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Tricho-taxonomic Studies for Identification of Indian Cervids, *Axis axis* (Erxleben, 1777), Axis porcinus Zimmermann, 1780, *Muntiacus muntjak* Zimmermann, 1780 and *Rusa unicolor* (Kerr, 1792) (Artiodactyla: Mammalia) by Dorsal guard Hairs

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ABSTRACT: A combination of characters of hairs of cervids are much required in the field of forensic science and predator diet analysis for species identification. The dorsal guard hairs of four species of Indian cervids *i.e.* Spotted Deer, *Axis axis* (Erxleben, 1777), Indian Hog Deer, Axis porcinus **Zimmermann, 1780, Barking Deer, *Muntiacus muntjak* Zimmermann, 1780 and Sambar Deer, *Rusa unicolor* (Kerr, 1792) were examined using optical light and scanning electron microscopes. Both morphological and microscopic characters of hairs had showed differences among the four species. Based on a combination of characters of dorsal guard hairs, the four cervid species can be identified. Further, this study supports other published hair identification studies, as an appropriate reference of microscopic images and descriptions of hairs of four cervid species presented here for species identity.**

Key words: forensic science, mammals, illegal trade, morphological and microscopic characters.

INTRODUCTION

Identification of mammals based on the morphological and microscopic structure of hairs are relatively significant in the study of mammalian identification along with diet-ecology and control of illegal trade of wildlife and its derivatives. While general morphology alone is not suitable for accurate identification, details of hair structure based on a combination of characters (morphological & microscopic) are helpful in the identification of species (Stains 1958; Brunner & Coman 1974; Koppikar & Sabins 1976; Teerink, 1991; Chakraborty & De 2010; Sahajpal *et al*. 2010).

Identification of hairs of some ungulates have been carried out by Gopal *et al.* (1993, Hard-ground Barasingha); Chatterjee *et al*. (2005, Yak); Bahuguna & Mukherjee (2000, Tibetan Antelope); Bahuguna *et al.* (2010, selected carnivores & artiodactyls); Sahajpal *et al*. (2010, Tibetan Antelope & *Capra* sp.); Joshi *et al.* (2012, 4 species of deer); Dharaiya & Soni (2012, cross-section of ungulates). However, a combination of characters for identification of species based on hair characteristics for family Cervidae is more appropriate for species identification. (Brunner & Coman 1974; Teerink 1991). Out of seven cervid species listed in India, four species namely Spotted Deer *Axis axis* (Erxleben 1777), Hog Deer *Axis porcinus* (Zimmermann 1780), Barking Deer *Muntiacus muntjak* (Zimmermann 1780) and Sambar Deer *Rusa unicolor* (Kerr 1792) were addressed in the present study. All the four species are listed under **Schedule III** of the Indian Wildlife (Protection) Act, 1972. As per the IUCN Red List category, *Axis porcinus* is **Endangered**, *Rusa unicolor* is **Vulnerable**, *Axis axis* and *Muntiacus muntjak* are **Least Concern**. The present study was carried out to provide a complete combination of characters such as morphological, cuticular, medullary and cross-sectional characteristics of hair with high on images and detailed descriptions using optical light and scanning electron microscopes for the identification of above mentioned four cervid species.

MATERIAL AND METHODS

The guard hairs were collected from the mid-dorsal region of three dry skins of each species present in the National Zoological Collections, Mammal & Osteology Section, Zoological Survey of India, Kolkata, India. The samples were washed thoroughly with Acetone ($(CH_3)_2CO$ = 58.08) and Carbon tetrachloride (CCl_4 = 153.82) to remove the dirt of exogenous materials. The morphological characters of hairs (n = 20) such as shape, colour, bands were recorded, and total lengths (mm) and diameters (µm) were measured using Dial calliper (Mitutoyo) and optical microscope (Olympus BX41), respectively. The cuticular characters of hair such as scale position, scale patterns, structure of scale margins and distance between scale margins and medullary characters such as width composition, structure and form of margins of the medulla, and shape of cross-section of hair were examined under 200 X and

400 X magnification with help of the digital camera fitted on optical microscope (Olympus BX41) and the observed microscopic characters of hair were photographed. To obtain the three dimensional structure and a more detailed examination of cuticular scales of hair, the scanning electron microscope (ZEISS Evo18 - Special edition) was used. The cuticular structures of hairs were observed under the high magnification from 1150 to 4080 X and the observed cuticular structures of hairs were photographed.

All the measurements of cuticular scales were through the optical microscope and the mean values were taken into the consideration for analysis. The methodology and nomenclature of morphological, cuticular, medullary and cross sectional characteristics of dorsal guard hairs were followed according to the descriptions provided by Ridgway (1886); Brunner & Coman (1974); Moore *et al*. (1974); Teerink (1991); Chakraborty *et al*. (1995). The description of different terms of patterns used in the results and discussion have been given herewith were followed from the Brunner & Coman (1974); Teerink (1991).

***Cuticle characteristics*:** *Transversal*– the position of scales lie at right angles and their width is greater than their length. *Regular wave*– the pattern of scales are non-overlapping, wavy in appearance and usually continuous, with the length of the waves on the same and different scales equal. *Smooth*– the structure of scale margins have no interruptions, irregularities or indentations. *Near*– the distance between the margins of the two consecutive scales are neither too close nor too away from each other.

***Medulla characteristics*:** *Multicellular in rows*– the medulla is composed of a continuous two or more columns of cells. *Wide aeriform lattice*– the structure of medulla is filled with air spaces appear as a network or lattice enclosing aggregations of shrunken wide medullary cells. *Straight*– the form of the medulla margins have a smooth and straight line. *Scalloped*– the form of the medulla margins have a series of convex and rounded projections.

RESULTS AND DISCUSSION

The dorsal guard hairs of the four species studied under family Cervidae can be identified on the basis of following combination of morphological and microscopic characters of dorsal guard hair.

(a) Morphological characters. The shape of studied species found as straight in both *A. axis* and *A. porcinus*, whereas the shape was slightly wavy and undulated between the *M. muntjak* and *R. unicolor* respectively. The coat colour of cervids had showed different shades of brown. The dorsal guard hairs of all the four species were unbanded. The mean length of hair significantly varied between two cervids, the *R. unicolor* (49.8±8.7 mm) had the maximum and the *M. muntjak* (21.2±3.3 mm) had the minimum length. Similarly, the maximum and minimum of mean diameter of hair were observed in the *R. unicolor* (89.4±14.7 µm) and *M. muntjak* (40±6.6 µm), respectively. The mean length and diameter were almost equal in size in the *A. axis* (length= 29.1±4.7 mm, diameter=84.7±2.9 µm) and *A. porcinus* (length= 30±5.9 mm; diameter=87.4±2.4 µm), but they were highest than the *M. muntjak* and lowest than the *R. unicolor* (**Table 1**). The morphological characters of hairs of mammals may differ due to the age, sex, season, climate, geographical variations, etc. However, the shape, colour, length and diameter of dorsal guard hair are presented here may be helpful for the preliminary investigations of hair and can be used as one of the combination of characters of hair for the identification of species.

(b) Microscopic characters

(i) ***Cuticular characteristics.*** The cuticular characteristics of the four species studied had showed no variations except the measurements of cuticular scales. The scale position, scale patterns, structure of scale margins and distance between scale margins were as 'transversal', 'regular wave', 'smooth' and 'near', respectively in relation to the longitudinal direction of the hair of all the four species. However, measurement data had showed variations among the species, the scale count per millimetre mean length of hair was highest in the *M. muntjak* (150.3±11 µm) and lowest in the *R. unicolor* (80±9.3 µm), and the *A. axis* and *A. porcinus* values were 102±13.4 and 117.2±11.1 µm, respectively. The minimum mean length of cuticular scales (38.9±1.7 µm) was observed in the *M. muntjak* and the maximum (79.5±6.3 µm) was observed in the *R. unicolor*, whereas the *A. axis* and *A. porcinus* values were 71±6.8 and 73.5±1.8 µm, respectively. The mean width of cuticular scales of the all the four species were slightly varied: 10.4±1.2 and 11.7±1.1 µm in the *A. axis* and *A. porcinus*, respectively, and 9.4±1.9 and 12.9±11.9 µm in the *M. muntjak* and *R. unicolor*, respectively (Table 2).

(ii) ***Medullary and Cross-sectional characteristics.*** The width composition of medulla had showed no variations and it was filled with as 'multicellular in rows' in all the four species. The structure of medulla of the species was observed as 'wide aeriform lattice' in both *A. axis* and *M. muntjak* and as 'wide medulla lattice' in both *A. porcinus* and *R. unicolor*. The form of the medulla margins were as 'straight' in all the species except in the *M. muntjak*, where it was as 'scalloped'. The shape of cross-section of the species had showed significant variation among the four species. The shape of the cross-section was observed as: 'oval large medulla' in both *A. axis* and *M. muntjak*, 'oval medium medulla' in the *A. porcinus* and 'biconvex large medulla' in the *R. unicolor* (Table 3).

Joshi *et al*. (2012) have done a comparative hair study on four species of deer namely *Moschiola indica, Axis axis, Muntiacus muntjak* and *Rusa unicolor* only on the basis of the hair colour and medulla structures of the species. Dharaiya & Soni (2012) have documented only the transverse sections of hairs of *Axis axis* and *Rusa unicolor* in the family Cervidae. Some studies of species under family Cervidae are mainly based on the predator's scat-hair (diet) analysis. Methods of hair identification need exact identification keys (Rajaram & Manon 1975; Dharaiya & Soni 2012).

The single character of hair does not help for the species identification, as the hair characters often show high variance, but the combination of characters may give significant values for identification of species (Brunner & Coman 1974; Teerink, 1991). Among the four studied species, both *A. axis* and *A. porcinus* had showed having almost similar characters except the coat colour, colour of dorsal guard hair, structure of medulla and shape of cross-section, however, the *M. muntjak* and *R. unicolor* showed significant variations in both morphological and microscopic characters of dorsal guard hairs. On the basis of morphological and microscopic characters of dorsal guard hairs, the key characters of hair for the four species studied under the family Cervidae is presented in Table 1-3 along with microscopic photographs (Figs. 1-16). The four species of cervids studied here such as the *A. axis, A. porcinus, M. muntjak* and *R. unicolor* are the deer species highly trafficked in the illegal trade after carnivores, used mainly for local bushmeat consumption, and for their skin and other derivatives. On the other hand, they are chief prey of large carnivores. Therefore, the identification keys along with the microphotographs presented here can be used in animal forensic science as well as the cross-section of hair-structure can be used for predator diet analysis, as an appropriate reference for species identification.

Table 1: Morphological characteristics of the dorsal guard hairs of species.

Species	Morphological characteristics					
	Shape	**Coat colour**	**Colour of dorsal guard hair**	**No. of bands**	**Length (mm)**	**Diameter (µm)**
Axis axis	Straight	Reddish brown coat with white spots	Fallow	Unbanded	23-35.9 (29.1±4.7)	81.1-89.5 (84.7±2.9)
Axis porcinus	Straight	Olive brown	Chamoisee	Unbanded	21.0-37.6 (30.0±5.9)	83.4-93.4 (87.4±2.4)
Muntiacus muntjak	Slightly wavy	Glossy reddish brown	Sepia	Unbanded	14.1-26.1 (21.2±3.3)	32.1-48.1 (40.0±6.6)
Rusa unicolor	Undulated	Dark brown	Camel	Unbanded	31.8-60.3 (49.8±8.7)	57.1-98.3 (89.4±14.7)

Table 2: Cuticular characteristics of the dorsal guard hairs of species.

Species	Cuticular characteristics						
	Scale position	**Scale patterns**	**Structure of scale margins**	**Distance between scale margins**	**Scale count/mm length of hair**	**Length of cuticular scale**	**Width of cuticular scale**
Axis axis	Transversal	Regular wave	Smooth	Near	68-146 (102±13.4)	64.3-83.6 (71±6.8)	8.3-12.4 (10.4±1.2)
Axis porcinus	Transversal	Regular wave	Smooth	Near	96-132 (117.2±11.1)	78.1-82.1 (73.5±1.8)	8.8-14.1 (11.7±1.1)
Muntiacus muntjak	Transversal	Regular wave	Smooth	Near	132-168 (150.3±11)	36.1-42.1 (38.9±1.7)	7.1-10.6 (9.4±1.9)
Rusa unicolor	Transversal	Regular wave	Smooth	Near	65-95 (80±9.3)	69.1-86.1 (79.5±6.3)	11.7-15.2 (12.9±11.9)

Table 3: Medullary characteristics and cross-section of the dorsal guard hairs of species.

Species	Medullary characteristics			Cross-section
	Width composition of medulla	**Structure of medulla**	**Form of medulla margins**	**Shape**
Axis axis	Multicellular in rows	Wide aeriform lattice	Straight	Oval- large medulla
Axis porcinus	Multicellular in rows	Wide medulla lattice	Straight	Oval- medium medulla
Muntiacus muntjak	Multicellular in rows	Wide aeriform lattice	Scalloped	Oval-large medulla
Rusa unicolor	Multicellular in rows	Wide medulla lattice	Straight	Biconvex- large medulla

Microscopic structure of dorsal guard hair of *Axis axis* (Erxleben, 1777)

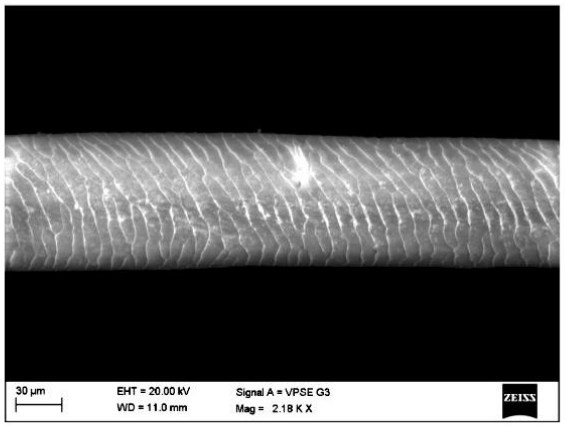

Fig. 1. Scanning electron micrograph of cuticle

Fig. 2. Cuticle 200 X

Fig. 3. Medulla 200 X

Fig. 4. Cross-section 200 X

Microscopic structure of dorsal guard hair of *Axis porcinus* Zimmermann, 1780

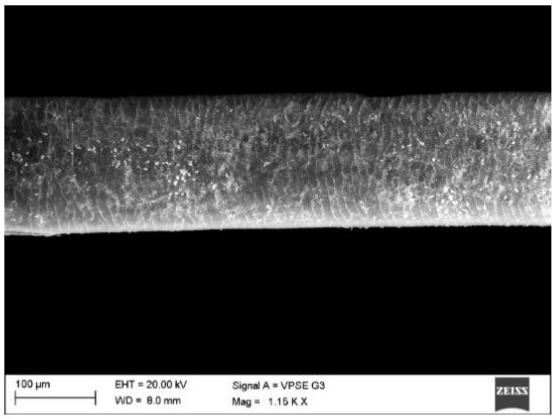

Fig. 5. Scanning electron micrograph of cuticle

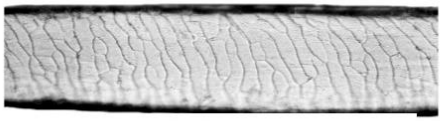

Fig. 6. Cuticle 200 X

Fig. 7. Medulla 200 X

Fig. 8. Cross-section 200 X

Microscopic structure of dorsal guard hair of *Muntiacus muntjak* Zimmermann, 1780

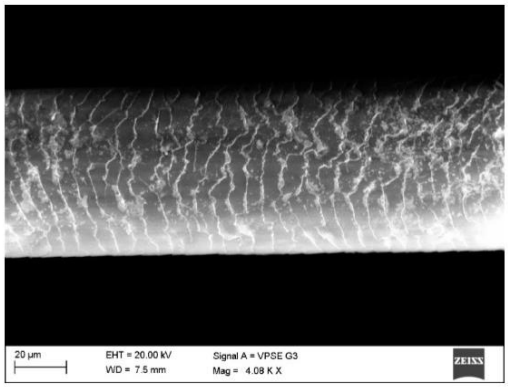

Fig. 9. Scanning electron micrograph of cuticle

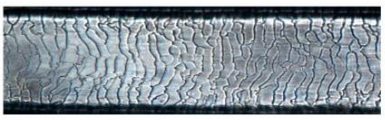

Fig. 10. Cuticle 400 X

Fig. 11. Medulla 400 X

Fig. 12. Cross-section 400 X

Microscopic structure of dorsal guard hair of *Rusa unicolor* (Kerr, 1792)

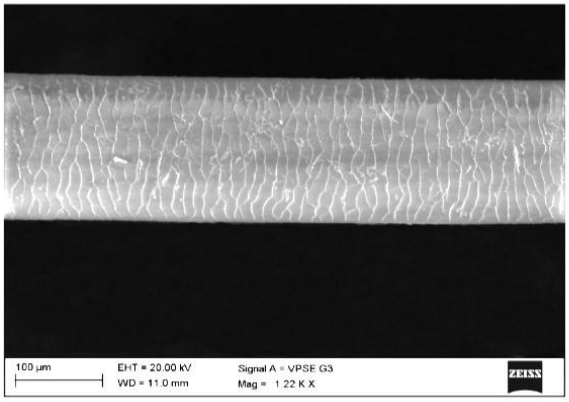

Fig. 13. Scanning electron micrograph of cuticle

Fig. 14. Cuticle 200 X

Fig. 15. Medulla 200 X

Fig. 16. Cross-section 200 X

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