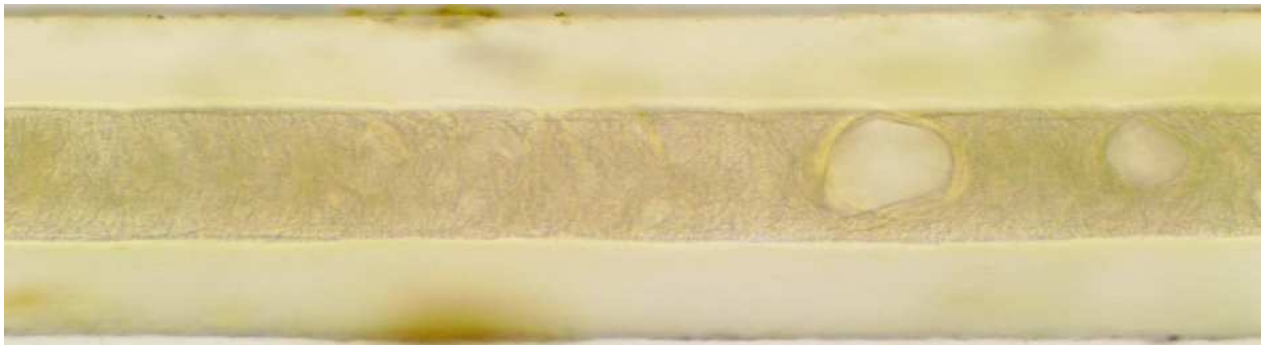
Distal

Family: HYAENIDAE

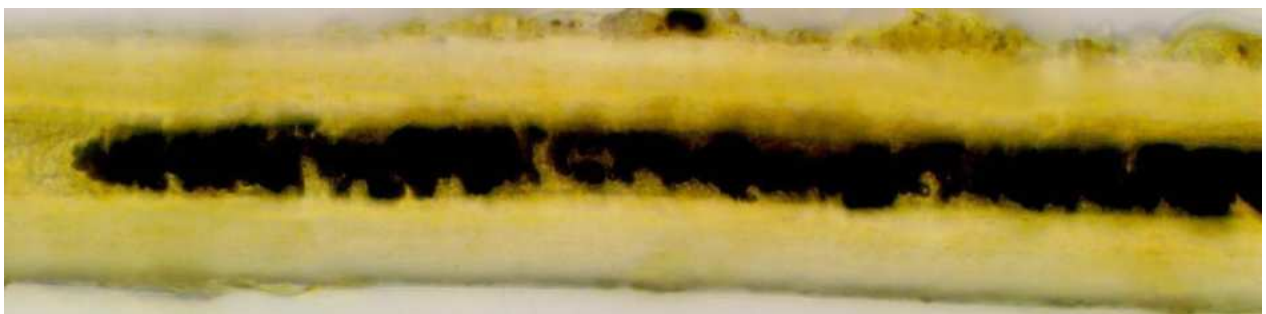
1. Striped Hyena (*Hyaena hyaena*)



Proximal



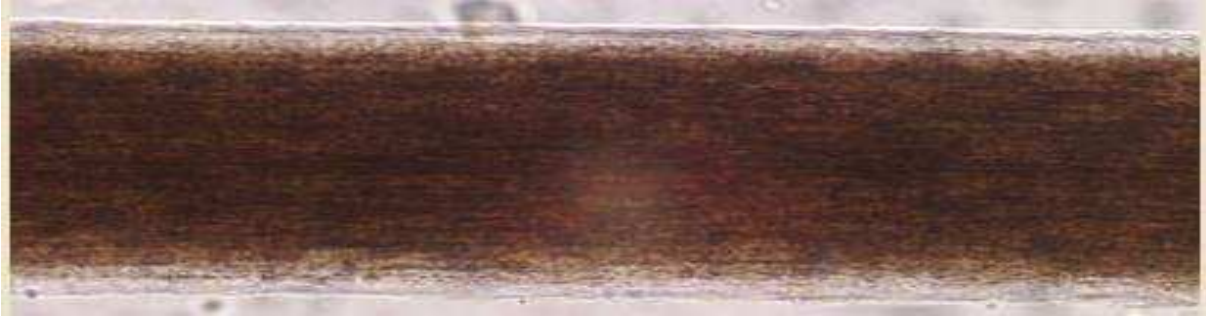
Medial



Distal

Family: URSIDAE

1. Sloth bear (*Melursus ursinus*)



Proximal



Medial



Distal

Family: HERPESTIDAE

1. Indian grey mongoose (*Herpestes edwardsii*)



Proximal



Medial

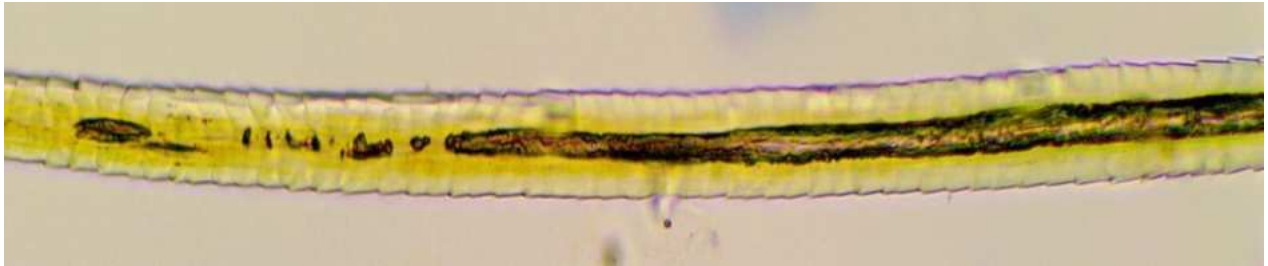


Distal

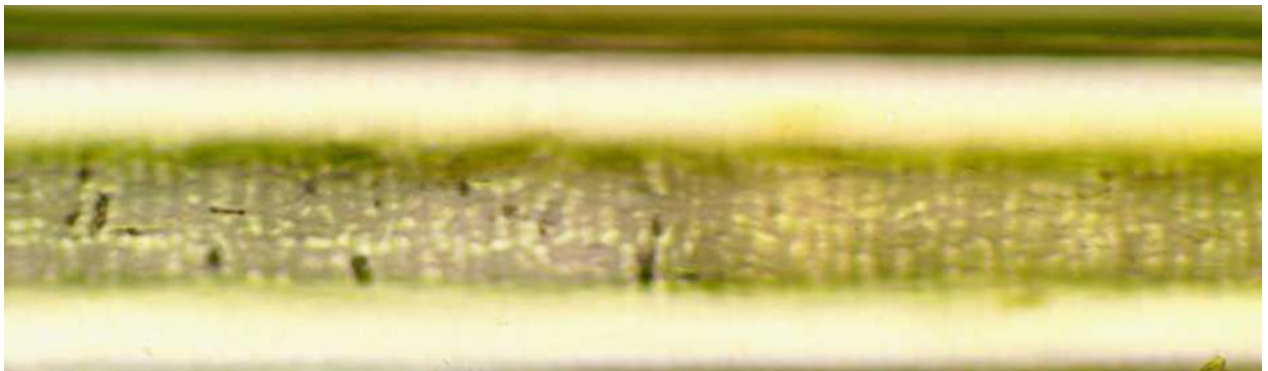
Order: RODENTIA

Family: SCIURIDAE

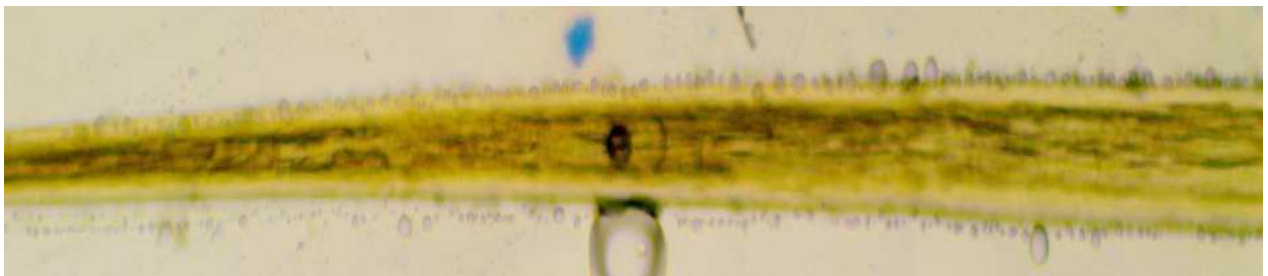
1. Malabar Giant Squirrel (*Ratufa indica*)



Proximal

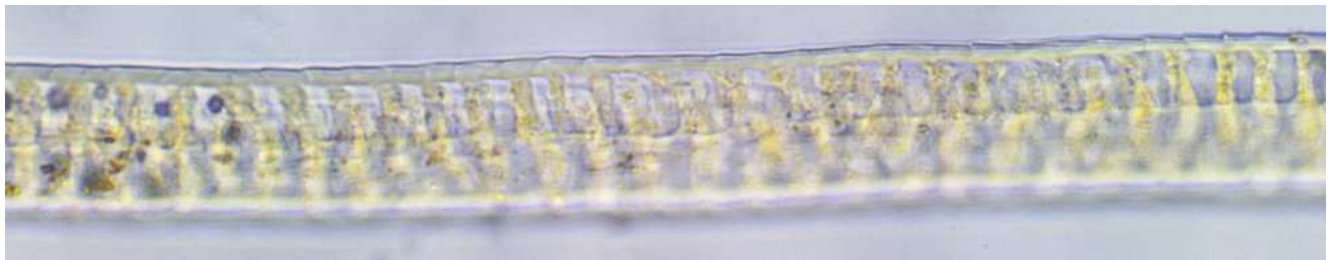
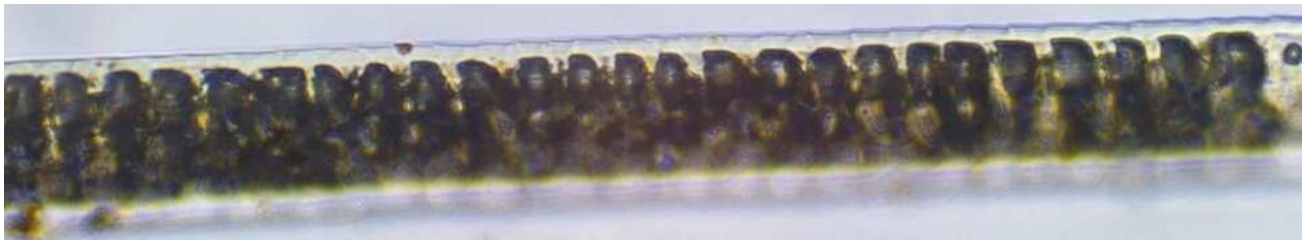


Medial

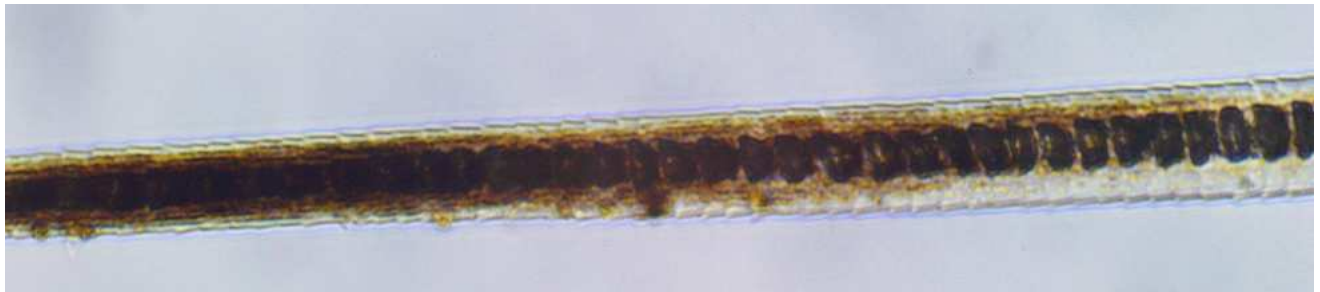


Distal

2. Indian palm squirrel (*Funambulus palmarum*)



Medial

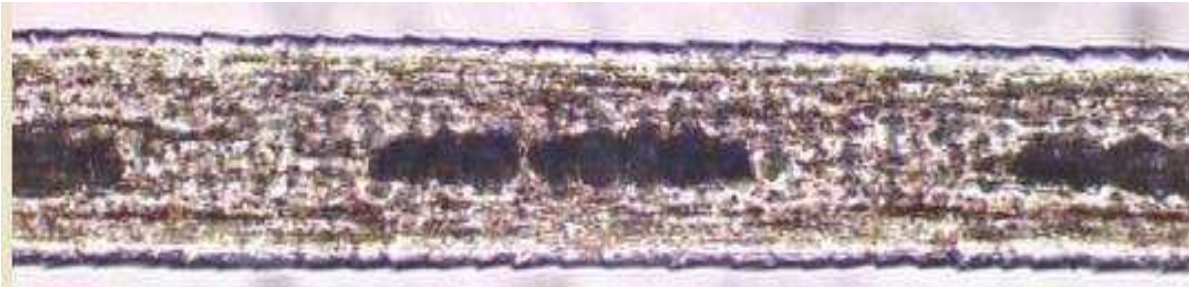


Distal

Order: PRIMATES

Family: CERCOPITHECIDAE

1. Nilgiri Langur (*Semnopithecus johnii*)



Proximal

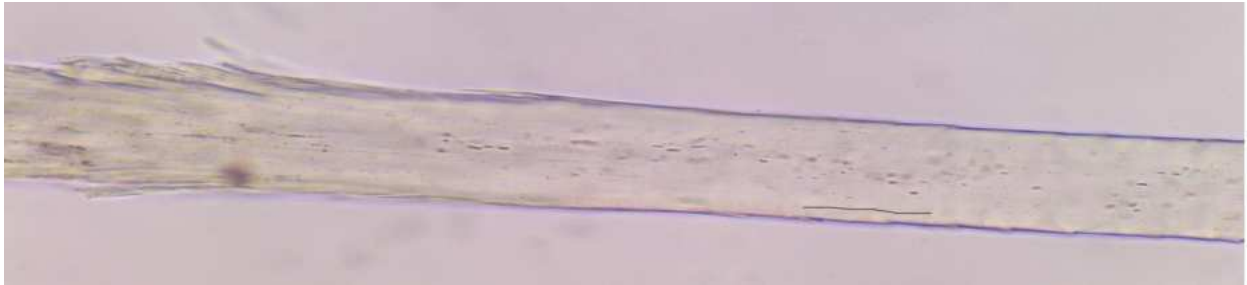


Medial

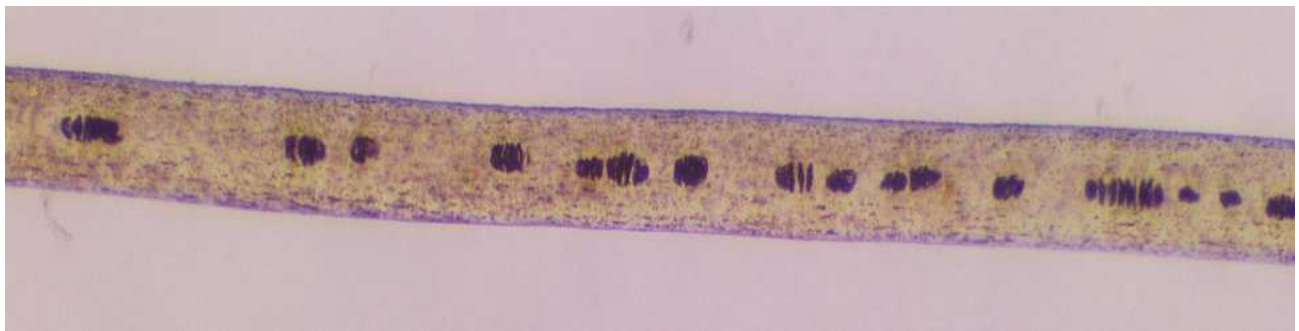


Distal

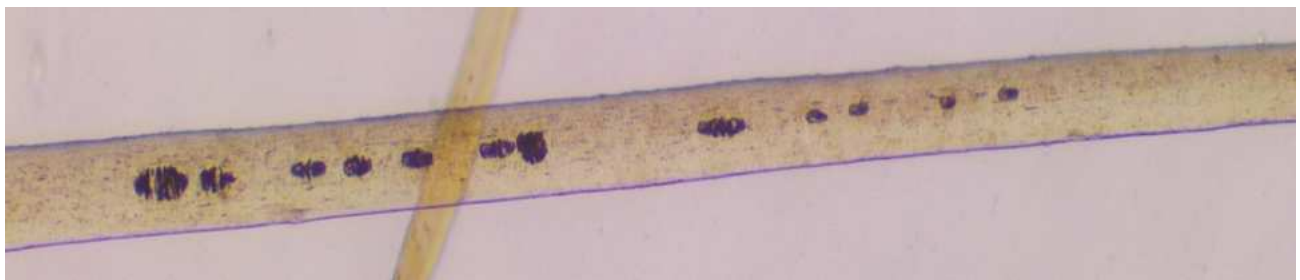
2. Bonnet macaque (*Macaca radiata*)



Proximal



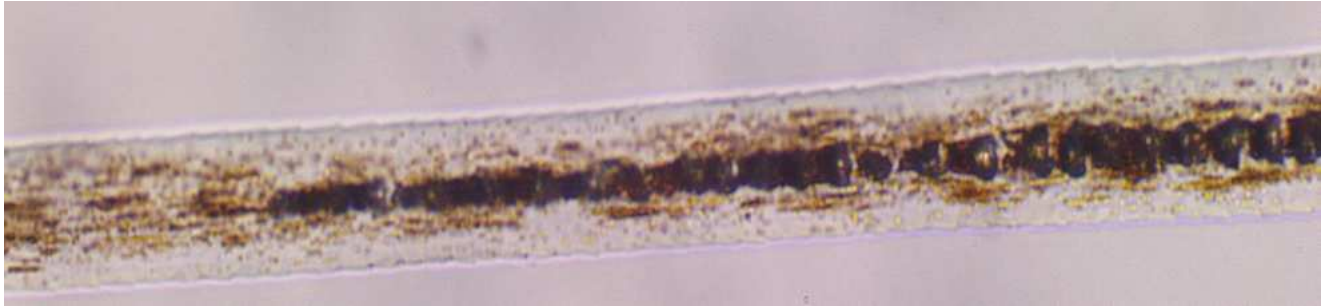
Medial



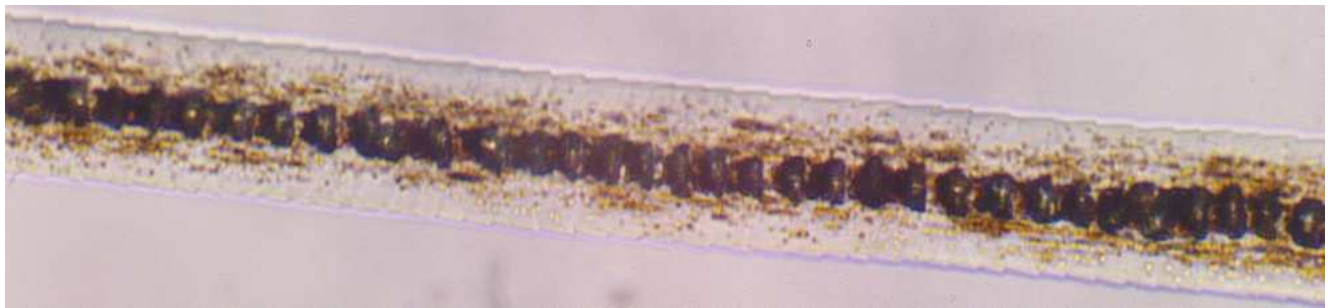
Distal

Family: LORISIDAE

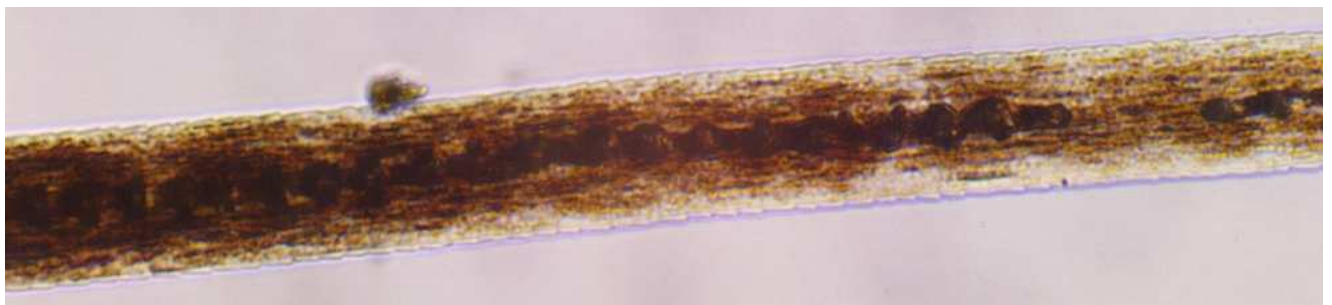
1. Slender loris (*Loris*)



Proximal



Medial



Distal

CONCLUSION

The trico-taxonomic reference database of guard hairs has emerged as a crucial tool in combating wildlife crime and supporting species identification. The current study attempted to compile a comprehensive collection of guard hair samples from 28 species, including wild and domestic species from various families such as Cervidae, Bovidae, Felidae, Canidae, Hyaenidae, Ursidae, Herpestidae, Viverridae, Sciuridae, Cercopithecidae and Lorisidae and created a reference database to identify species from illicit wildlife articles received at AIWC, Morphometry Lab. The inclusion of domestic species in this study was prompted by the prevalence of wildlife cases received at AIWC, which often involved fake wildlife products from domestic animals, such as domestic cats and dog skins.

This study mainly focused on the medullary patterns of guard hairs of selected species and created a digital database as a reference. By providing a reliable means of identifying species involved in wildlife crimes, such as illegal trade and poaching, the database has empowered law enforcement agencies and conservation organizations to protect endangered wildlife. Identifying the species becomes challenging when seized wildlife articles lack guard hairs, as the medullary patterns can vary across different body regions. The utilization of tricotaxonomic techniques, coupled with advancements in molecular techniques, has significantly enhanced the accuracy and efficiency of species identification from guard hair samples. This reference database's continuous expansion and improvement will continue to play a pivotal role in the fight against wildlife crime.

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