Forensic Science International 189 (2009) 34–45

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Microscopic hair characteristics of a few bovid species listed under Schedule-I of Wildlife (Protection) Act 1972 of India

Vivek Sahajpal [a], S.P. Goyal [a,*], M.K. Thakar [c], R. Jayapal [b]

[a] Wildlife Forensic Lab, Wildlife Institute of India, Dehradoon 248001, India
[b] Wildlife Institute of India, Dehradoon 248001, India
[c] Forensic Science Dept. Punjabi University, Patiala 147002, India

ARTICLE INFO

Article history:
Received 6 May 2008
Received in revised form 2 March 2009
Accepted 6 April 2009
Available online 19 May 2009

Keywords:
Wildlife forensics
Microscopy
Bovid
Hair

ABSTRACT

Dorsal guard hairs of 10 bovid species of India, listed under Schedule-I of Wildlife (Protection) Act 1972 of India and some of them quite frequently encountered in illegal trade, were studied using light microscopy. We discuss characteristics including colour, hair thickness, cuticular pattern, medulla pattern, medulla index, cross-section and scale count index for species characterisation/identification to deal wildlife offence cases. Although some species could be identified very easily based on one or few microscopic hair characteristics, however there were some overlaps of few hair characteristics among some species. Species like *Pantholops hodgsonii* could be characterised most easily, just based on cuticular pattern and similarly *Capricornis sumatraensis* could be characterised simply by medulla pattern. For species showing overlaps in some of the microscopic hair characteristics, a combination of all the characteristics was most useful. We suggest the use of maximum number of parameters for distinguishing sympatric and closely related species. In addition to wild species, hair characteristics of three domestic species have been examined and compared with the selected wild species.

1. Introduction

India being one of the 12 mega biodiversity nations of the world [1] has the rare blend of Palaearctic, Oriental and Afrotropical fauna [2]. More than 400 mammal species have been reported from India [3]. Of these 129 mammal species are protected under the Wildlife (Protection) Act 1972 (India) and their poaching amounts to an offence. India is further a party to the Convention on International Trade in Endangered Species (CITES). Trade in wildlife parts and products is a major threat to conservation and is causing local extinction of these species, and according to some estimates, illegal trade in wildlife parts is the third largest illegal trade after illegal trade in narcotics and firearms [1]. The wild animals are being poached for meat, antlers, horns, musk pods, biles, ivory, bones, skin, fur, shells, claws, teeth, wool, etc. and some of these may be found in processed and finished form like Traditional Chinese medicines (TCM), leather goods, Shahtoosh shawls, trophies, ornaments, combs, brushes, glass frames, etc. [4].

Bovids form one of the major group of mammals protected under the Wildlife (Protection) Act 1972 (India) with 19 bovid species listed under Schedule-I of the Act [5]. Bovids are mostly poached for their meat, horns and skin such as Black buck (*Antilope cervicapra*), Indian Gazelle (*Gazella bennettii*), Serow (*Capricornis sumatraensis*), Indian Bison (*Bos gaurus*), Blue sheep (*Pseudois nayaur*), Ladakh Urial (*Ovis vignei vignei*), Argali or the Great Tibetan sheep (*Ovis ammon hodgsonii*), Four Horned antelope (*Tetracerus quadricornis*), Nilgiri Tahr (*Hemitragus hylocrius*), etc. and for wool like Tibetan antelope (*Pantholops hodgsonii*) for Shahtoosh.

Hair is one of the major physical evidence found invariably in cases related to poaching of these species. This evidence if utilized properly can be used to identify the species with the help of microscopic examination and hence can be used for the successful conviction of the poachers in India under the Wildlife (Protection) Act 1972. Thus it becomes imperative to undertake a study on hair characterisation of these species through microscopy to develop a data base to deal such cases.

Many investigators have worked on species identification from hair using microscopic techniques using light microscopy. A detailed study of the microscopic hair characteristics of indigenous mammals for Australia and techniques involved has been reported [6]. Another detailed study on the microscopic hair characteristics has been reported for the mammalian species of North America [7].

* Corresponding author at: Wildlife Forensic Lab, Wildlife Institute of India, Chandrabani, Post Box No. 18, Dehradoon 248001, Uttrakhand, India.
Tel.: +91 135 2640111; fax: +91 135 2640117.
E-mail addresses: viveksahajpal@gmail.com (V. Sahajpal), goyalsp@wii.gov.in (S.P. Goyal).

0379-0738/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2009.04.008

Similarly, a key atlas on the microscopic hair characteristics of West-European mammals has been published [8].

In addition to light microscopy, use of scanning electron microscopy for characterisation of cuticular scale patterns for some species has also been reported [9]. Microscopic characterisation of Shahtoosh wool, derived from Tibetan antelope (*P. hodgsonii*), Cashmere wool derived from Pashmina goat (*Capra hircus*), Yak (*Bos grunniens*) wool/hair and Ibex (*Capra ibex*) wool/ has been reported by using scanning electron microscopy [10–12].

However, very little work has been done on the Indian mammals for microscopic characterisation of hairs and is mostly related to felid, primate and few herpestid species to understand prey composition for carnivore species [13–19]. A detailed forensic study on the microscopic hair structure of four protected Indian bear species has been reported [20]. Similarly, a study on cuticle of the guard hair structure for Tibetan antelope (*P. hodgsonii*) with scanning electron microscopy has been reported [21].

The Wildlife Forensic Laboratory at the Wildlife Institute of India has hither to received about 400 cases related to identification of species from hair with large number of them suspected to be those of bovid species. Identifying, if those hairs are from a particular protected bovid species becomes imperative for criminal investigation. In the present study we characterised guard hair (dorsal) of the 10 wild bovid species viz. *A. cervicapra*, *G. bennettii*, *T. quadricornis*, *B. gaurus*, *H. hylocrius*, *C. sumatraensis*, *P. nayaur*, *O. ammon hodgsonii*, *O. vignei vignei*, and *P. hodgsonii* found in India. The former five are found in plains and peninsular part of India at low altitudes except for *H. hylocrius* which is found at relatively higher altitude (1600–2200 m) in the Nilgiri Hills of Southern Peninsular India. *C. sumatraensis* is an exceptional bovid found at altitudes as low as 200 m and up altitudes of 3000 m. The latter four are found at higher altitudes (Greater Himalayas and Trans Himalayas). The study was aimed to serve the purpose of species identification from various wildlife seizures involving these species. In addition to these hair characteristics of three domesticates viz. *Bos taurus* (domestic cattle), *C. hircus* (domestic goat) and *Ovis aries* (domestic sheep) were examined and compared with selected wild species.

2. Materials and methods

Reference hair samples for the 10 selected bovid species (from 3 individuals for each species) were obtained from the reference collection of the Wildlife Forensic Laboratory, of the Wildlife Institute of India. Hair samples of the three domesticates (from 3 to 4 individuals of each species), were collected from the local livestock farms. The samples were marked serially and stored in plastic zip-lock bags to prevent cross-contamination. 10 hairs were taken for examination from each individual.

Prior to examination, hair samples were cleaned thoroughly with distilled water and then with isopropyl alcohol to remove the inorganic and organic dirt from the surface of the hair samples. The texture, colour and thickness of the hair samples were noted and microscopic features were examined using light microscopy (Comparison Microscope Leica DMR, Leica Microsystems, Germany). Images were taken with a digital camera (Leica F 300, Leica Microsystems, Germany).

The hair thickness was measured by using ocular micrometer fitted to the comparison microscope. For cuticular studies, the cuticular impressions of the hair surface were taken on a thin film of saturated gelatin solution in water, made on a microscopic glass slide. The thin film of gelatin was made on a microscopic glass slide with the help of glass rod and hair samples were placed gently on it keeping their ends free from the glass slide surface [20]. The slides dried in about 25 min and the hairs were plucked off with forceps. The impressions of the hair left behind on the gelatin film were examined using a comparison microscope at magnifications of 100–400× for cuticular characteristics. Scale count index values were determined for the hairs of 10 species at proximal, medial, and distal position as described Kirk and Gamble [22]. For studying the medulla patterns, the hair samples were chopped into small pieces (0.5 cm approx.) and were dipped in xylene overnight. These were then mounted in D.P.X. mounting media and examined at magnifications of 100–400×. Cross-sections were obtained by mounting the hair tufts in wax and the cross-sections were cut manually using a shaving blade [20]. After dewaxing with xylene the cross-sections were observed at magnifications of 100–400×. Medulla thickness was determined and medullary index values were calculated accordingly for the 10 species using the following formula:

$$\text{medullary index} = \frac{\text{medulla thickness (MT)}}{\text{hair thickness (HT)}}$$

To describe the microscopic hair characteristics terminology given by Brunner and Coman [6] was used. The scientific names of the species studied have been mentioned according to the list of mammal species of the world given by Wilson and Reeder [23]. Test for analysis of variance (ANOVA) was carried out to study the intraspecies and interspecies variation of scale count index and medullary index values of hairs of the 10 bovid species. In case of significant differences in group means, pair wise multiple comparisons (Tukey's HSD and Dunnett's *C* test) were done to ascertain which of the bovid species differed with respect to their hair structure. If homogeneity of variance in the data was indicated by Levene's test, Tukey's HSD (honestly significantly different) was adopted for post hoc test; if not, Dunnett's *C* test was opted to test for the differences in hair structure between a pair of bovid species. All the analysis was done using the statistical software SPSS Release 12.0.0.

3. Results

The comparative summary of the hair characteristics for ten wild bovid species and three domesticates is given in Tables 1 and 2. The close ups of hair and the light photomicrographs for the 10 wild bovid species are given in Plates 1–11. Physical examination showed that the hair of high altitude (Greater Himalayas and Trans Himalayas) dwelling bovid species viz. *P. hodgsonii*, *P. nayaur*, *O. ammon* and *O. vignei* analysed in this study were mostly kinky (Plate 1) and brittle. Among these bovids, *P. hodgsonii* and *O. vignei* hair were soft. In sharp contrast to this, the hair of bovids dwelling in plains and peninsular parts of India had straight hair (Plate 1) which were not brittle as observed in all such species studied viz. *A. cervicapra*, *G. bennettii*, *T. quadricornis* and *B. gaurus*. *H. hylocrius* and *C. sumatraensis* also showed straight hair which was hard like the hair of low altitude dwelling bovid species of Plains and Peninsular India. Similarly, domesticates also showed straight and hard hair. The mean hair thickness among the high altitude mammals was the highest ranging from 120 μm (*P. hodgsonii*) to 360 μm (*O. ammon*); where as in case of bovids dwelling in Plains and Peninsular parts of India (including *H. hylocrius*) the mean hair thickness was comparatively less ranging from 50 μm (*T. quadricornis*) to 200 μm (*H. hylocrius*). In case of *C. sumatraensis* the mean hair thickness was 120 μm. Hair thickness for domesticates ranged from 68 μm (*B. taurus*) to 95 μm (*C. hircus*) (Table 1).

The high altitude bovids showed smooth margin of the scales in all three (proximal, medial and distal) regions except for *P. nayaur* and *O. ammon* which showed a crenate margin and rippled margins respectively at the distal region (Plates 8 and 9). The scale pattern for *O. vignei*, *P. nayaur* and *P. hodgsonii* was very typical at proximal region with the former two showing broad petal and the latter one showing a regular mosaic structure respectively. *P. hodgsonii* showed regular mosaic pattern even at medial position which is in conformity with findings of Bahuguna and Mukherjee [21] however at distal region it showed a regular wave pattern (Plate 11). Bovids dwelling in plains and peninsular parts of India showed a regular wave pattern with nearly placed smooth scales at the proximal and medial region for *A. cervicapra*, *G. bennettii* and *T. quadricornis* (Plates 2–4). However, *H. hylocrius* and *B. gaurus* showed crenate scale margins and irregular wave patterns at the proximal and medial regions (Plates 5 and 7). *C. sumatraensis* also showed crenate scale margins with irregular wave patterns at the proximal and medial regions (Plate 6). The scale patterns were very similar in gross appearance in case of *A. cervicapra*, *G. bennettii* and *T. quadricornis*. Domesticates showed mostly irregular wave pattern.

Interesting observations were made in scale count index values and analysis of variance (one way ANOVA) in them. The range of scale count index and the mean scale count index values for all the

Table 1
Observed hair characteristics among the 10 Indian bovid species.

Species	Physical observations			Cuticular characteristics				Medullar Characteristics		
	Texture	Colour	Thickness mean ± S.D.	Region	Scale margin	Scale distance	Scale pattern	Scale count index range, mean ± S.E.	Medulla pattern	Medullary index range, mean ± S.E.
Antilope cervicapra	Rough	Black to light buff	70 ± 3.2	Proximal Medial Distal	Smooth Smooth Crenate	Near Near Near	Regular wave Regular wave Irregular wave	56–75, 66.90 ± 0.88 74–95, 83.87 ± 0.92 88–109, 98.07 ± 0.95	Wide cellular lattice	0.85–0.89, 0.8707 ± 0. 0.002
Gazella bennettii	Rough	Light yellowish brown to white	83 ± 5.4	Proximal Medial Distal	Smooth Smooth Smooth	Near Near Near	Regular wave Regular wave Regular wave	70–88, 79.27 ± 0.93 91–114, 101.43 ± 1.16 98–121, 110.13 ± 1.23	Wide cellular lattice	0.76–0.82, 0.8103 ± 0.003
Tetracerus quadricornis	Rough	Light brown to brown	50 ± 6.2	Proximal Medial Distal	Smooth Smooth Crenate	Near Near Close	Regular wave Regular wave Irregular wave	98–124, 112.07 ± 1.4 110–138, 121.50 ± 1.28 133–156, 146.77 ± 1.05	Wide medulla lattice	0.70–0.74, 0.7100 ± 0.002
Hemitragus hylocrius	Very rough	Dark brown	210 ± 13	Proximal Medial Distal	Crenate Crenate Crenate	Near Near Near	Irregular wave Irregular wave Irregular wave	93–111, 101.07 ± 0.97 86–107, 96.57 ± 1.02 138–162, 151.33 ± 1.03	Wide medulla lattice	0.78–0.83, 0.8207 ± 0.002
Capricornis sumatraensis	Rough	Dark to reddish brown or white	120 ± 9.1	Proximal Medial Distal	Crenate Crenate Crenate	Near Near Near	Irregular wave Irregular wave Irregular wave	100–125, 114.97 ± 1.19 108–128, 118.63 ± 1.03 105–130, 119.40 ± 1.07	Narrow medulla lattice	0.41–0.46, 0.4371 ± 0.002
Bos gaurus	Rough	Black or dark brown	100 ± 7.3	Proximal Medial Distal	Crenate Crenate Crenate	Near Near Near	Irregular wave Irregular wave Irregular wave	78–96, 87.3 ± 0.89 96–119, 106.63 ± 1.05 105–132, 117.30 ± 1.32	Medium width amorphous medulla	0.58–.065, 0.6100 ± 0.003
Pseudois nayaur	Rough and brittle	Slate bluish brown to white	210 ± 10	Proximal Medial Distal	Smooth Smooth Crenate	Distant Distant Near	Broad petal Broad petal Irregular wave	34–49, 41.90 ± 0.64 47–61, 53.07 ± 0.73 112–132, 122.43 ± 1.08	Wide Cellular lattice	0.95–0.97, 0.9628 ± 0.001
Ovis ammon hodgsonii	Rough and brittle	Reddish brown to grey	364 ± 12	Proximal Medial Distal	Smooth Smooth Rippled	Near Near Near	Regular wave Regular wave Irregular wave	42–60, 50.83 ± 0.9 36–50, 42.27 ± 0.63 54–66, 60.33 ± 0.52	Wide medulla lattice	0.96–0.98, 0.9711 ± 0.001
Ovis vignei vignei	Soft	Copper red to white	170 ± 10	Proximal Medial Distal	Smooth Smooth Smooth	Distant Distant Near	Broad petal Regular wave Regular wave	41–57, 50.03 ± 0.82 57–77, 66.50 ± 0.98 86–109, 95.8 ± 1.25	Wide medulla lattice	0.96–0.98, 0.9774 ± 0.001
Pantholops hodgsonii	Soft, kinky and very brittle	Light tan to white	120 ± 6.9	Proximal Medial Distal	Smooth Smooth Smooth	Distant Distant Near	Regular mosaic Regular mosaic Regular wave	23–36, 28.90 ± 0.67 24–36, 30.30 ± 0.62 61–80, 70.47 ± 0.96	Wide cellular lattice	0.96–0.98, 0.966 ± 0.001
Bos taurus	Hard, rough and straight	Light to dark (variable shades)	68 ± 5	Proximal Medial Distal	Crenate Crenate Crenate	Near Near Close	Irregular wave Irregular wave Irregular wave	95–110, 102.4 ± 0.81 108–130, 117.8 ± 1.41 120–150, 139.8 ± 1.43	Wide amorphous medulla	0.68–0.72 0.715 ± 0.011
Capra hircus	Soft and straight	Light to dark (variable shades)	95 ± 5.2	Proximal Medial Distal	Smooth Crenate Crenate	Near Near Close	Regular wave Irregular wave Irregular wave	50–70, 56.07 ± 1.06 70–90, 79.1 ± 1.11 92–110, 98.8 ± 0.98	Wide medulla lattice	0.83–0.89, 0.854 ± 0.003
Ovis aries	Soft and straight	Light to dark (variable shades)	84.7 ± 8	Proximal Medial Distal	 Crenate Crenate	Near Distant Close	Irregular wave Irregular wave Irregular wave	50–62, 57.01 ± 0.57 60–76, 71.6 ± 0.81 110–140, 126.9 ± 1.74	Narrow medulla with vacuoles	0.54–0.62., 0.581 ± 0.004

Table 2
Observed variations in cross-sections and pigments among the ten Indian Bovid species.

Species	Hair cross-section			Cortical pigments		
	Shape	Medulla shape	Shape of margin	Pigment colour	Pigment size	Intensity of distribution
Antilope cervicapra	Kidney shaped	Kidney shaped	Smooth	Dark to brown	Large	Distributed in clumps to outer cortex
Gazella bennettii	Oblong to dumb bell	Oblong to dumb bell	Smooth	Brown	Large	Distributed more towards extremities
Tetracerus quadricornis	Oval to oblong	Granular with vacuoles	Smooth	Light brown	Large	Distributed in clumps across cortex
Hemitragus hylocrius	Dumb bell with a thicker cortex towards extremities and narrow cortex near medial constriction	Dumb bell with irregular margins	Smooth	Dark brown	Large	Distributed in clumps towards extremities
Capricornis sumatraensis	Oval	Irregular with large cortical intrusions.	Smooth	Light brown	Large	More towards medullar side of cortex
Bos gaurus	Circular	Circular and granular	Smooth	Dark	Large	Pigment distributed in clumps
Pseudois nayaur	Circular	Circular	Irregular	Mostly absent	–	–
Ovis ammon hodgsonii	Oval	Oval	Irregular	Mostly absent	–	–
Ovis vignei vignei	Circular	Oval	Smooth	Mostly absent	–	–
Pantholops hodgsonii	Circular	Circular	Irregular	Mostly absent	–	–
Bos taurus	Oval	Oval	Smooth	Absent to light brown and dark (varying shades of brown)	Large if present	Distributed in clumps
Capra hircus	Oval to oblong	Oval to oblong	Smooth	Absent to light brown and dark (varying shades of brown)	Large if present	Distributed evenly in cortex
Ovis aries	Oval	Oval	Not very regular margin	Absent to light brown and dark (varying shades of brown)	Large if present	Distributed evenly in cortex

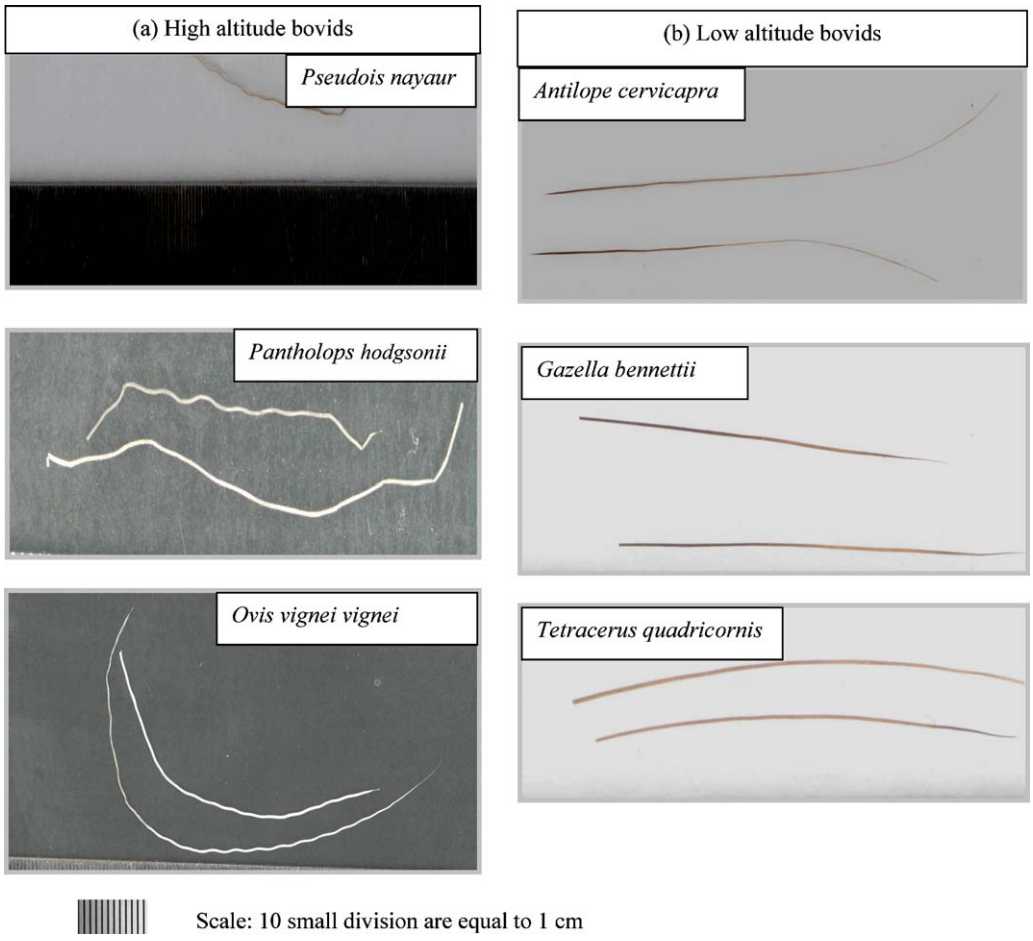

Plate 1. Variation in the hair outline of high altitude bovids and low altitude bovids.

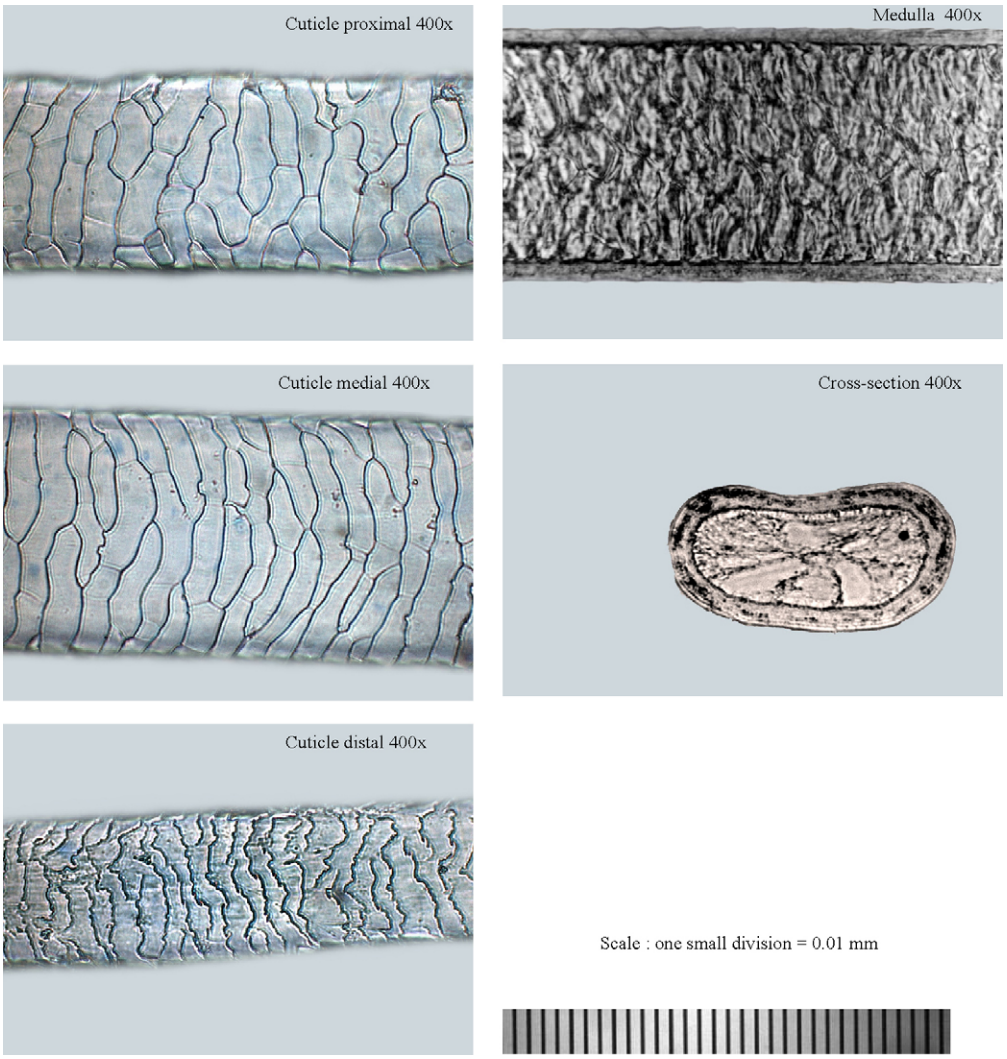

Plate 2. *Antilope cerivcapra* (photomicrographs showing cuticle, medulla and cross-section).

species, along with standard error (S.E.) are given in Table 1. Scale count index values from the high altitude mammals (Greater and Trans Himalayas) were comparatively low at the proximal and medial regions as seen in case of *P. nayaur*, *O. ammon*, *O. vignei* and *P. hodgsonii*. Among all the species studied, *P. hodgsonii* showed the lowest scale count index values at the proximal and medial regions.

The analysis of variance (one way ANOVA) for scale count index values between different individuals of the same species showed no significant ($P > 0.05$) differences. Levene's test for homogeneity of variance in the data for scale count index values (proximal, medial and distal) of individuals from same species indicated a significant homogeneity of variance ($P > 0.05$) for all species except *A. cervicapra* which showed insignificant ($P < 0.05$) homogeneity of variance in scale count index values at the distal region. Tukey's HSD test also showed insignificant variation in scale count index across individuals of same species ($P > 0.05$). Dunnett's *C* test for variance in scale count index of *A. cervicapra* at distal region also showed low variation in scale count index across the individuals of the same species ($P > 0.05$).

Interspecies analysis of variance (one way ANOVA) for scale count index values showed significant ($P < 0.05$) differences in the scale count index values between different species as a group at the proximal, medial and distal region. Levene's test for homogeneity of variance in the data for scale count index values (proximal, medial and distal) between different species showed insignificant homogeneity of variance ($P < 0.05$). Dunnett's *C* test (post hoc test) also showed significant differences ($P < 0.05$) in scale count index values at the proximal region for all species except for the insignificant differences ($P > 0.05$) between *O. ammon* and *O. vignei*, between *T. quadricornis* and *C. sumatraensis*, between *B. taurus* and *H. hylocrius* and, between *C. hircus* and *O. aries*. For medial region Dunnett's *C* test showed significant differences ($P < 0.05$) between all the species except for the insignificant differences ($P > 0.05$) in the scale count index values among *G. bennettii*, *H. hylocrius* and *B. gaurus* and among *T. quadricornis*, *C. sumatraensis* and *B. taurus*. For the distal region although one way ANOVA showed significant differences ($P < 0.05$) but post hoc test (Dunnett's C) showed insignificant differences ($P > 0.05$) in the scale count index values among, *A. cervicapra*, *O. vignei* and *C. hircus*; among *P. nayaur*, *C. sumatraensis*, *B. gaurus* and *O. aries* and between *T. quadricornis* and *Hemitragus jemlehicus*.

The medulla pattern and medullary index values (Table 1) were found to be interesting and showed good group specificity and in certain cases species specificity. The medulla pattern in all the species was wide lattice medulla except for *C. sumatraensis* which showed a narrow lattice medulla (Plate 6) and *B. gaurus* where a medium width amorphous medulla was observed (Plate 7). The high altitude mammals showed medulla index values greater than 0.95 (Table 1). Medulla index values were least in *C. sumatraensis*

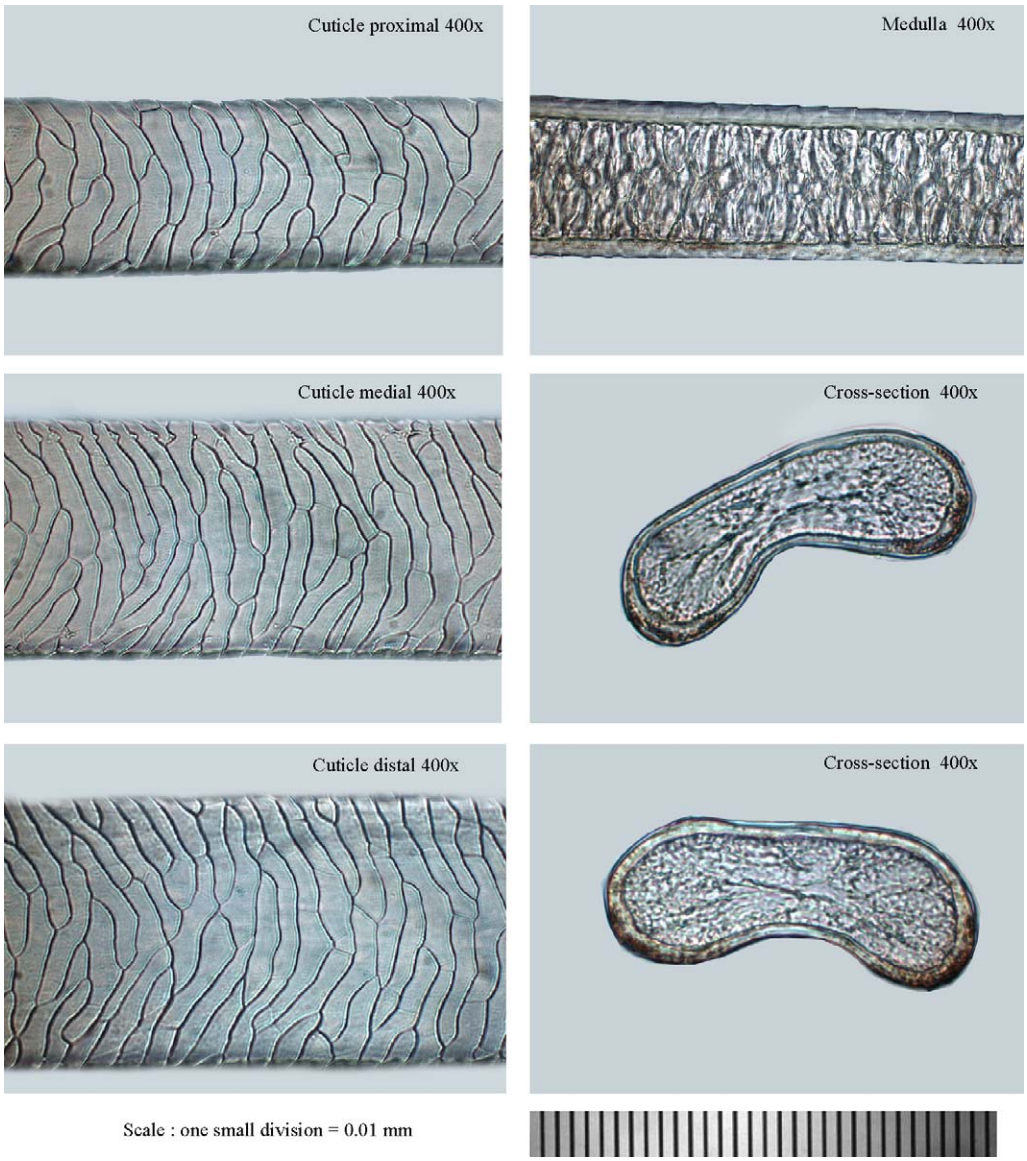

Plate 3. *Gazella bennettii* (photomicrographs showing cuticle, medulla and cross-section).

(0.437) whereas little higher medulla index (0.61) was observed in *B. gaurus*. The remaining four species medulla index values ranged from 0.71 (*T. quadricornis*) to 0.87 (*A. cervicapra*). Analysis of variance (one way ANOVA) for medullary index values between individuals of same species indicated insignificant ($P > 0.05$) variation. Levene's test for homogeneity of variance in the data for medullary index values from individuals of the same species indicated a significant homogeneity of variance ($P > 0.05$) in all species except for *A. cervicapra* and *G. bennettii* which revealed insignificant homogeneity ($P < 0.05$) in the data for medullary index. Tukey's HSD test also indicated low variation in medullary index across individuals of same species ($P > 0.05$) and similarly, Dunnett's *C* test revealed low intraspecies variation ($P > 0.05$) in medullary index values for *A. cervicapra* and *G. bennettii*.

Interspecies analysis of variance (one way ANOVA) for medullary index values revealed significant ($P < 0.05$) differences in the medullary index values between different species as a group. Levene's test for homogeneity of variance in the data for medullary index values between different species indicated insignificant homogeneity of variance ($P < 0.05$). Dunnett's *C* test also revealed significant differences ($P < 0.05$) in medullary index values for all the species except for the insignificant differences ($P > 0.05$) observed between *G. bennettii* and *H. hylocrius*, between *P. nayaur* and *P. hodgsonii*, between *O. ammon* and *P. hodgsonii* and between *T. quadricornis* and *B. taurus*.

The cross-section patterns were mostly circular and oval in high altitude mammals with a very wide medulla and inconspicuous pigment (Table 2). The cross-section shapes were found to be unique for some species. A reniform cross-section and similar configuration of medulla, with dark pigment distributed towards outer cortex was observed in case of *A. cervicapra* (Plate 2). A dumb-bell shaped cross-section with light pigment distributed towards extremities was observed in case of *G. bennettii* (Plate 3). A circular cross-section was observed in *B. gaurus* with a granular medulla and dark pigment granules evenly distributed in the cortex (Plate 7). *H. hylocrius* revealed a dumb-bell shaped cross-section with a thicker cortex towards the two extremities and narrow cortex near medial constriction (Plate 5). The dark pigment was distributed in clumps towards extremities. The most unique cross-section was observed in *C. sumatraensis* with an oval outline but an irregular medulla outline (big cortical intrusions) and pigment distribution more towards the medullar side of cortex (Plate 6).

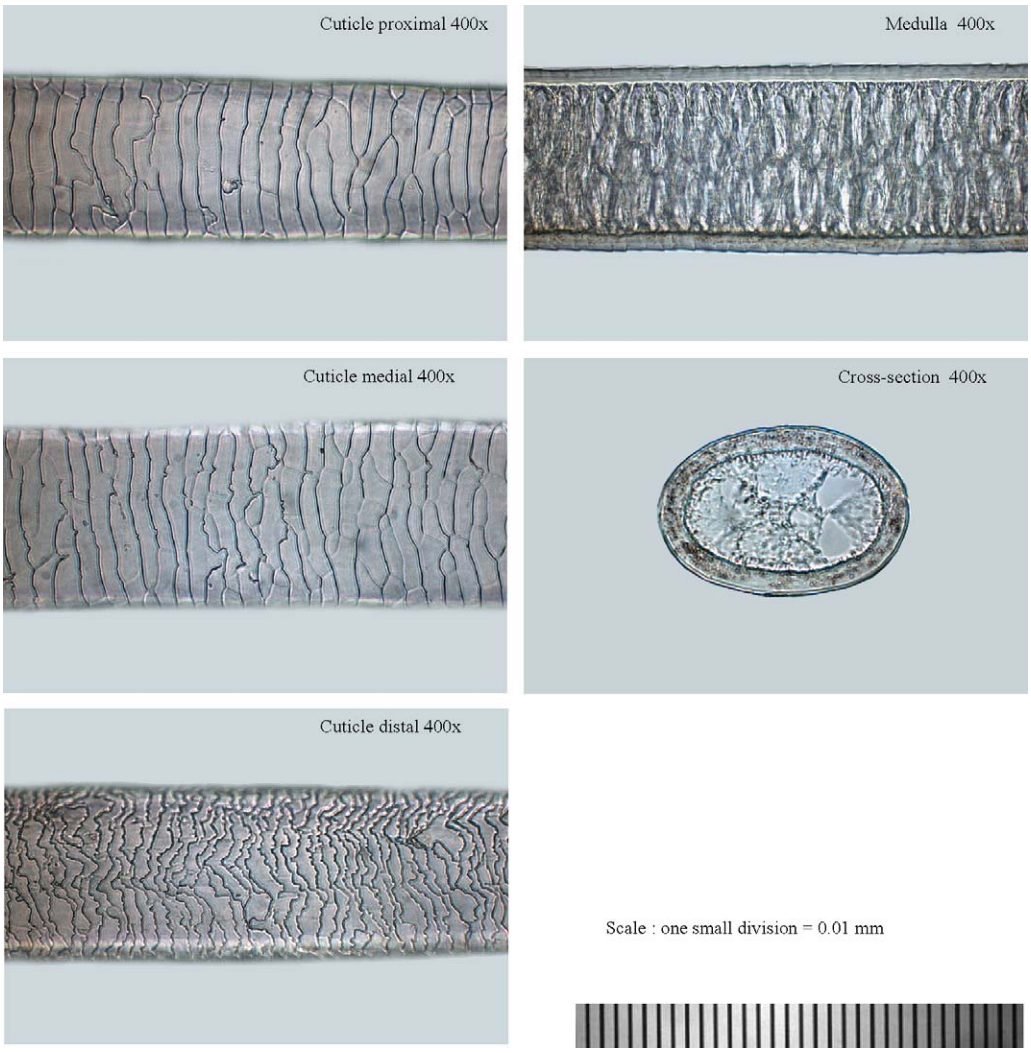

Plate 4. *Tetracerus quadricornis* (photomicrographs showing cuticle, medulla and cross-section).

4. Discussion

It becomes evident from the study that hairs of high altitude bovids (*P. nayaur*, *P. hodgsonii*, *O. vignei* and *O. ammon*) have a distinct kinky shape with a peculiar brittleness. In contrast to this, bovids from plains and peninsular parts of India (*A. cervicapra*, *G. bennettii*, *B. gaurus*, *T. quadricornis*) lack brittleness of hair. The domesticates studied also showed hard and straight hair. The hair of high altitude bovids (*P. nayaur*, *P. hodgsonii*, *O. vignei* and *O. ammon*) are mostly thicker than those of bovids from plains and peninsular India, but there is an overlap for some species (*H. hylocrius* where hair thickness is $\geq$200 μm), hence it cannot be used as alone as a species specific marker. Hence, thick, kinky and brittle hair may be used as an indicative of high altitude bovids. Cuticular patterns do set apart high altitude bovids a they mostly show broad petal and regular mosaic patterns at the proximal and medial region. Other bovids mostly show regular wave and irregular wave pattern. Hence it cannot provide a correct judgement about the species except for *P. hodgsonii* which gets distinguished from all other species examined, on the basis of regular mosaic cuticular pattern at proximal and medial position. The scale count index values although highly consistent within species where the analysis of variance (one way ANOVA) showed *P* values greater than 0.05, however show some overlap in case of some species, as revealed by post hoc test (Dunnett's *C*) with *P* values greater than 0.05. Hence alone scale count index values cannot be used for species characterisation.

Medulla type and medullary index values appear to be quite useful for characterisation of some species. *C. sumatraensis* and *B. gaurus* are most distinct due to their medulla patterns, narrow medulla lattice and medium width amorphous medulla respectively. High altitude bovids can be distinguished from other bovids owing to their high medullary index values ($\geq$0.95). Medullary index values for *C. sumatraensis* (0.43) and *B. gaurus* (0.61) were again most peculiar among all species studied. Although there is a significant consistency in the medullary index values among the individuals of same species with *P* values greater than 0.05, but there are some overlaps when compared with other species as revealed by Dunnett's *C* test. Hence, alone medulla type or medulla index values cannot be used as species identification characteristic. The cross-sections are also good markers for group identification with high altitude bovids mostly having circular and oval cross-sections and they lacked visually appreciable amount of pigments. Bovids from plains and peninsular India showed a vivid range of cross-sections and may be used as a useful parameter for species characterisation. Domesticates showed a great range of pigment shades (Table 2).

From the results it becomes evident that no single characteristic can be used to characterise/identify a species, except some characteristics that were found to be unique for some species

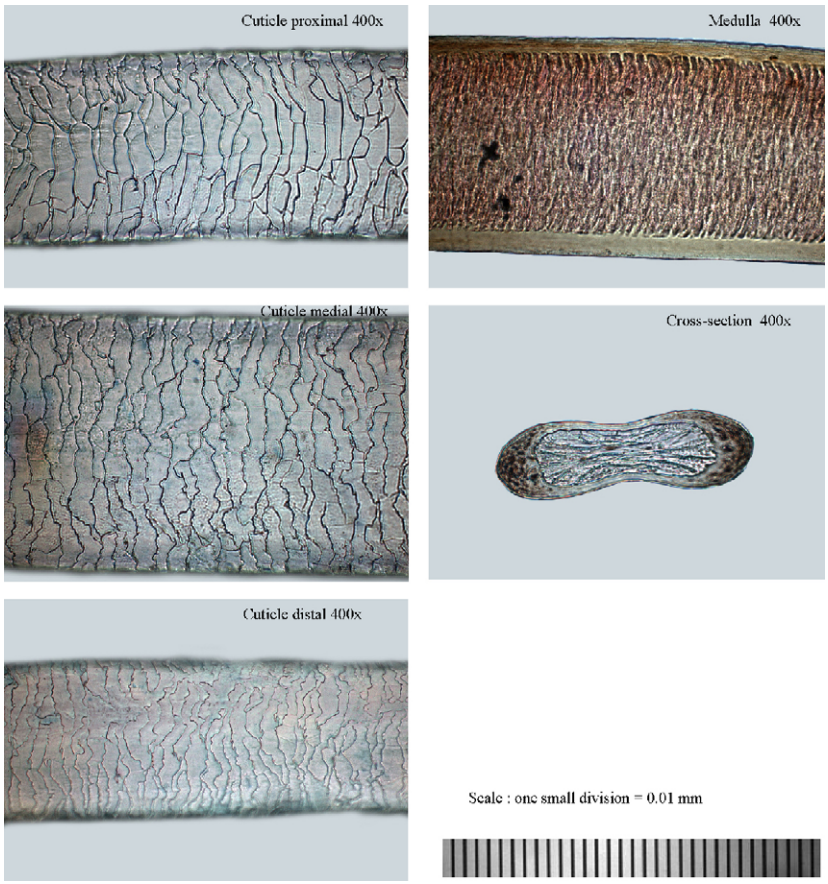

Plate 5. *Hemitragus hylocrius* (photomicrographs showing cuticle, medulla and cross-section).

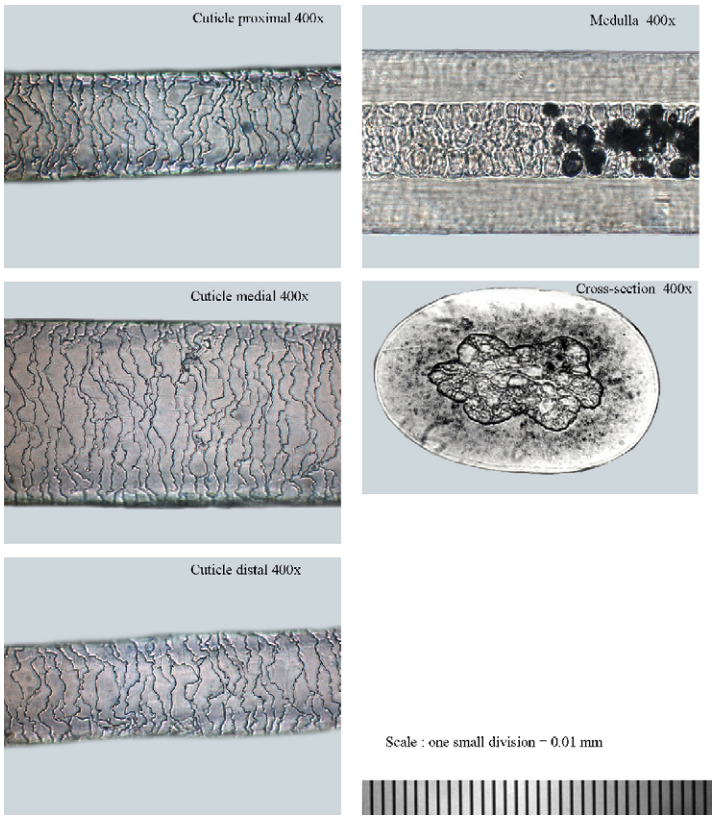

Plate 6. *Capricornis sumatraensis* (photomicrographs showing cuticle, medulla and cross-section).

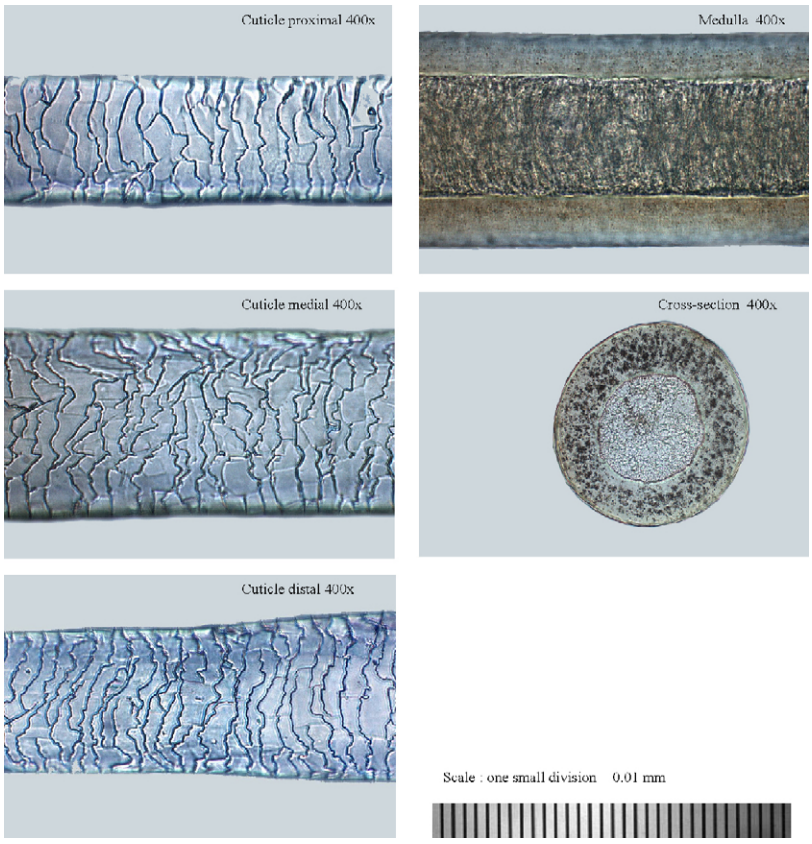

Plate 7. *Bos gaurus* (photomicrographs showing cuticle, medulla and cross-section).

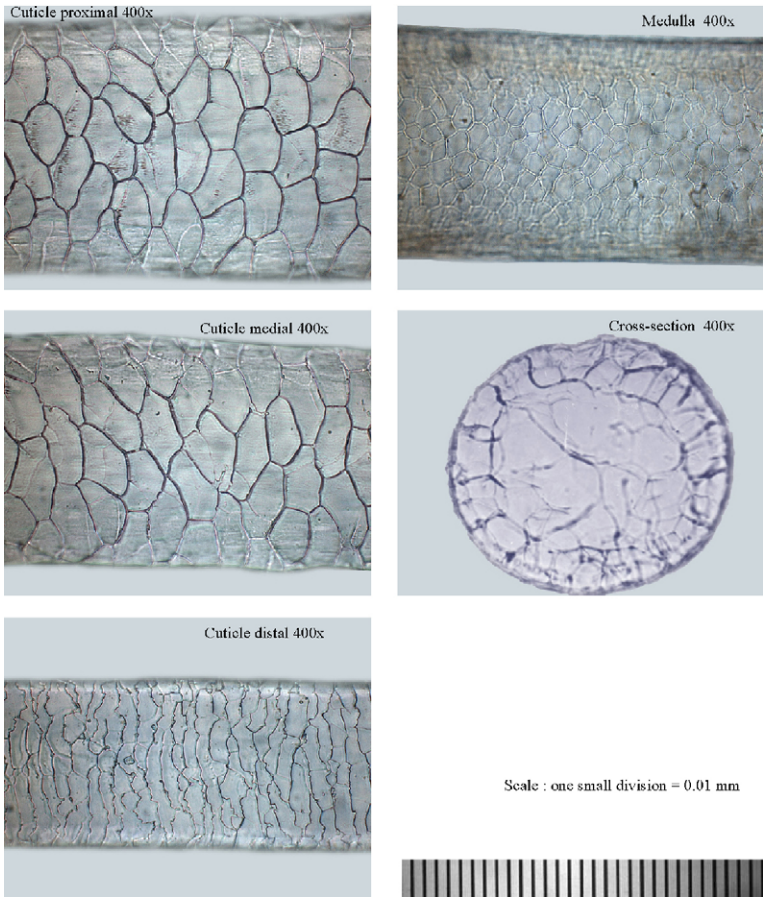

Plate 8. *Pseudois nayaur* (photomicrographs showing cuticle, medulla and cross-section).

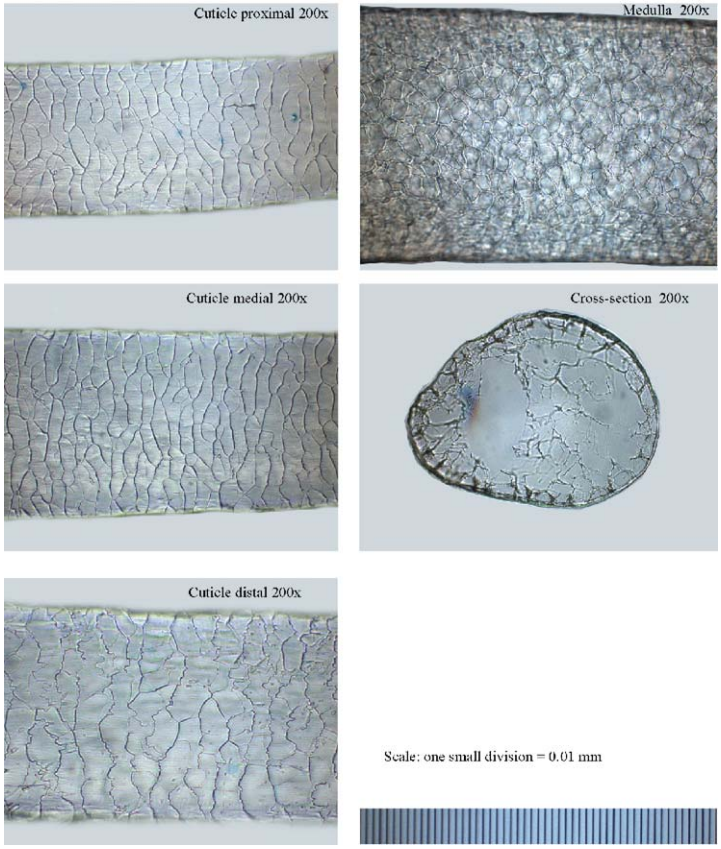

Plate 9. *Ovis ammon hodgsonii* (photomicrographs showing cuticle, medulla and cross-section).

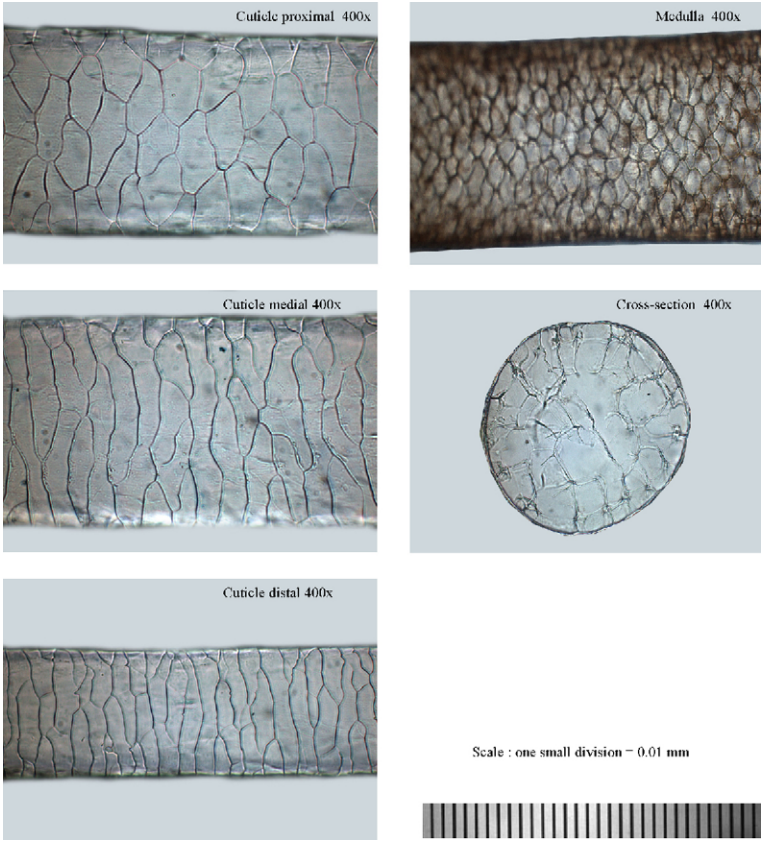

Plate 10. *Ovis vignei vignei* (photomicrographs showing cuticle, medulla and cross-section).

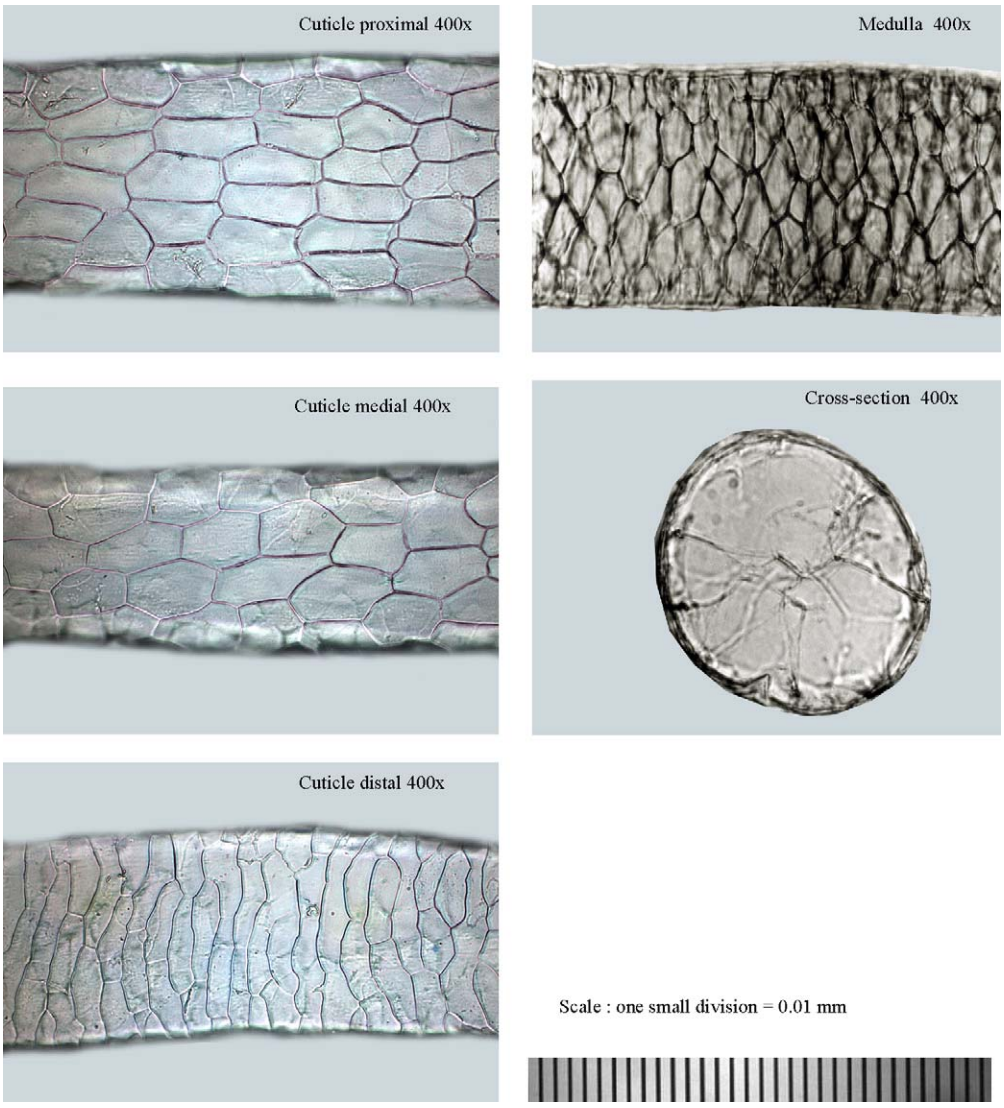

Plate 11. *Pantholops hodgsonii* (photomicrographs showing cuticle, medulla and cross-section).

like the regular mosaic scale pattern found in *P. hodgsonii*. Similarly, *B. gaurus* and *C. sumatraensis* can be distinguished from others on the basis of their unique medulla patterns and medulla index values. Some species show unique cross-sections (*C. sumatraensis* and *H. hylocrius*). However in some species there is a great deal of overlap in most of the characteristics. *A. cervicapra* and *G. bennettii*, which are sympatric in their habitat, show similarity in most of the microscopic hair characteristics but differ in medullary index values. However in case of high altitude bovids (*P. nayaur*, *P. hodgsonii*, *O. vignei* and *O. ammon*) the medulla index values become least useful for species characterisation due to the great deal of overlap in medullary index values among species. In such cases hair thickness and cuticular patterns appear more important for species characterisation. In case of *P. nayaur* and *O. vignei*, *hair* thickness and cuticular pattern at the medial region help in distinguishing them from each other. Likewise, in case of *O. vignei* and *O. ammon*, the hair thickness and cuticular pattern at the proximal and distal region help to distinguish them. As the animals have a distinct coat colour which is reflected in their guard hair, it would be always worthwhile to consider colour of hair as corroborative characteristic in species identification from hair. Comparison with some commonly encountered domesticates shows that there is no single characteristics that can distinguish them from wild bovids but, a combination of characteristics is useful.

5. Conclusion

For a few species (*P. hodgsonii*, *C. sumatraensis*, and *H. hylocrius*) a single parameter, such as visual characteristics, can be diagnostic of the genus. As there are many more mammalian species is India than those in this study it is not reliable to use a single parameter for species identification as there can be overlaps between some species when such a single parameter is considered. This study highlights the need for a maximum number of parameters in hair comparison to be used to increase the confidence in species identification. Closely related species or those with sympatric phenotypes should be included in such comparisons. Dorsal guard hairs of domesticates can also be distinguished from wild bovids using a combination of all the characteristics detailed in this paper. We conclude that by a combination of all the microscopic characteristics detailed, it may be possible to differentiate between the 10 wild species studied in this paper and further they can be distinguished from the domesticates. The findings may help in identification of species from wildlife offence case exhibits related to ten protected species studied, thus leading to conservation of

Indian bovids by successful implementation of the Wildlife (Protection) Act 1972 in India and achieving the aims of CITES. Further, studies are however required to cover all the possible species in wildlife trade such that a better comparison can be made.

Acknowledgements

The authors are grateful to the Director and Dean, Wildlife Institute of India, for the great support provided during the study. Help and support provided by the staff of the Wildlife Forensic Laboratory, Wildlife Institute of India is greatly acknowledged.

References

[1] S.C. Dey, Wildlife trade: global perspective and the Indian scenario, Central Bureau of Investigation, Bulletin 4 (1996) 6–8.
[2] M.S. Mani, Ecology and Biogeography in India, vol. 23, Dr. Junk Publisher, The Hague, 1974, p. 727.
[3] P.O. Nameer, Cheklist of Indian Mammals, KFRI (Wildlife Wing) and Kerala Agricultural University, 2000, pp. 1–90.
[4] F. Hanfee, Wildlife Trade: A Handbook for Enforcement Staff, vol. 4, WWF Tiger Conservation Program, 1998,, p. 56.
[5] Indian Wildlife (Protection) Act-1972 (with amendments), A Hand Guide with Case Law and Commentaries, Natraj Publishers, Dehradun, pp. 215.
[6] H. Brunner, B.J. Coman, The Identification of Mammalian Hair, Inkata Press, Victoria, Australia, 1974, pp. 1–196.
[7] T.D. Moore, L.E. Spence, E.E. Dugnolle, Identification of the Dorsal Guard Hairs of some Mammals of Wyoming, Wyoming Game and Fish Dept, 1974, pp. 1–77.
[8] B.J. Teerink, Hair of West-european Mammals, Cambridge University Press, Cambridge, 1991, pp. 1–223.
[9] H.L. Short, Analysis of cuticular scales on the hair using the scanning electron microscope, J. Mammal. 59 (1978) 261–268.
[10] K.H. Phan, F.J. Wortmann, Aspects of morphology of Cashmere and Yak fibres, Schriftenreihe des DWI 101 (1987) 137–140.
[11] C.K. Rollins, D.M. Hall, Using light and scanning electron microscopic methods do differentiate Ibex Goat and Tibetan antelope fibers, Text. Res. 69 (1999) 856–860.
[12] K.H. Phan, G. Wortmann, F.J. Wortmann, Microscopic characteristics of Shahtoosh and its differentiation from Cashmere/Pashmina, in: 10th International Wool Research Conference, vol. 2, Aachen, Germany, (2000), pp. 1–16.
[13] B.R. Koppikar, J.H. Sabnis, Identification of hairs of some Indian Mammals, J. Bombay Nat. Hist. Soc. 73 (1976) 5–20.
[14] B.R. Koppikar, J.H. Sabnis, Further studies on the identification of hairs of some Indian Mammals, J. Bombay Nat. Hist. Soc. 74 (1977) 50–59.
[15] J.K. De, Study of surface structure of hair of some primates of Indian Subcontinent, Rec. Zool. Surv. India 93 (1993) 31–34.
[16] J.K. De, R. Chakraborty, S. Chakraborty, Identification of dorsal guard hairs of Indian species of genus *Panthera* Oken, Mammalia 60 (1996) 473–480.
[17] J.K. De, S. Chakraborty, R. Chakraborty, Identification of dorsal guard hairs of five Indian species of Mongoose, *Herpestes* Illiger (Mammalia: Carnivora), Mammalia 62 (1998) 285–295.
[18] R. Chakraborty, S. Chakroborty, J.K. De, Identification of dorsal guard hairs of three species of Indian Lesser Cats (Carnivora: Felidae), Mammalia 65 (1999) 93–104.
[19] R. Chakraborty, J.K. De, Structure of mid-dorsal guard hairs of Hunting Leopard, *Acinonyx jubatus venaticus* (Griffith) and Lesser Panda, *Ailurus fulgens* (Mammalia: Carnivora), Rec. Zool. Surv. India 100 (2002) 131–136.
[20] V. Sahajpal, S.P. Goyal, R. Jayapal, K. Yoganand, M.K. Thakar, Hair characteristics of four Indian bear species, Sci. Justice 48 (2008) 8–15.
[21] A. Bahuguna, S.K. Mukherjee, Use of SEM to recognize Tibetan antelope (Chiru) hair and blending in wool products, Sci. Justice 40 (2000) 177–182.
[22] P.L. Kirk, J.H. Gamble, Further investigation of the scale count of human hair, J. Crim. Law Criminol. 33 (1942) 276–280.
[23] D.E. Wilson, D.M. Reeder (Eds.), Mammal Species of the World, Smithsonian Institution Press, Washington, 1993, p. 1206.