Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(6): 900-904

Received: 19-09-2019
Accepted: 23-10-2019

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Light and scanning electron microscopy analysis of hair samples of some wild animals for individual identification

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Abstract
Hair samples from Four-horned antelope (*Tetracerus quadricornis*), Blackbuck (*Antilope cervicapra*), Hog deer (*Axis porcinus*), Spotted deer (*Axis axis)* and Sambar deer (*Rusa unicolor* and Indian mouse deer (*Moschiola indica*) were collected for light and scanning electron microscopy analysis. The medullary index of hair samples for each animals were calculated by using a light microscope and the results were 0.84± 0.01, 0.75±0.02, 0.84±0.02, 0.79±0.02, 0.65±0.02, 0.77±0.01 respectively. Under the scanning electron microscope the respective mean interscale distance of hair samples were 16.21 ±1.16 µm, 12.85±1.4 µm, 14.05±0.79 µm, 9.07±0.3 µm, 11.21±0.54 µm, and 10.97±0.46 µm. These parameters were important for individual species identification.

Keywords: Medullary index, interscale distance, species identification

1. Introduction
Hair can be taken as physical evidence as it is strongly resistance from decomposition. Analysis of animal hair could be used as an effective tool in veterinary forensic investigation and biology allowing for the identification of illegal trade, slaughter and poaching of animals including endangered species. The mammalian hair fibers could easily be collected, preserved and transported to the lab for species identification through microscopy [1, 2]. Hair sample can be examined by direct observation of whole mounts using light microscopy [3, 4, 5, 6] where as scanning electron microscopy provides higher range of magnification [7, 8] and coupled Energy Dispersive Spectra (EDS) lead to the identification of geographical region by elemental analysis can be used further for other examinations [9]. This study aims at using light and scanning electron microscopy to obtain some features of hair samples from the dorsum of some wild animals mostly from Bovidae, Cervidae and Tragulidae in trying to differentiate between them. Hair Microstructure is composed of three layers of keratin: medulla, cortex and cuticle, from the innermost to the outermost. The medullary index may provide an important tool for the species identification. The interscale distance on the cuticular layer also used as a tool for the species identification.

2. Materials and Methods
Hairs of Four-horned antelope (*Tetracerus quadricornis*), Blackbuck (*Antilope cervicapra*) belong to family Bovidae, Hog deer (*Axis porcinus*), Spotted deer (*Axis axis)* and Sambar deer (*Rusa unicolor*) belong to family Cervidae and Indian mouse deer (*Moschiola indica*) of Tragulidae family were collected from Nandankanan Zoological Park, Bhubaneswar-754005, Odisha, India with the help of centre of wild life, C.V. Sc. and A.H., OUAT, Bhubaneswar. 10 hair strands from each animal i.e. 6 different animals from each of the six species were taken in two sets for the study. One set for light microscopy and the other for Scanning electron microscopy. The dorsum hair were taken from the enclosure while restraining the animal for giving medication by using forceps with gloves to avoid contamination and were packed in zip-lock bags followed by paper envelops.

Sample preparation for light microscopy studies
The hair samples were subjected to treatment with 30% hydrogen peroxide overnight (12 hours) for light microscopic examination. Each hair was then mounted on microscopic slide in a drop of DPX (Xylene and Din Butyl Phthalate) and covered with cover slip and allowed to

dry for 48 hours for light microscopic observation (10X). The numerical measurement in millimeter for both cortex and medulla width were taken using a calibrated ocular micrometer. The measurements were converted to micrometer. The medullary index (MI) of each samples were calculated by using the formula described below.

$$\text{Medullary index (MI)} = \frac{\text{Width of medulla}}{\text{Width of cortex}}$$

Sample preparation for scanning electron microscopy studies
For Scanning electron microscopy the hair samples were dissected in pieces of 5 mm size leaving 3 mm from root side and placed on sample holder followed by plasma Gold-Palladium coating at 5 milibar vacuum and 5 mA current for 20 seconds. The samples were focused under 1000X magnification under scanning electron microscope. The inter scale distance of the hair cuticle for each animal species were measured using SEM software digital scale.

3. Results and Discussions

Hair identification is not employed solely by forensic scientists. Hair identification is an important tool used by wildlife biologists, archaeologists, anthropologists, and textile conservators. Many researchers have investigated the morphological characteristics of hair, devised keys and reviewed the science of animal-hair identification [10, 11]. The study revealed that the medullary index of individuals from the same species showed very small or no variations by changing the body region but comparing medullary index on the species level showed significant differences [12]. The medulla of hairs valuable for species identification [13, 14] and differences in medullary index and patterns observed between different species [15].

The hair samples were put under light microscope (Fig.1-6) to record the medullary index. The hair samples of Indian mouse deer and Blackbuck were 0.84± 0.01 & 0.79±0.02, nearly similar to previous records [16, 17] respectively. For Spotted deer it was 0.75±0.02, higher than the earlier report of 0.63 ±00 [17]. We observed medullary index of Hog deer was 0.84±0.02, Four-horned antelope and Samber deer were 0.65±0.02, 0.77±0.01 respectively. The medullary index of dorsum region hair middle portion of American black bear,, Blue Nile monkey, Barbary sheep, Llama, Bacterian camel were 41.0± 0.57, 48.16± 0.60,86.5± 0.10, 33.96± 0.31, 16.63± 0.49 respectively [13]. These observations were different from ours which may be due to the species difference. The medullary index of domestic animals such as cow (*Bos taurus*: 0.22-0.43), buffalo (*Bubalus bublis*: 0.53-0.78), cat (*Felis indicus*: 0.52-0.78), dog (*canis lupus familaris*: 0.54-0.75) were also studied [18] showing different values which attributed to the fact that the medullary index could be an important tool for species identification. Statistically there were existences of significant difference with respect to medullary index of the hair samples among all the six species with observed F-value of 8.35 ($p<0.05$) by performing single factor ANOVA test (Table1).

Table 1: The medullary index (Mean ± SE) of hair samples for different wild animals

Species	Four-horned Antelope	Blackbuck	Hog Deer	Spotted Deer	Sambar Deer	Indian Mouse deer
Mean ± SE	0.65^{a}±0.02	0.79^{b}±0.02	0.84^{c}±0.02	0.75^{b}±0.02	0.77^{b}±0.01	0.84^{c}± 0.01

* Mean with different superscripts differ significantly ($p<0.05$)

The scanning electron microscopy could be used in wildlife forensic for species identification [19]. The surface cuticular pattern, cross section and medullary index provides the information of the sample which could further be used as a geographical region and species identification tool. The cuticle was a useful tool to discriminate species among wild ungulates and to distinguish young from adult and winter from summer coat in deer [20]. The interscale distance of hair samples were observed at 1000X magnifications with the help of scanning electron microscope (Fig 7-12). The cuticular scale patterns of all the six wild ruminant species used in the above studies were revealed an imbricate type consisting of overlapping scales with narrow margins. This may be due to the common species character of all the animal used i. e a ruminant type as per the earlier interpretation [21]. We observed the mean interscale distance of Four-horned antelope, Blackbuck, Hog deer, Sambar deer, Indian mouse deer and Spotted deer were 11.21 ±0.54 µm, 9.07±0.3 µm, 14.05±0.79 µm, 10.97±0.46 µm, 16.21±1.16 µm, and 12.85±1.4 µm respectively. The scale difference between each layers range from 6.367 µmeter to 6.948 µmeter in Asiatic lion, from 7.758 µmeter-7.992 µmeter for tiger while it ranges from 8.63-9.884 µmeter in case of leopard [9]. The mean scale distance of wool hair and primary hair during preruminant transitional and ruminant age group 9.0375±0.56143µ, 6.7±0.301 µ and 6.589±0.301 and 6.9575±0.44µ, 3.76±0.244 µ and 3.7075±0.3636 µ respectively [22]. Species wise different interscale scale distance indicated that it might be used a tool for species identification under ultramicroscopic level. Significant difference in interscale distance between two adjacent scale layers among all six species of the animals, were observed with estimated F- value of 15.787 ($p< 0.05$) by performing single factor ANOVA test (Table 2).

Table 2: The interscale distance (Mean ± SE) of hair samples for different wild animals

Species	Four-horned Antelope	Blackbuck	Hog Deer	Sambar Deer	Indian Mouse deer	Spotted Deer
Mean ± SE (µm)	11.21^{b}±0.54	9.07^{a}±0.3	14.05^{c}±0.79	10.97^{b}±0.46	16.21^{d}±1.16	12.85^{bc}±1.4

* Mean with different superscripts differ significantly ($p<0.05$)

4. Conclusions

In conclusion, the medullary index and the interscale distance of the hair samples are specific for all species that should be recorded for easy identification of species during forensic investigation. The cuticular scale patterns of all the six wild ruminant species used in the above studies were of imbricate type. Statistically with respect to medullary index samples of black buck, Samber and Spotted deer showed significant difference with four horned antelope, Hog deer and Indian mouse deer. Four horned antelope showed significant difference with all the species which were taken into account. Hog deer and Indian mouse deer significantly different to all species except to each other. When the interscale distance was taken into account the statistical analysis revealed that the

four horned antelope and spotted deer showed significant difference with Black buck, Hog deer and Indian mouse deer. Black buck and Indian mouse deer samples significantly differed with all the five species. Interscale distance of Hog deer significantly differed with all four species except spotted deer. Spotted deer showed significant difference with Black buck and Indian mouse deer. Samber deer Interscale distance significantly differed with Blackbuck, Hog deer and Indian mouse deer.

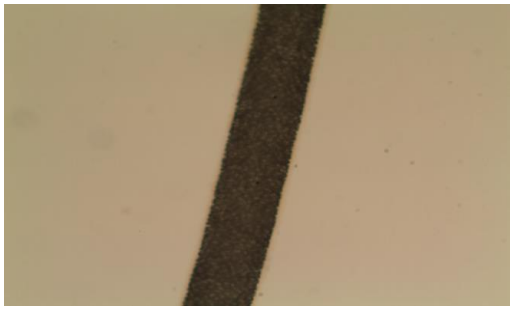

Fig 1: Light microscopic structure of Indian mouse deer hair (10X)

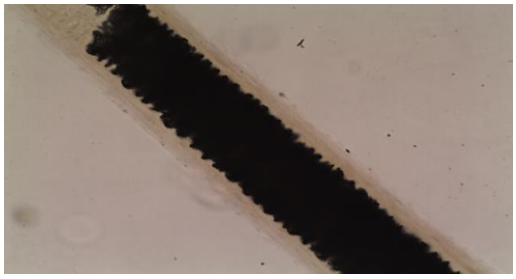

Fig 2: Light microscopic structure of spotted deer hair (10X)

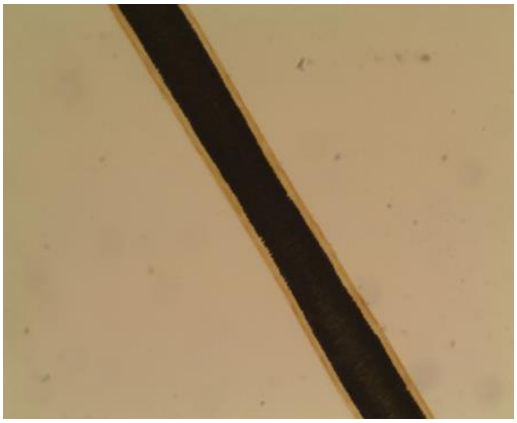

Fig 3: Light microscopic structure of Hog deer hair (10X)

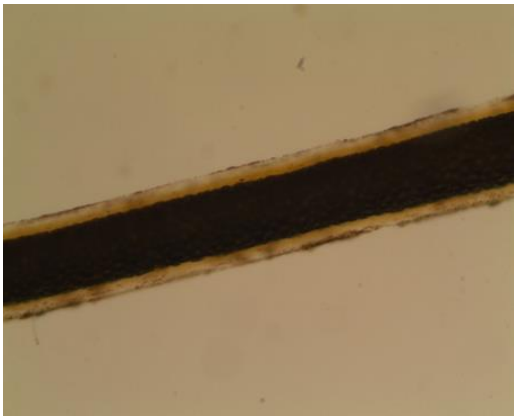

Fig 4: Light microscopic structure of Blackbuck hair (10X)

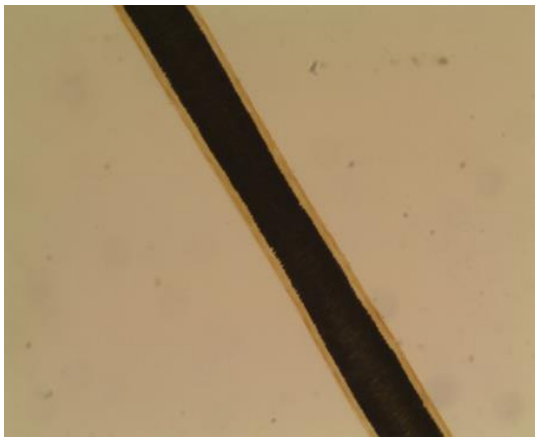

Fig 5: Light microscopic structure of Four-horned Antelope hair (10X)

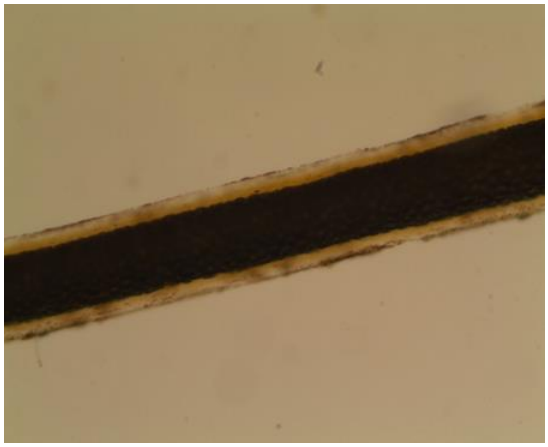

Fig 6: Light microscopic structure of Samber deer hair (10X)

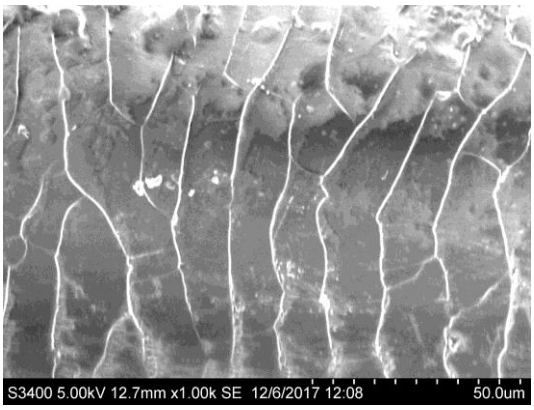

Fig 7: Scanning electron microscopic structure of 4 horned antelope (1000X)

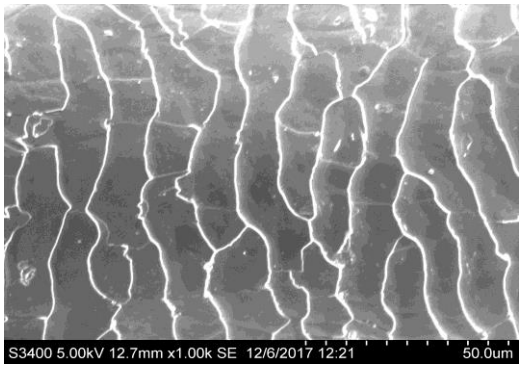

Fig 8: Scanning electron microscopic structure of Blackbuck (1000X)

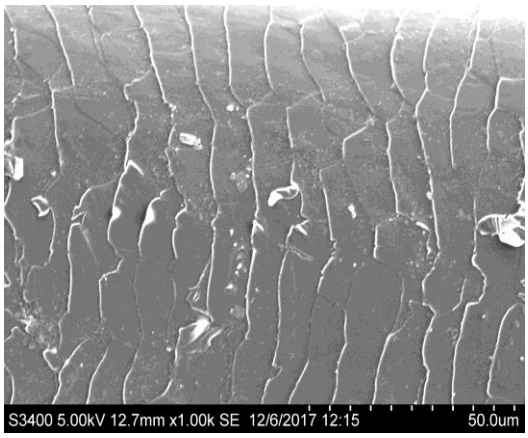

Fig 9: Scanning electron microscopic structure of Hog deer (1000X)

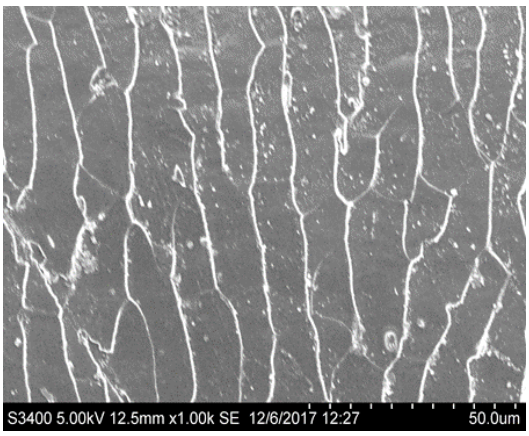

Fig 10: Scanning electron microscopic structure of Sambar deer (1000X)

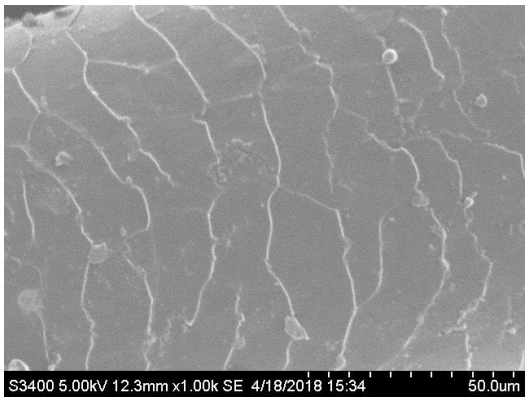

Fig 11: Scanning electron microscopic structure of Indian mouse deer (1000X)

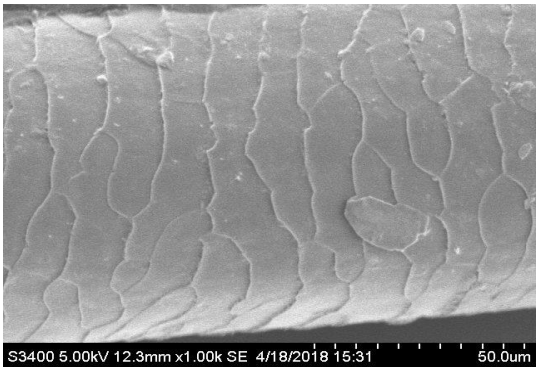

Fig 12: Scanning electron microscopic structure of spotted deer (1000X)

5. Acknowledgement
Authors are thankful to Director of Nandankanan Zoological Park, Bhubaneswar-754005, Odisha and Centre of wild life, C.V. Sc. and A.H., OUAT, Bhubaneswar for providing all necessary facilities and supports for smooth conduct of this research work.

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