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Cover: Common Silverline *Spindasis vulcanus vulcanus* in poster colours adapted from photograph by Kalpesh Tayade. © Pooja R. Patil.

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SHORT COMMUNICATION

Comparative study of morphology and keratin levels in hair from deer and goat

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Abstract: Hair is a defining character of mammals. In the present study, the hair samples of Chital *Axis axis*, Sambar Deer *Rusa unicolor*, and goat *Capra hircus* were collected from the back, neck, abdomen and tail regions of carcasses brought to the forensic laboratory for necropsy examinations. Cross-sections of hair, cuticle scale, and medullary patterns were analyzed to establish indices for species identification. Keratin levels were also analyzed by protein electrophoresis (SDS-PAGE). We determined that both microscopic and SDS-PAGE analysis of guard hair is useful for identifying species, particularly in forensic applications.

Keywords: *Axis axis, Capra hircus,* domestic animals, guard hair, protein electrophoresis, *Rusa unicolor,* SDS-PAGE, wild herbivores.

Abbreviations: kDa–kilo Dalton | MALDI-TOF–matrix-assisted laser desorption/ionization–time of flight | PMF–peptide mass fingerprinting | SD–standard deviation | SDS-PAGE–sodium dodecyl sulphate

No other animal possesses hair except mammals, and these hairs have the capability to resist putrefaction and may keep unpreserved for a long time. Hair being the most common biological material found at the scene of a crime, plays a crucial role in criminal investigations related to wildlife, taxonomy, investigative dermatology, pathology, and other fields of forensic science (Sahajpal et al. 2009; Bahuguna et al. 2010). Guard hairs are usually procured for wildlife forensics, particularly species identification of wild animals (Tridico 2005; Knecht 2012). The hair has three internal parts: cuticle, cortex, and medulla, covered with a thin coating of derived proteins and tilted scales. Hair coloring is based on the presence of keratin protein in the hair cortex, scales of keratin overlapped by the cuticle layer (Deedrick & Koch 2004). The high content of cysteine and dead keratinocytes helps to protect the hairs from putrefaction and keep its chemical composition intact (Knecht 2012). Studies on human hair keratin show that it constitutes approximately 80% of the total mass of the hair and consists primarily of keratins having 40–65 kDa (molecular weight) and 6–30 kDa keratin-associated with proteins (KAPs) and may be isolated using SDS-electrophoresis (Gillespie 1990; Langbein et al. 2001; Nakamura et al. 2002). There are two subfamilies of

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keratin protein, type I (acidic; 40–50 kDa) and type II (neutral/basic; 55–65 kDa). These keratin proteins are also classified into high-sulfur proteins, ultra-high-sulfur proteins and high-glycine / tyrosine proteins based on their amino acid content (Fuji et al. 2013). Meager information is only available about the comparative morphology of guard hairs of domestic goats and deer families. Thus in many instances, poachers get the advantage of insufficient evidence of poaching for uncertainty regarding the seized hair, whether it belongs to goat or deer family. However, since illustrative research on morphological aspects of ungulates and carnivores has been done at the Wildlife Institute of India, the present study focused on the analysis of the hair of wild herbivores to generate hair index to identify and differentiate between the hair of domestic and wild animals for forensic uses. This will be helpful in the prosecution and conviction of poachers to overcome the wildlife crime.

Materials and Method

Hair samples are regularly brought to the School of wildlife forensic and health, NDVSU, Jabalpur, to identify whether the seized hair belongs to a wild animal or not. In the present study, hair samples of Chital *Axis axis*, Sambar Deer *Rusa unicolor*, and goat *Capra hircus* were collected and processed for identification and differentiation for forensic uses. Histological study of the hair cuticle, scale pattern, type of medulla, medullary index, and cross-section morphology was performed in the present study (Table 1) following the standard protocols of Trimori et al. (2018). The hairs of each animal's dorsal and ventral regions were collected in a sterilized container, washed separately using 95% ethanol, and dried before further analysis. Hair samples were examined under a light microscope after whole-mount and scale cast preparation.

Microscopic examination of hair

The cuticle scale pattern was examined using the nail polish method described by Brunner & Coman (1974). The nail polish method is very convincing and quick. For cuticle scale examination, nail polish was spread on a clear glass slide and hair was placed on it and kept until dried. Then the hair was removed, and the impression was examined under a compound microscope at 40x magnification. The cuticle scale pattern was also examined using the gelatin casting method described by Cornally & Lawton (2016). For this, 20% gelatin was mixed in boiling water, and a thin gelatin film was spread on a clean glass slide. The hair shafts were superficially placed in the gelatin film and left at room temperature overnight. The hairs were subsequently removed, leaving the scale imprint on the gelatin cast, which was examined under the microscope. Further, the same cleaned and washed hairs were kept in xylene for 72 h before examination of the medullary pattern under the compound microscope. The camera lucida drawings were made to compare the cuticular and medullary patterns of deer and goats.

Extraction of keratin

Guard hair of Chital, Sambar, and goat were washed with ethanol and a mixture of chloroform-methanol (2:1, v/v) for 24 h to remove lipid molecules on the surface of the hair. The washed hair (20 mg), dispensed in a solution (5 ml) containing 25 mM tris–HCl, pH 8.5, 2.6M thiourea, 5M urea and 5% 2-mercaptoethanol (2-ME), was kept at 50°C for 48 h in a hot air oven. The mixture was filtered using a muslin cloth and centrifuged at 15000 rpm for 20 min at room temperature. The light to dark brownish supernatant was further processed following the protocols of Nakamura et al. (2002). The Protein amounts were estimated using Bradford colorimetric method, and further SDS-PAGE electrophoresis process was done at a refrigerated temperature of 40°C to protect the electrophoresis chamber from excess heat. To differentiate the hair matrix protein (HMP) area by the position and intensity of the polypeptide band, the isolated proteins gel was stained with 0.1% Coomassie brilliant blue R-250 (dissolved in 10% acetic acid and 40% ethanol) for 24 h, then de-stained by adding acetic acid, methanol, and distilled water (1:3:6 ratio) following the method of Folin et al. (1996).

Results

The cuticle pattern of the wild herbivores Chital and Sambar are smooth and irregular, whereas, in the goat, it is rough with a marginal gap within the cuticle and medulla. The margin and distance between the cuticular pattern and medullary pattern of the hair from the various regions, including proximal and distal regions, were also examined (Table 1), and it was seen that the cuticle scale pattern varied from species to species. While the medullary pattern of both the domestic and wild herbivores looks similar, the goat's hair medulla was a more compact mass than that of chital and sambar (Image 1, Table 2). The keratin extracted through SDS-PAGE revealed no remarkable differences between protein bands (40–65 kDa) of wild and domestic herbivores (Image 2).

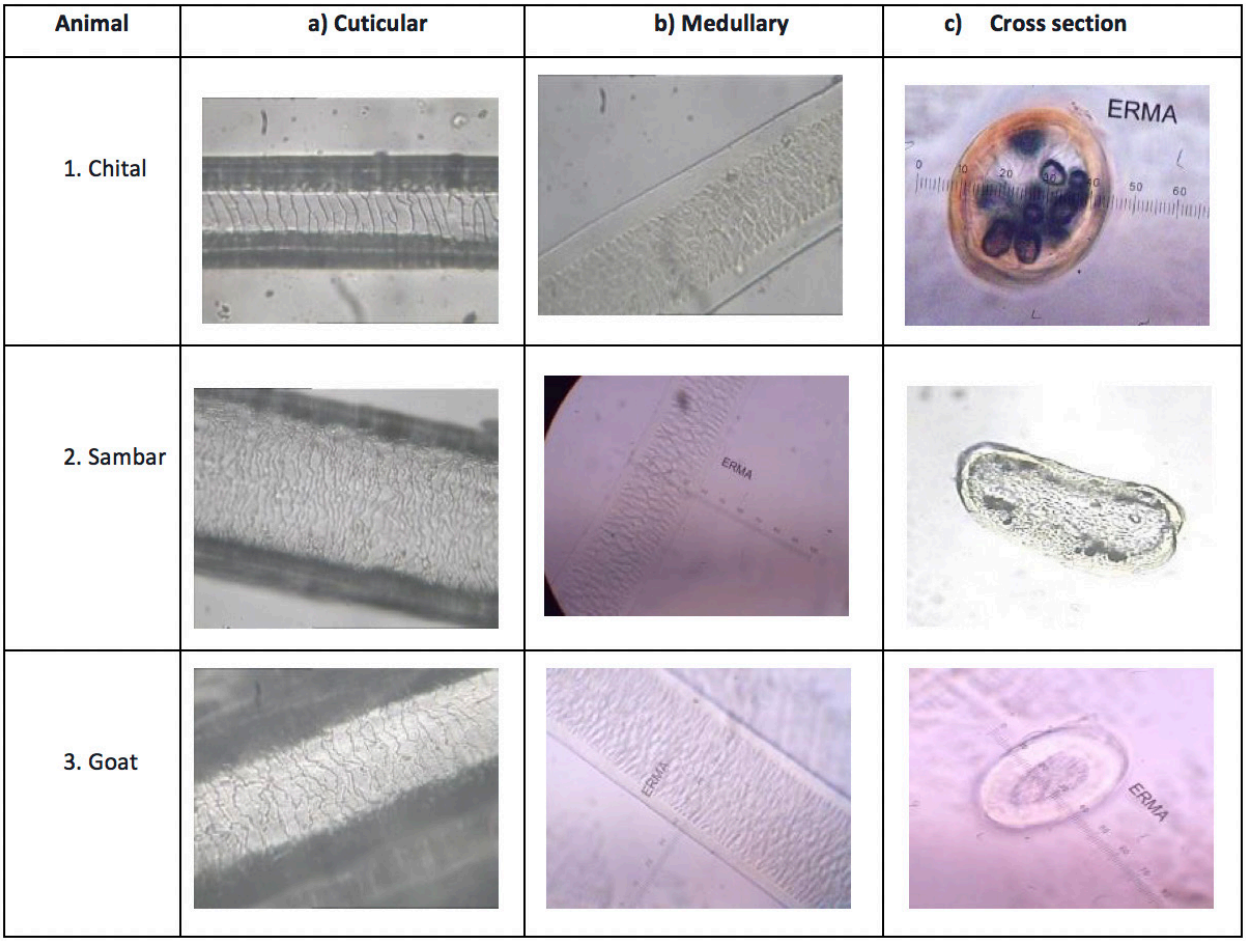

Image 1. Hair of wild and domestic herbivores.
Microscopic images of different hair: 1–Chital | 2–Sambar | 3–Domestic Goat showing (a) cuticular pattern, (b) medullary pattern, and (c) transverse section of hair using Leica DM 3000 compound microscope(40 X).

Table 1. Micrometry of wild and domestic animal's hair.

	Species	Hair length (mm)		Diameter of hair T.S. (mm)		Cuticle scale pattern			Medullary pattern
		Max	Min	Max	Min	Margin	Distance	Pattern	
1	**Chital**	30	15	0.087	0.025	Regular wave	Distant	Smooth	Multicellular in rows Cloisonné
2	**Sambar**	96	23	0.077	0.012	Rippled	Near	Irregular	Multicellular in rows Cloisonné
3	**Goat**	40	15	0.10	0.005	Irregular	Close	Rippled	Packed with cell

Discussion

The results of the present study showed that the irregular pattern of hair cuticle has distinctive characteristics for certain animals sufficient to determine its origin. The distribution of the medulla is also an important characteristic feature; the medulla along the hair shaft differs in its continuous or discontinuous texture, showing species to species variations. In the present study, the hair index value of the goat was found greater than that of the Chital and Sambar, the values varying between 92.5±0.100–44.6±0.200, (44.4±0.100) mean ± SD.

Keratin proteins and their variations have also opened a means to recognize species through keratin protein molecular weight. SDS-PAGE technique helps to isolate the protein that can be validated using the western blot technique with specific antibodies raised in a particular species and by two-dimensional gel

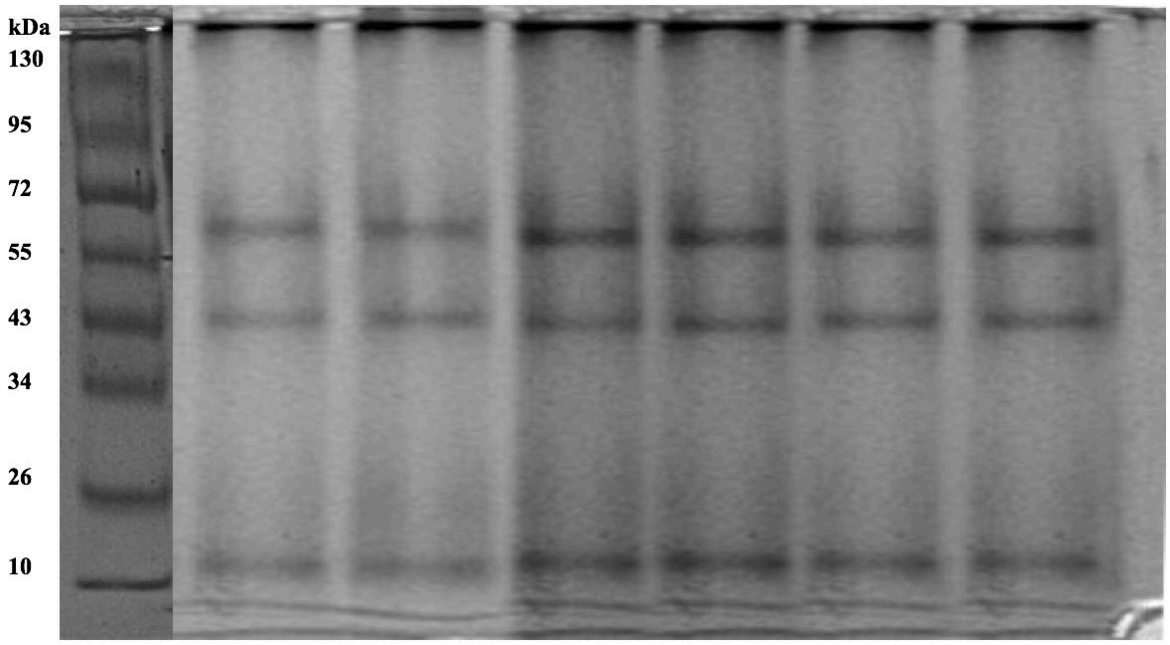

Image 2. Isolated keratin pattern of Chital *Axis axis*, Sambar *Cervus unicolor*, and goat *Capra hircus* by SDS-PAGE. SDS-PAGE data represent the isolation pattern of keratin protein based on their molecular weight of Chital, Sambar, and goat. Keratin separation was done in between 40–60 kDa.

Table 2. Hair index of wild and domestic animal's hair.

Species	Chital	Sambar	Goat
Scale count index	9.72–9.90 (9.81±0.100)	1.58–1.56 (1.57±0.010)	6.04–6.0 (6.02±0.020)
Medullary index	0.83–0.82 (0.825±0.005)	0.92–0.94 (0.93±0.010)	0.51–0.52(0.51±0.010)
Hair index	44.4–44.8 (44.6±0.200)	44.5–44.3(44.4±0.100)	92.6–92.5 (92.5±0.100)

electrophoresis. Nakamura et al. (2002) also reported similar results as in the present study. Another protein validation method is based on specific peptide markers by using peptide mass fingerprinting (PMF) with the MALDI-TOF technique to accurately identify amino acid sequences in a particular sample (Caroline et al. 2013; Carnally & Lawton 2016; Cortellini et al. 2019). The keratin extracted consisted of hard keratin with a molecular mass of 40–60 kDa, matrix proteins with 12–18 kDa, and minor components with 110–115 kDa & 125–135 kDa (Nakamura et al. 2002). Our study supports the fact that the keratin band separated (40–60 kDa) in the present study may be further categorized through serological tests for species identification by gel precipitation tests. The methods, morphological data, and molecular characterization may help to study the genetic variation and post-translational modification among the species in the matured keratinized tissues, hairs, and horns. The medullary index, cuticular pattern and cross-section thickness of the hair of different wild animals and domestic animals also could be used as an identical feature for species differentiation.

Conclusion

The morphological study of Chital, Sambar and goat hair reveals the variations in cuticular scale pattern, medullary structure and shape of medulla visible in the cross-transverse sections. It is evident from the study that there are definite differences regarding the diameter, scale type, scale margin and medullary configuration of the dorsal guard hair of the three species. Further confirmatory species identification is also possible through species-specific antibodies that can be raised in a specific animal. The microscopic hair characteristics corroborated with keratin pattern studies are a competent basis for species identification and successful implementation of the Indian Wildlife (Protection) Act 1972 as scientific evidence for prosecution and conviction of wild animal poachers.

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