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Bipin S. Phal Desai, Avelyno H. D'Costa & S.K. Shyama

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A comparative analysis of hair morphology of wild and domestic ungulate prey species of Leopard *Panthera pardus fusca* (Mammalia: Carnivora: Felidae) from Goa, India

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PLATINUM OPEN ACCESS

Bipin S. Phal Desai [1], **Avelyno H. D'Costa** [2] & **S.K. Shyama** [3]

[1] Goa Forest Department, Government of Goa, Goa 403001, India.
[2] Department of Zoology, Dhempe College of Arts & Science, Miramar, Goa 403001, India.
[3] Department of Zoology, Goa University, Goa 403206, India.
[1] phaldesaibipin00@gmail.com, [2] avelynodc@gmail.com, [3] skshyama@gmail.com (corresponding author)

Abstract: Guard hairs were collected from four live domesticated ungulate species and shed guard hairs of six wild ungulate species from zoo captive animals from five individuals each. Photographic reference was prepared showing analytic features of hair characteristics. Study results were analysed and cuticle and medulla patterns were identified along with pigmentation features from the literature available for wild and domestic ungulates from India and abroad. Clear and easily distinguishable morphological characters of hair medulla and cuticle were used in the present study. Scat analysis of big cats used in this study is easy, speedy and efficient which can be used in routine investigations related to wildlife, crime forensics as well as human animal conflicts by studying carnivore feeding habits. In a majority of the animal species, the distal part of the hair showed maximum variation from the rest of the hair portions. The cuticle scales were imbricate in all tested animals. Scale position in almost all the tested animals was transversal except in goat (proximal part and medial part) and mouse deer (Distal part). Majority of the species showed smooth margins at proximal and medial part. Whereas the distal part scale margin was crenate and rippled in appearance the proximal part and medial part of hair of the majority of sampled animals showed a regular wave -type scale pattern whereas the distal part of hair showed irregular wave-type scale pattern in dominance. The composition of the medulla was multicellular in all the sampled deer species. Only the cow calf's hair medulla was unicellular and uniseriate in appearance. A comparison of the hair of the domestic pig with that of the wild boar and gaur hair with that of cow calf and buffalo calf hair was made for the first time in the present study. Similarly goat hair morphology can also be differentiated from other cervids in this study. Medulla and cuticle characters in combination with each other can help differentiate wild ungulate species from the domestic ones since these wild ungulate species are frequently involved in hunting crime investigations. Therefore, the photographic reference presented in this study can be used in wildlife forensic science as well as predator diet analysis as an appropriate reference for prey species identification.

Keywords: Carnivore prey identification, hair pigmentation, medulla, ungulate.

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Author details: Mr. Bipin S. Phal Desai is a Range Forest Officer of the Goa Forest Department, Government of Goa. He is also pursuing his PhD in the Department of Zoology, Goa University. His research interests include wild cat biology and man-wildlife interaction studies. Dr. Avelyno H. D'Costa is an Assistant Professor in the Department of Zoology, Dhempe College of Arts & Science which is affiliated to Goa University. He is an ecotoxicologist and is interested in research related to genetics, ecology and conservation. Dr. S.K. Shyama is a Professor (Retd.) and ex. head of the Department of Zoology, Goa University. His area of expertise is genotoxicology and interests include ecotoxicology and wildlife biology. He has guided several PhD students in toxicology and wildlife and conservation.

Author contribution: BSPD collected the hair specimens, analyzed them and wrote the manuscript. AHD'C assisted with the analysis of the specimens by providing protocols and revision of the manuscript. SKS supervised the study and helped in the revision of the manuscript.

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INTRODUCTION

The presence of hair on the body is one of the important characteristics of class Mammalia. Mammalian hair is structurally unique in different mammal species which is a result of adaptation and evolution in response to the environment. This characteristic is widely used to identify animals at species level (Menike et al. 2012). Identification of mammals using hair morphology has also been used in diet analysis of predator species using scat analysis. In southern India several studies have documented feeding habits of Leopard *Panthera pardus fusca* during the last two decades (Ramakrishnan et al. 1999; Athreya et al. 2014). All these studies suggest that wild as well as domestic animals form major components of leopard diet especially in areas of human dominated landscapes. Predation of livestock is one of the most important human- leopard conflict scenarios in India and is the primary cause for leopard persecution. This in turn can lead to major threats to leopard conservation goals. Therefore, understanding the leopard diet can play an important role for its conservation in human dominated landscapes of India.

Photographic references of the hair structures of wild and domestic ungulate prey species of leopard has effective practical applications in understanding leopard feeding habits. Hair morphology is an important tool that can be used to identify animal species (ENFSI 2015); however, identification of species from hair structure is not a straightforward process and practitioners need to develop expertise to identify and to be able to distinguish hair characteristics especially in closely related species. Thus, the understanding and comparison of hair structures is important to help distinguish one species from another.

Mammals have four types of body hair, of which guard hair are the most important in differentiation between various animal species (Tridico 2005; Knecht 2012). A typical mammal hair consists of a hair root and hair shaft. The root is embedded in the epidermis and the shaft is the part which extends above the epidermis as a cylindrical structure. The hair shaft is made up of three distinct morphological layers, i.e., medulla (central layer), cortex (intermediate layer) and cuticle (outer layer) (Deedrick & Koch 2004a; Debelica & Thies 2009; Knecht 2012). The medulla, which is the innermost layer of the hair shaft, is a honeycomb-like keratinous structure which can be continuous, discontinuous or fragmented with vacuoles in between (Deedrick & Koch 2004a). The cortex contains keratin fibers and pigment granules which are responsible for the coloration of the hair. The cuticle, the outermost layer, consists of overlapping keratin scales (Deedrick & Koch 2004a). Two main patterns of cuticle scales are: (i) imbricate, and (ii) coronal. The distance between every two successive scale margins can be close, intermediate or wide, depending on the animal species (Debelica & Thies 2009). The pattern of the cuticle scales, the type and the diameter of the medulla and/or the characteristics of pigmentation can be used for animal species identification (Brunner & Coman 1974).

The presence of high content of cysteine-containing keratin and dead keratinocytes delays postmortem changes and chemical decomposition in mammal hair (Harkey 1993; Knecht 2012). This property of mammalian hair has helped in carrying out forensics investigations.

Although several studies have reported hair structure identification for wild and domesticated mammal hairs (Dharaiya et al. 2012), very few of them deal with ungulates. Further, such studies are also required to create database for different geographical areas. In the present study we provide a comprehensive comparative database about wild and domestic ungulate prey species of the leopard in Goa.

MATERIALS AND METHOD

Sample collection and preparation

For preparing the photographic reference of cuticle and medulla characters, shed hairs were collected from six wild ungulate species from the night shelter enclosures of the Bondla Zoo located at Bondla Wildlife Sanctuary, Ponda Goa and from four live domestic ungulate species from five individuals each from Goa during January 2016 to December 2016. Guard hairs were separated from other hairs based on their properties before analysis as given by De Marinis & Asprea (2006). Hairs were then immersed in 70% ethanol solution for 5–10 minutes to remove any debris and non-hairy sticky materials. The hair strands were then dried and cleaned on a blotting paper. Each hair length was divided into three parts: proximal (base), medial (middle), distal (apical). Longer hairs were cut into these three respective parts for comparative analysis of each strand at different lengths whereas shorter hairs were used as a whole.

Examination of hair cuticle scale pattern

Cuticle scales of individual hairs were analyzed using the methodology of Mukherjee et al. (1994b). A 20% gelatin solution was prepared by boiling the gelatin powder in distilled water. The solution was cooled and

two drops of Leishman's stain was added to the solution to obtain a pale blue colour. One to two drops of this solution was used to prepare a smooth film on a clean dry glass slide and immediately cleaned hair shafts were superficially placed on the film. The glass slide was then covered with a glass petri plate and left for 15–20 minutes at room temperature. The hair shafts were then slowly separated from the gelatin film using forceps such that an imprint of the scales was formed on the glass slide. These imprints were observed and photographed under (400X) magnification using a light microscope (Olympus microscope BX 53).

Examination of hair medulla and pigmentation

For hair medullary pattern and pigment analysis, hairs were immersed in xylene for 24 hours. The hairs were then dried and then mounted on glass slides using a drop of DPX and then covered with a coverslip (Mukherjee et al. 1994b). The slides were allowed to dry for an hour and then observed and photographed under a light microscope at 400X magnification.

RESULTS

Hair structures from different species were identified and compared for their cuticle and medulla patterns along with pigmentation features from the literature available for wild and domestic ungulates from India and abroad (Mukherjee et al. 1994; Dharaiya & Soni 2012; Joshi et al. 2012; Ghallab et al. 2018).

Comparative analysis of cuticle

Cuticle scale position, scale margin, scale margin distance and scale patterns were analyzed from guard hairs collected from six wild and four domestic animal hairs at different hair lengths (Table 1; Images 1–10). Analysis of hair at three different levels revealed considerable variations in scale margin, scale margin distance and scale patterns in all the sampled animals whereas scale position showed no major variations at different hair lengths. In a majority of the animal species, the distal part of the hair showed maximum variation from the rest of the hair portions. The cuticle scales were imbricate in all the species studied. Scale position in almost all the studied animals was transversal except in Goat *Capra aegagrus hircus* (proximal part and medial part) and Mouse Deer *Moschiola indica* (distal part).

Based on scale margin type, pattern and distance, the species of animals could be clearly differentiated.

Table 1. Comparative analysis of the Cuticle characteristics of wild and domestic ungulate species from Goa.

Species	Proximal part of hair				Medial part of hair				Distal part of hair			
	Scale position	Scale margin	Scale margin distance	Scale pattern	Scale position	Scale margin	Scale margin distance	Scale pattern	Scale position	Scale margin	Scale margin distance	Scale pattern
Cow calf	Transversal	Smooth	Distant	Regular wave	Transversal	Smooth	Near	Regular and irregular wave	Transversal	crenate	Near	Single chevron
Buffalo calf	Transversal	Smooth	Near	Regular wave	Transversal	Rippled	close	Double chevron	Transversal	Rippled, crenate	Near	Irregular wave
Pig	Transversal	Crenate	Near	Irregular wave	Transversal	Rippled, crenate	Close	Double Chevron	Transversal	Crenate	Close	Irregular wave
Goat	Intermediate	Crenate	Distant	Irregular wave	Intermediate	Smooth	Distant	Irregular mosaic	Transversal	Rippled	Near	Single chevron
Spotted deer	Transversal	Smooth	Near	Regular wave	Transversal	Smooth	Near	Regular wave	Transversal	Smooth, crenate	Distant	Regular, irregular wave
Wild boar	Transversal	Rippled	Close	Irregular wave	Transversal	Rippled	Near	Irregular wave	Transversal	Rippled	Near	Irregular wave
Sambar deer	Transversal	Smooth	Distant	Regular wave	Transversal	Smooth, Rippled	close	Regular wave	Transversal	Rippled	Near	Irregular wave
Mouse deer	Transversal	Smooth	Distant	Regular wave	Transversal	Smooth	Distant	Regular wave	Intermediate	smooth	Distant	Irregular mosaic
Barking deer	Transversal	Smooth	Near	Regular wave	Transversal	Smooth	Near	Regular wave	Transversal	Smooth, crenate	Distant	Regular wave
Gaur	Transversal	Rippled	Near	Irregular wave	Transversal	Crenate	Close	Single Chevron	Transversal	Crenate	Near	Single Chevron

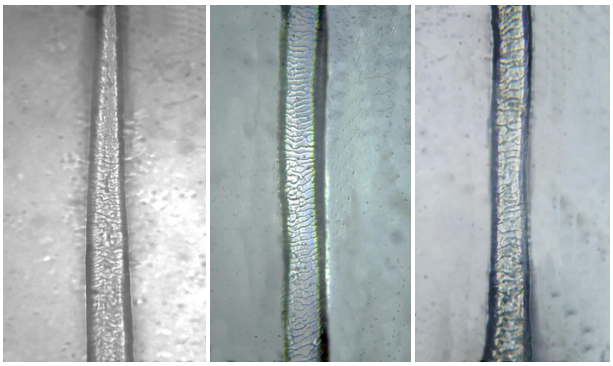

Image 1. *Bos taurus* (calf) medulla; distal, medial and proximal part of the hair (400 X).

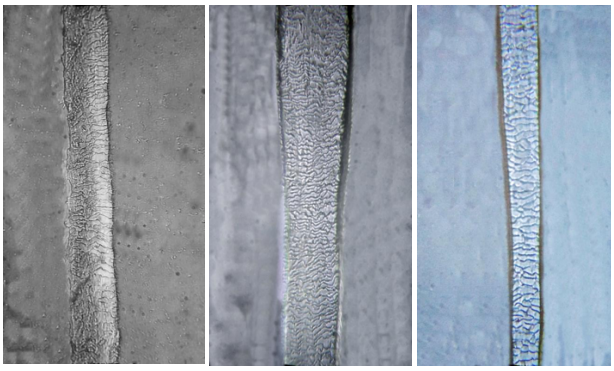

Image 2. *Bubalus bubalis* (calf) medulla; distal, medial and proximal part of the hair (400X).

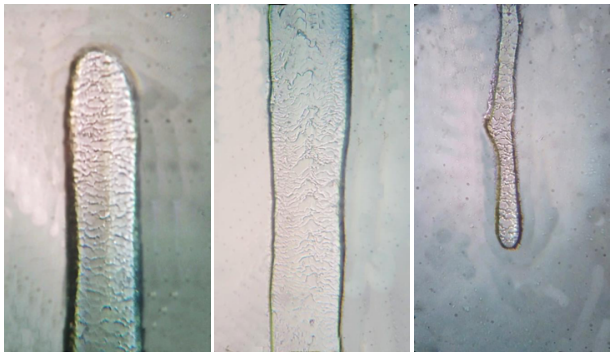

Image 3. *Sus scrofa domesticus* (adult) medulla; distal, medial and proximal part of the hair (400 X).

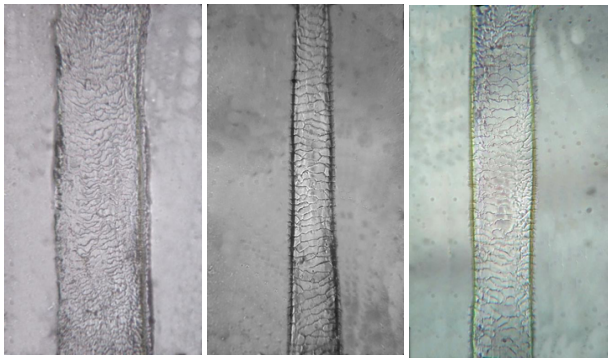

Image 4. *Capra aegagrus hircus* (adult) medulla; distal, medial and proximal part of the hair (400 X).

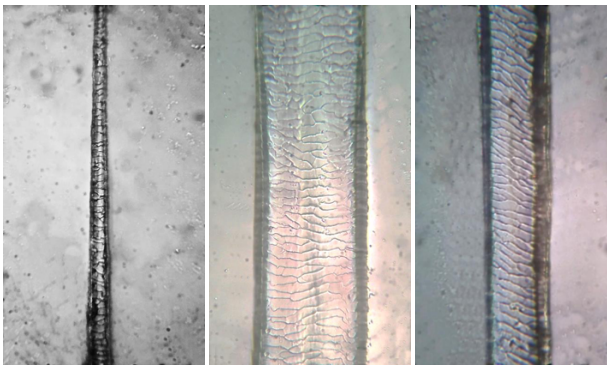

Image 5. *Axis axis* (adult) medulla; distal, medial and proximal part of the hair (400 X).

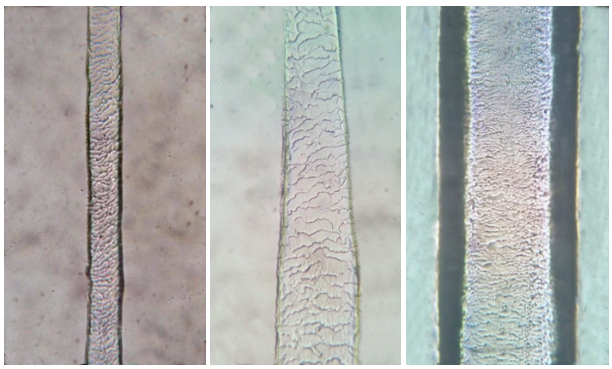

Image 6. *Sus scrofa* (adult) medulla; distal, medial and proximal part of the hair (400 X).

A majority of the species showed smooth margins at proximal and medial part, whereas the distal scale margin was crenate and rippled in appearance. The proximal and medial parts of the hair of a majority of the sampled animals showed regular wave type scale pattern whereas the distal part of hair showed irregular wave type of scale pattern. A single chevron type of scale pattern was seen in the medial part and distal part of gaur hair (*Bos gaurus*) and distal part of cow calf (*Bos taurus)* and goat (*Capra aegagrus hircus*). Buffalo calf (*Bubalus bubalis*) and domestic pig (*Sus scrofa domesticus*) both showed double chevron type of scale pattern in the medial part.

Therefore, the hair of Wild Boar *Sus scrofa* and domestic pig can be very well differentiated from the analysis of cuticle characteristics. Goat hair also can be

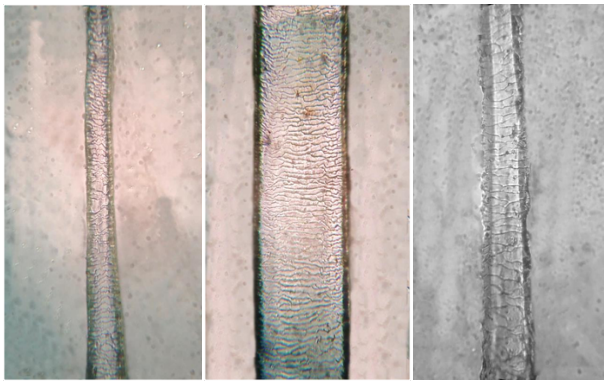

Image 7. *Rusa unicolor* (adult) medulla; distal, medial and proximal part of the hair (400 X).

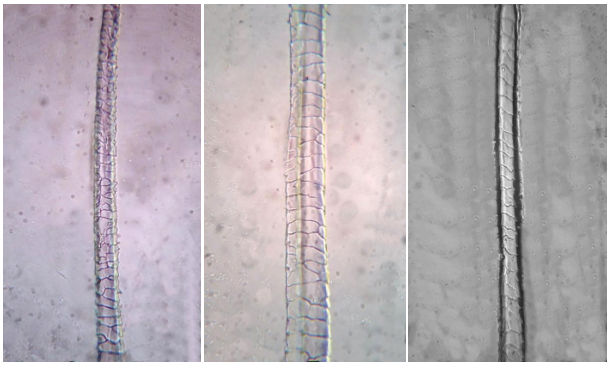

Image 8. *Moschiola indica* (adult) medulla; distal, medial and proximal part of the hair (400 X).

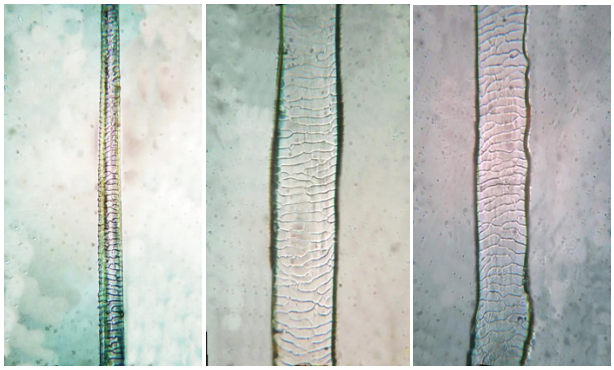

Image 9. *Muntiacus muntjac* (adult) medulla; distal, medial and proximal part of the hair (400 X).

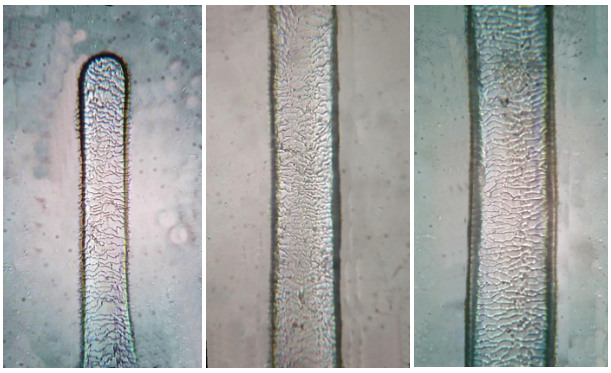

Image 10. *Bos gaurus* (adult) medulla; distal, medial and proximal part of the hair (400 X).

differentiated from other sampled deer species using cuticle pattern analysis. In case of the sampled deer species such as Sambar Deer *Rusa unicolor,* Spotted Deer *Axis axis* and Barking Deer *Muntiacus muntjac,* including Mouse Deer *Moschiola indica*—all exhibited very similar cuticular scale patterns.

Comparative analysis of hair medulla morphology and pigmentation

In addition to cuticle scale characteristics, hair medulla morphology, composition, structure, pattern and margins as well as the pigmentation has been used to identify and compare the sampled wild and domestic ungulate prey species of leopard. The composition of medulla was multicellular in all the sampled deer species. Only cow calf hair medulla was unicellular and uniseriate in appearance. Medulla cell type could not be differentiated in buffalo calf, domestic pig and goat. Amorphous type medulla structure was observed in buffalo calf, domestic pig, goat, Spotted Deer, Wild Boar and Gaur, whereas filled lattice type medulla was observed in Sambar Deer, Mouse Deer and Barking Deer. Vacuolated and fragmented medulla was observed only in case of spotted deer. A majority of the sampled ungulate hairs showed irregular type margin type except in case of Sambar Deer, Mouse Deer, Barking Deer and Gaur. Pigmentation was not observed in hair cortex of cow calf, Spotted Deer and Mouse Deer (Table 2; Images 11–12

Morphometric analysis of ungulate hair

Total hair diameter as well as medullary thickness was measured using binocular microscope with camera attachment using ProgRes software. Total diameter of hair was maximum at the proximal part compared to the rest of the portion of the hair in all the sampled ungulate species, whereas thickness of medulla was maximum at the medial part in a majority of the species with the exception of cow calf, buffalo calf and goat. Medulla did not extend to the distal end in case of wild boar and to the proximal end in case of gaur whereas in case of cow calf and spotted deer medulla was absent at both the

Table 2. A comparative analysis of medulla and pigmentation features of wild and domestic ungulate species from Goa.

Comparative analysis of hair medulla morphology and pigmentation in different animal species					
Animal species	**Composition**	**Structure**	**Pattern**	**Margin type**	**Pigmentation**
Cow calf	Unicellular	Uniseriate	Continuous	Irregular	No pigments
Buffalo calf	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Domestic pig	Cells not visible	Amorphous	Continuous	Irregular	Streak like pigments
Goat	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Spotted Deer	Multicellular	Amorphous, vacuolated	Fragmented	Irregular	No pigments
Wild Boar	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Sambar	Multicellular	Filled lattice	Continuous	scalloped	Streak pigments
Mouse Deer	Multicellular	Filled lattice	Continuous	Scalloped	No pigments
Barking Deer	Multicellular	Filled lattice	Continuous	Scalloped	Streak like pigments
Gaur	Multicellular	Amorphous	continuous	Scalloped	Streak like pigments

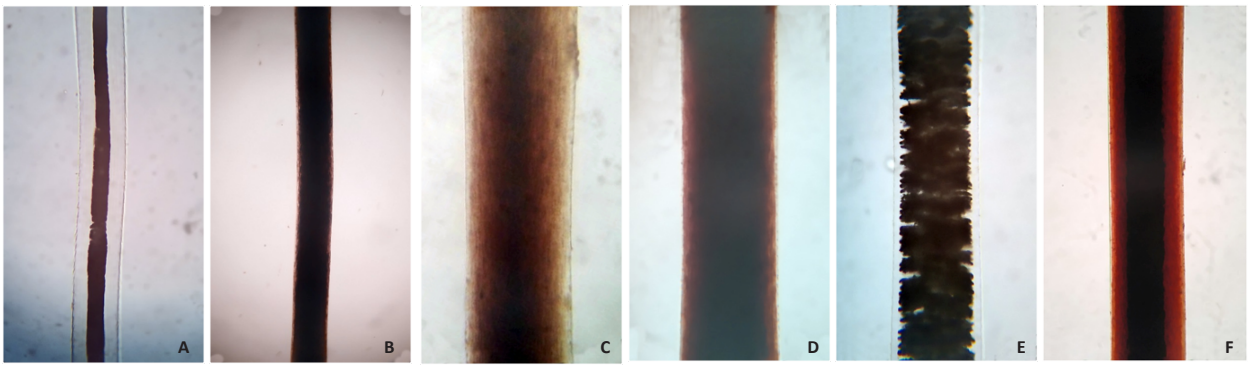

Image 11. Morphology of the hair medulla in different ungulate prey species of Leopard.
A—*Bos taurus* (calf) | B—*Bubalus bubalis* (calf) | C—*Sus scrofa domesticus* | D—*Capra aegagrus hircus* | E—*Axis axis* | F—*Sus scrofa* at medial part of the hair (400x).

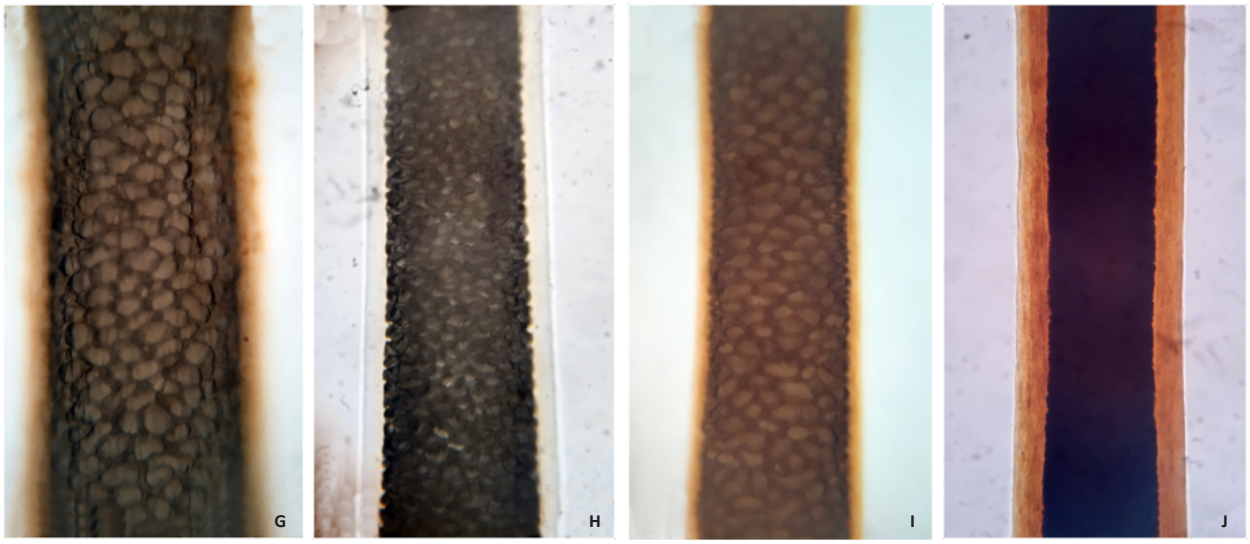

Image 12. Morphology of the hair medulla in different ungulate prey species of Leopard.
G—*Rusa unicolor* | H—*Moschiola indica* | I—*Muntiacus muntjac* | J—*Bos gaurus* bison, at medial part of the hair (400x).

Table 3. Morphometric analysis of total hair and medulla of wild and domestic ungulate species from Goa.

Morphometric analysis of ungulate animal Hair												
	Total hair thickness (µm)					Medulla thickness (µm)						
	Proximal part	SD	Medial part	SD	Distal part	Proximal part	SD	Medial part	SD	Distal part	SD	Remarks
Cow calf	70.17	±16.88	55.76	±2.78	31.06	50.20	±9.37	30.42	±2.50	15.14	±2.57	Medulla absent in distal end and proximal end
Buffalo calf	266.88	±18.96	181.19	±2.57	37.21	151.89	±8.29	149.68	±2.10	26.94	±8.29	-
Domestic pig	120.08	±3.93	185.86	±16.90	38.26	57.79	±16.90	163.85	±13.05	0.00	±4.07	Medulla absent at distal part
Goat	153.75	±4.57	137.97	±2.35	34.03	111.85	±12.43	106.70	±6.82	14.78	±1.27	±4.94 -
Spotted Deer	95.90	±19.73	134.30	±2.63	17.23	53.77	±4.99	110.03	±32.48	12.39	±4.03	±4.48 Medulla absent at proximal and distal end
Wild Boar	363.80	±4.43	372.30	±17.05	41.16	278.08	±5.33	313.18	±5.14	29.99	±10.33	±10.28 Medulla absent at distal end
Sambar	240.57	±6.31	228.82	±1.55	20.33	169.28	±4.75	193.50	±37.04	9.49	±2.88	±1.56 -
Mouse Deer	135.77	±5.19	159.04	±2.64	13.94	97.03	±5.79	124.26	±31.79	9.19	±3.22	±2.99 -
Barking Deer	118.15	±61.11	180.22	±9.38	23.21	129.02	±5.09	166.23	±33.99	25.12	±2.39	±5.70 -
Gaur	120.68	±23.91	162.74	±2.00	46.15	46.77	±14.55	104.22	±21.22	23.86	±7.38	±7.77 Medulla absent at proximal end

distal far and proximal end. Only domestic pig hair was devoid of medulla completely in the distal part (Table 3).

DISCUSSION

Our results showing hair morphological characters of wild and domestic ungulates were similar to the available literature (Brunner & Coman 1974; Knecht 2012), but with a few exceptions. Our medulla analysis results in the case of Spotted Deer showed fragmented amorphous medulla. During the study period since there was no reports of leopard attacks on adult cow and buffalo in Goa, only the hairs of the young one of these domestic ungulates were used in the study. Cuticle as well as medulla characters of adult and young individuals of these species did not show any variation when compared with the literature (Ghallab et al. 2018). Hair characteristics of domestic pig have been compared with that of wild boar as well as gaur with that of cow calf and buffalo calf for the first time in the present study. Similarly goat hair morphology can also help differentiate it from other cervids in the present study. This information can be put to best use when identifying carnivore species in human-carnivore conflict situations where they attack livestock and other domesticated animals. Unlike hair cuticle character, medulla of all the studied species did not show variation along the hair length. Cuticle of hair at different lengths in all the studied species showed variation in scale position, pattern, spacing and margin. This is helpful in microscopic analysis of hair fragments which are usually the case when studying the diet of predators through scat analysis. Medulla and cuticle characters in combination can help differentiate wild ungulate species from the domestic ones since these wild ungulate species are mostly involved in crime investigations such as illegal hunting.

Characters of hair when used in isolation may not be of much value in species identification as they show high variation; however, when these characters are analyzed in combination these they may provide significant information for identification of species (Brunner & Coman 1974; Teerink, 1991). Several studies are available on the combined analysis of hair characters for species identification. Joshi et al. (2012) have done a comparative hair study only on the basis of the hair colour and medulla structures in Mouse Deer, Spotted Deer, Barking Deer and Sambar Deer. Dharaiya & Soni (2012) have documented characters of transverse section of Spotted Deer, Sambar Deer, buffalo, cow,

and goat, but have not explored cuticle and medulla characters. The remaining studies on ungulate hair are mainly based on the predator's scat-hair (diet) analysis.

Analysis and examination of animal hair plays a vital role in wildlife forensics investigation. Analysis of hair collected from the crime scene can provide essential information about the species involved (Soni et al. 2003). Hair analysis has even proved beneficial in tracing chemical poisoning cases in animals (Harkey 1993; Krumbiegel et al. 2014) and hence the same can be utilized in wildlife forensic investigation where the carcass has decomposed and tissues cannot be collected.

This photographic reference in the present study will help to identify the ungulate prey species of leopard and other wild carnivores from the scat analysis from such localities. Therefore, the photographic reference presented in this study can be used in wildlife forensic science as well as predator diet analysis, as an appropriate reference for prey species identification.

In conclusion, the present study provides a first-step towards preparation of local photo reference database of hair of wild and domesticated ungulate species which can be used in forensic investigations as well as to study human carnivore conflict scenarios arising out of livestock depredation. Further studies using more advanced techniques such as electron microscopy can be used to prepare a complete local atlas for all wild and domestic animal species' identification.

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