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The identification of pika and hare through tricho-taxonomy (Mammalia: Lagomorpha)

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Abstract: The macroscopic and microscopic characters of dorsal guard hairs of Indian lagomorphs (four species of pikas and three species of hare) are described; the cuticular and medullary characters are similar between the species studied. The cuticular and medullary characters, however, are dissimilar between the family Ochotonidae and Leporidae. The cross-section of hair of the species had shown two identical shapes between the family Ochotonidae and Leporidae. The cross-section was observed as an oval shape in all the four ochotonid species, whereas there was a dumb-bell shape in all three leporid species. The hair of the Indian lagomorphs can easily be differentiated up to the family level on the basis of their unique cuticula, medulla and cross-section of the dorsal guard hair. The high-resolution microphotographs and key characteristics of hair that are presented here can be used as an appropriate reference for family-level identification of Indian lagomorphs.

Keywords: Cuticular, dorsal guard hairs, lagomorphs, medullary character, microphotographs.

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Author contribution: MK conducted the laboratory examinations, designed the study and prepared the manuscript. KC directed and encouraged the study and provided the necessary facilities to accomplish the work. JKD and CV supervised the study.

INTRODUCTION

Mammalian hair characters are one of the important features that can be used to identify the species when the external morphology is unable to help with identification in case only of a small part of the skin of the mammal is available (Teerink 1991; Chakraborty & De 2010). Tricho-taxonomy (the study of hair) is relatively significant in the study of the food habit of carnivores and is supportive of controlling the illegal trade of wildlife and its derivatives (Chakraborty & De 2010; Sahajibal et al. 2010). There are many researchers, viz., Mayer (1952), Stains (1958), Brunner & Comman (1974), Moore et al. (1974), Koppiker & Sabins (1976), Teerink (1991), Wallis (1993), Chakraborty & De (2010), and Dharaiya & Soni (2012), who have documented the different hair characters of mammals well. Least importance has been given to the species belonging to the order Lagomorpha, except for a few studies by Moore et al. (1974) and Teerink (1991).

The order Lagomorpha comprises of two living families: Ochotonidae and Leporidae. The family Ochotonidae comprises the pikas, under the single genus *Ochotona*; out of a total of 30 species worldwide, India has seven species. The family Leporidae includes hares and rabbits consisting of 61 species under 11 genera, of which India has four species under two genera (Wilson & Reeder 2005).

Ochotonids are distinguished by a small-sized body (head-body length: average 15cm) and weighing 70–300 g, having greyish-brown silky fur. Unlike leporids, the pikas lack a visible tail and have short rounded ears, short limbs, with the hind limbs being barely longer than the forelimbs (Vaughn et al. 2000; Smith 2008; Sokolov et al. 2009).

The leporids are distinguished by a medium-sized body (head and body length: 40–70 cm), long hindlimbs and feet, a small visible tail, and relatively long ears (up to 20cm in length). Most leporids are counter-coloured, with dark-coloured dorsal pelage and light-coloured ventral pelage. Pelage texture can be thick and soft or coarse and woolly (e.g., Hispid Hare) and may become increasingly sparse along the length of the ears. Rabbits and hares have short bushy tales, which are sometimes conspicuously marked, and the soles of their hind limbs are covered with hair (Nowak 1999; Vaughn et al. 2000; MacDonald 2001; Sokolov et al. 2009).

The above-mentioned morpho-taxonomic characters have differentiated the families Ochotonidae and Leporidae. The present tricho-taxonomy study, however, helps to differentiate the two families only with the help of hairs when morpho-taxonomy is unable to offer the fruitful result (Teerink 1991; Chakraborty & De 2010).

METHODS

A bunch of dorsal guard hairs was collected from five, dry, preserved skins of four pika species, namely Ladakh Pika *Ochotona ladacensis* (Günther, 1875), Large-eared Pika *Ochotona macrotis* (Günther, 1875), Royle's Pika *Ochotona roylei* (Ogilby, 1839), and Moupin's Pika *Ochotona thibetana* (Milne-Edwards, 1871) of the family Ochotonidae, and three species of hare, namely, Hispid Hare *Caprolagus hispidus* (Pearson, 1839), Indian Hare *Lepus nigricollis* F. Cuvier (1823), and Woolly Hare *Lepus oiostolus* Hodgson (1840) of the family Leporidae, housed at the National Zoological Collections of Zoological Survey of India, Kolkata, India.

The morphological characters of hairs (n=20) such as colour, number of bands and profile of hairs were recorded, and the length and diameter of hairs were measured using a dial calliper (Mitutoyo). To study the cuticular characters, the acetone washed hair samples were placed over the varnish coated-microscopic glass slide and the dried hairs were dragged gently over it to leave the imprint of scales over the microscopic glass slide. To study the medulla characters, the hair samples were mounted over the microscopic glass slide using D.P.X. To study the shape of the cross section, the hair samples were hand sliced and mounted over the microscopic glass slide using D.P.X. The cuticular characters of hair such as scale position, scale patterns, structure of scale margins and distance between scale margins, the medullary characters such as width composition, the structure and form of margins of the medulla and the shape of cross-section of hairs were examined and photographed (400x magnifications) using a digital camera set onto an optical microscope (Olympus BX41).

To obtain the three-dimensional structure and a more detailed examination of cuticular scales of the hair, the scanning electron microscope (ZEISS Evo18 - special edition) was used. The cuticular structures of hairs were observed under the high magnifications 1630x and 2600x, and the observed cuticular structures of hairs were photographed.

The measurement data such as the maximum, minimum, mean and standard deviation of cuticular scales and medulla were obtained through the digital scale fitted on an optical microscope. The methodology was followed according to the descriptions provided by Brunner & Comman (1974) and Teerink (1991). The description of different terms of patterns used in the

results and discussion that have been given herewith were followed from Teerink (1991) and the nomenclature of colour was followed as per Ridgway (1886).

RESULTS

Family Ochotonidae

The pelage colour of four species of the family Ochotonidae show different shades of brownish-grey; however, the colour of single guard hairs that was observed was grey-buff. The hair of all four species were observed as bicoloured with two bands. The profile of the hair of all species had shown no variations and was observed as a wavy form (Table 1).

The mean length of hair significantly varied among the four species (range: 8.5–22.6 mm): the maximum length was recorded in *Ochotona roylei* (16.6±3.4 mm) and the minimum in *O. ladacensis* (11.7±1 mm), the mean length of hair of *O. macrotis* and *O. thibetana* were recorded as 15.6±4.7 and 16.3±3.1 mm, respectively (Table 1). The mean diameter of hair also significantly varied among the four species (range: 11.4–56.3 µm): The maximum diameter was recorded in *O. macrotis* (44.7±14.4 µm) and the minimum in *O. thibetana* (32.2±10 µm), the mean diameter of hair of *O. ladacensis* and *O. roylei* was recorded as 38.6±11.4 and 33.4±8.2 µm, respectively (Table 1).

The hair of four species had shown almost similar cuticular characters (Images 1 & 7) between the species: the scale position, scale patterns, the structure of scale margins and distance between scale margins were observed in all the four species as 'transversal', 'streaked' ('regular wave' in *O. thibetana*), 'smooth' and 'near', respectively (Table 2).

The measurement values had shown significant variations among the four species, the mean scale count per millimetre length of hair (range: 69–201 µm) was highest in *O. ladacensis* (158±34.8 µm) and lowest in *O. roylei* (82.8±11.7 µm). The mean length of cuticular scales (range: 20–37.9 µm) was observed; as a maximum (35.9±1.2 µm) in *O. thibetana* and as a minimum (21.8±1.7 µm) in *O. ladacensis*. The mean width of cuticular scales (range: 4.3–13.6 µm) was highest in *O. ladacensis* (10.3±2.1 µm) and lowest in *O. thibetana* (6.3±1.7 µm) (Table 1).

The medullary characteristics of hair (Image 2) showed no variations between four species: the composition of medulla, the structure of medulla and medulla margins were observed as 'multicellular', 'isolated' and 'scalloped', respectively (Table 3).

The mean width of medulla (range: 27.1–47.8 µm) showed slight variations among the species. *Ochotona ladacensis* had the highest (45.1±1.1 µm) mean medullary width while the lowest (34.3±2.8 µm) was in *O. thibetana*. The mean medullary width of *O. macrotis* and *O. roylei* were recorded as 39.3±3 and 34.6±1.1 µm, respectively (Table 3).

The cross-section of hair (Image 3) of the species showed similar shapes in the family Ochotonidae and was observed as an oval shape in all the four ochotonid species (Table 3).

Family Leporidae

The pelage colour of the three species of the family Leporidae had shown different shades of blackish-grey and the colour of a single guard hair had also shown various shades of black yellow. The hair of all three

Table 1. Macroscopic characteristics of dorsal guard hairs of the species of the order Lagomorpha.

Species	Coat colour	Colour of hair	Base	Tip	No. of Bands	Profile	Length (mm)	Width (µm)
Family Ochotonidae								
O. ladacensis	Orangeish, sandy brown or grey	Bicoloured	Slate gray	Buff	2	Wavy	10.3–13.5 (11.7±1)	17.4–49.1 (38.6±11.4)
O. macrotis	Pale brownish-grey with an ochre tinge	Bicoloured	Gray	Buff	2	Wavy	8.5–21.6 (15.6±4.7)	18.1–56.3 (44.7±14.4)
O. roylei	Rufous grey	Bicoloured	Gray	Earth yellow	2	Wavy	11.6–22.6 (16.6±3.4)	18.1–41.2 (33.4±8.2)
O. thibetana	Rich russet brown	Bicoloured	Slate gray	Earth yellow	2	Wavy	13.1–22.6 (16.3±3.1)	11.4–40.1 (32.2±10)
Family Leporidae								
C. hispidus	Brown with black grizzled hair	Bicoloured	Black	Yellow	4	Slightly wavy	14.9–34 (27.5±6.6)	71.5–166.5 (113.2±39.6)
L. nigricollis	Reddish-brown with black hair	Bicoloured	Cream	Black	3	Wavy	12–23.2 (18.5±3.4)	64.1–109.2 (78.6±13.4)
L. oiostolus	Black grizzled with brownish-grey	Bicoloured	Pale yellow	Black	4	Wavy	10.3–33.2 (21.8±7.5)	36.1–76.1 (65.8±14.8)

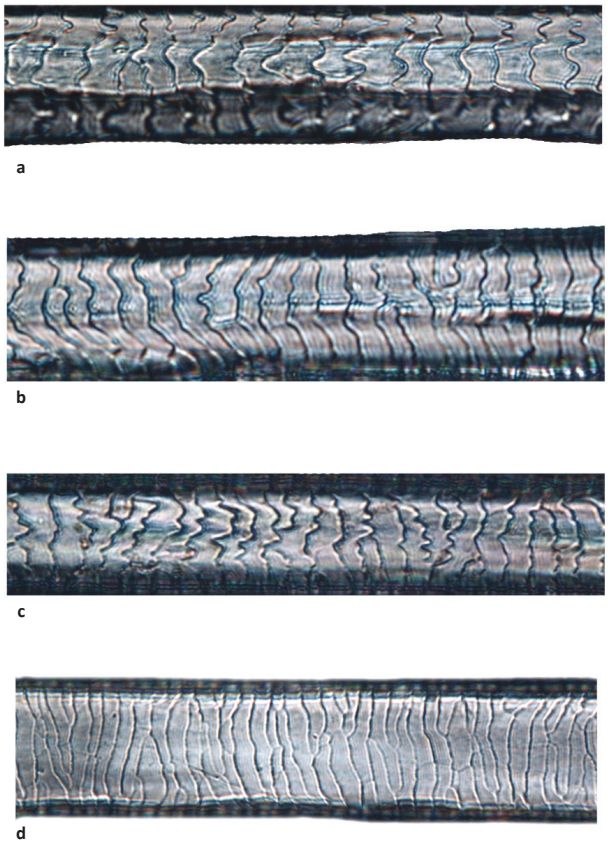


Image 1. Micro-photographs of cuticula (400 X) of dorsal guard hair: a—*O. ladacensis* | b—*O. macrotis* | c—*O. roylei* | d—*O. thibetana*

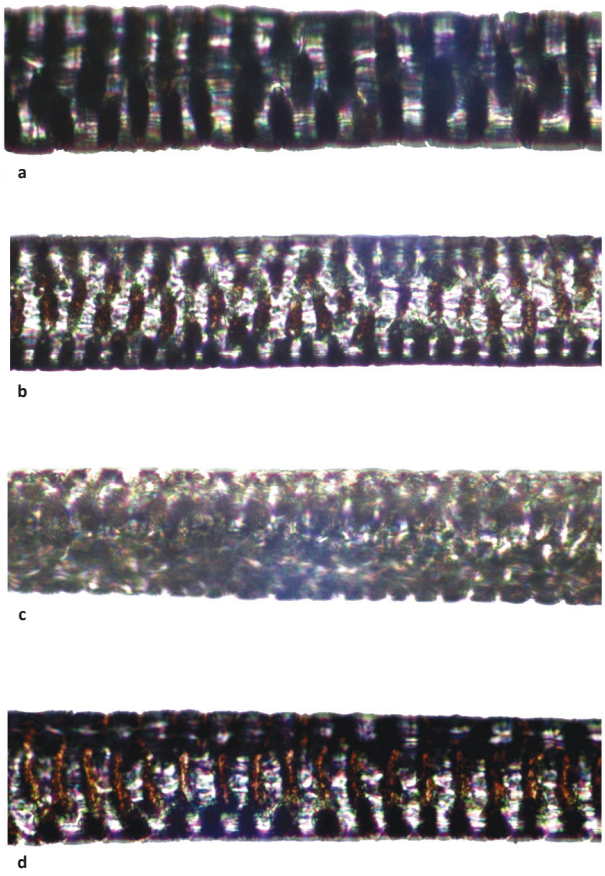


Image 2. Micro-photographs of medulla (400 X) of dorsal guard hair: a—*O. ladacensis* | b—*O. macrotis* | c—*O. roylei* | d—*O. thibetana.*

Table 2. Cuticular characteristics of dorsal guard hairs of the species of the order Lagomorpha.

Species	Scale position	Scale patterns	Structure of scale margins	Distance between scale margins	Scale count/mm length of hair	Length of scale (µm)	Width of scale (µm)
Family Ochotonidae							
O. ladacensis	Transversal	Streaked	Smooth	Near	104–201 (158±34.8)	20–26.3 (21.8±1.7)	6.7–13.6 (10.3±2.1)
O. macrotis	Transversal	Streaked	Smooth	Near	104–160 (139.6±18.5)	23.8–29.6 (27.4±1.6)	6.7–13.6 (9.4±2)
O. roylei	Transversal	Streaked	Smooth	Near	69–102 (82.8±11.7)	28.1–32.1 (30.6±1.4)	6.9–10.2 (8.1±1.4)
O. thibetana	Transversal	Regular wave	Smooth	Near	103–163 (125.8±18.3)	34.1–37.9 (35.9±1.2)	4.3–9.2 (6.3±1.7)
Family Leporidae							
C. hispidus	Transversal	Regular wave	Smooth	Near	176–226 (200.6±15.7)	88.7–116.2 (99.9±7.8)	7.4–11.4 (9.3±2.2)
L. nigricollis	Transversal	Regular wave	Smooth	Near	118–168 (137.9±14.2)	47.1–51.3 (49.3±1.3)	9.6–13.2 (12.2±1.3)
L. oiostolus	Transversal	Regular wave	Smooth	Near	135–160 (148.6±8.5)	34.6–40.8 (37.1±2.1)	14.2–15.1 (14.3±0.8)

species were observed as bicoloured with 3–4 bands. The profile of the hair had shown slight variations and was observed as slightly wavy in *C. hispidus*, and wavy in both *L. nigricollis* and *L. oiostolus* (Table 1).

The mean length of hair significantly varied among the three species (range: 10.3–34 mm): the maximum length was observed in *C. hispidus* (27.5±6.6 mm) and minimum in *L. nigricollis* (18.5±3.4 mm), whereas the mean length of hair of *L. oiostolus* was recorded as 21.8±7.5 mm (Table 1). The mean diameter of hair also significantly varied among the three species (range: 36.1–166.5 µm): the maximum diameter was observed in *C. hispidus* (113.2±39.6 µm) and minimum in *L. oiostolus* (65.8±14.8 µm), whereas the mean diameter of hair of *L. nigricollis* was recorded as

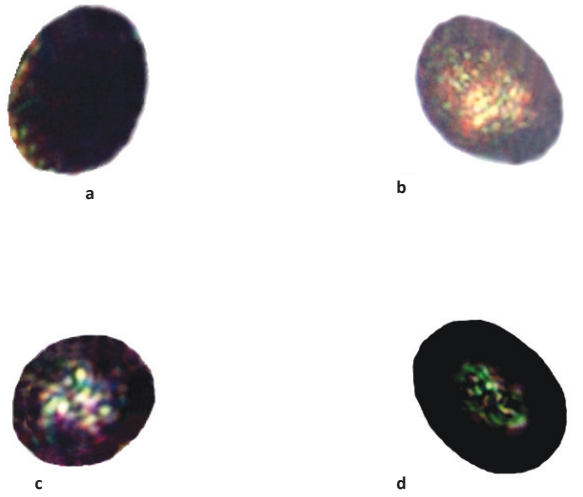


Image 3. Micro-photographs of cross-section (400 X) of dorsal guard hair: a—*O. ladacensis* | b—*O. macrotis* | c—*O. roylei* | d—*O. thibetana.*

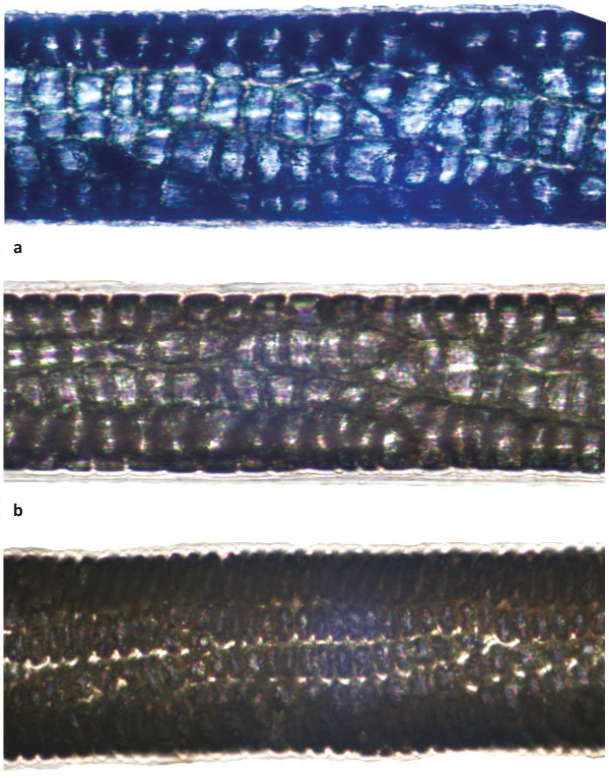


Image 5. Micro-photographs of medulla (400 X) of dorsal guard hair: a—*C. hispidus* | b—*L. nigricollis* | c—*L. oiostolus.*

78.6±13.4 µm (Table 1).

The cuticular characteristics of hairs of all the three leporid species (Images 4 & 8) had shown no variations between the species and were observed with

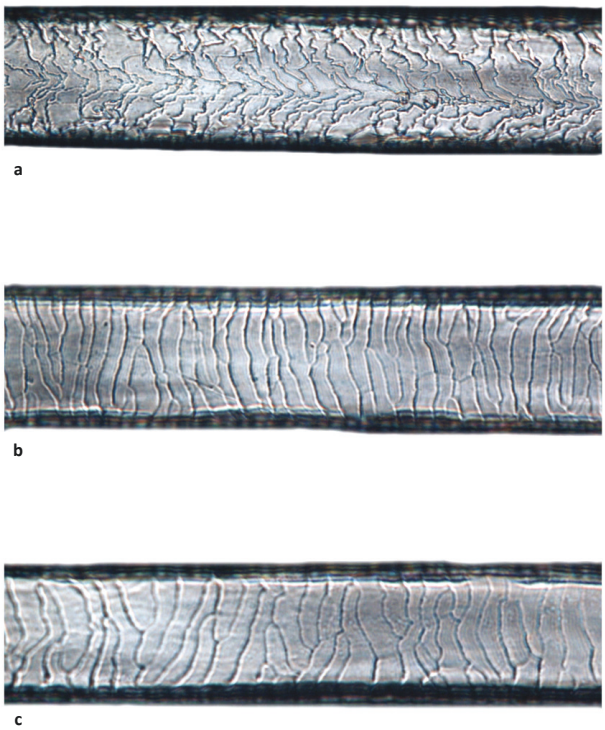


Image 4. Micro-photographs of cuticula (400 X) of dorsal guard hair: a—*C. hispidus* | b—*L. nigricollis* | c—*L. oiostolus.*

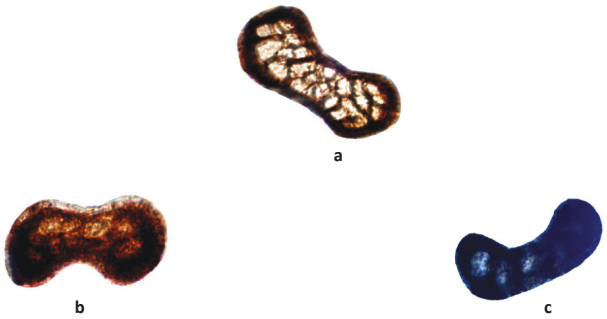


Image 6. Micro-photographs of cross-section (400 X) of dorsal guard hair: a—*C. hispidus* | b—*L. nigricollis* | c—*L. oiostolus.*

scale position- 'transversal', scale patterns- 'regular wave', the structure of scale margins- 'smooth' and distance between scale margins- 'near'. The measurement values had shown slight variations among the species, the mean scale count per millimetre length of hairs (range: 118-226 µm) were observed as maximum in *C. hispidus* (200.6±15.7 µm) and minimum in *L. nigricollis* (137.9±14.2 µm), whereas *L. oiostolus* was 148.6±8.5 µm. The mean length of scale (range: 34.6–116.2 µm) was observed to be the highest in *C. hispidus* (99.9±7.8 µm) and the lowest in *L. oiostolus* (37.1±2.1 µm), whereas *L. nigricollis* was 49.3±1.3 µm. The maximum and minimum of mean

Table 3. Medullary characteristics and shape of cross-section of dorsal guard hairs of the species of the order Lagomorpha.

Species	Composition of medulla	Structure of medulla	Margins of medulla	Width of medulla (µm)	Shape of cross-section
Family Ochotonidae					
O. ladacensis	Multicellular	Isolated	Scalloped	44.1–47.8 (45.1±1.1)	Oval
O. macrotis	Multicellular	Isolated	Scalloped	34.6–45.6 (39.3±3)	Oval
O. roylei	Multicellular	Isolated	Scalloped	33.1–36.1 (34.6±1.1)	Oval
O. thibetana	Multicellular	Isolated	Scalloped	27.1–38.1 (34.3±2.8)	Oval
Family Leporidae					
C. hispidus	Multicellular in rows	Multiserial ladder	Scalloped	7.4–11.4 (9.3±2.2)	Dumb-bell
L. nigricollis	Multicellular in rows	Multiserial ladder	Scalloped	64.1–68.1 (65.9±1.2)	Dumb-bell
L. oiostolus	Multicellular in rows	Multiserial ladder	Scalloped	64.1–69.8 (66.9±2)	Dumb-bell

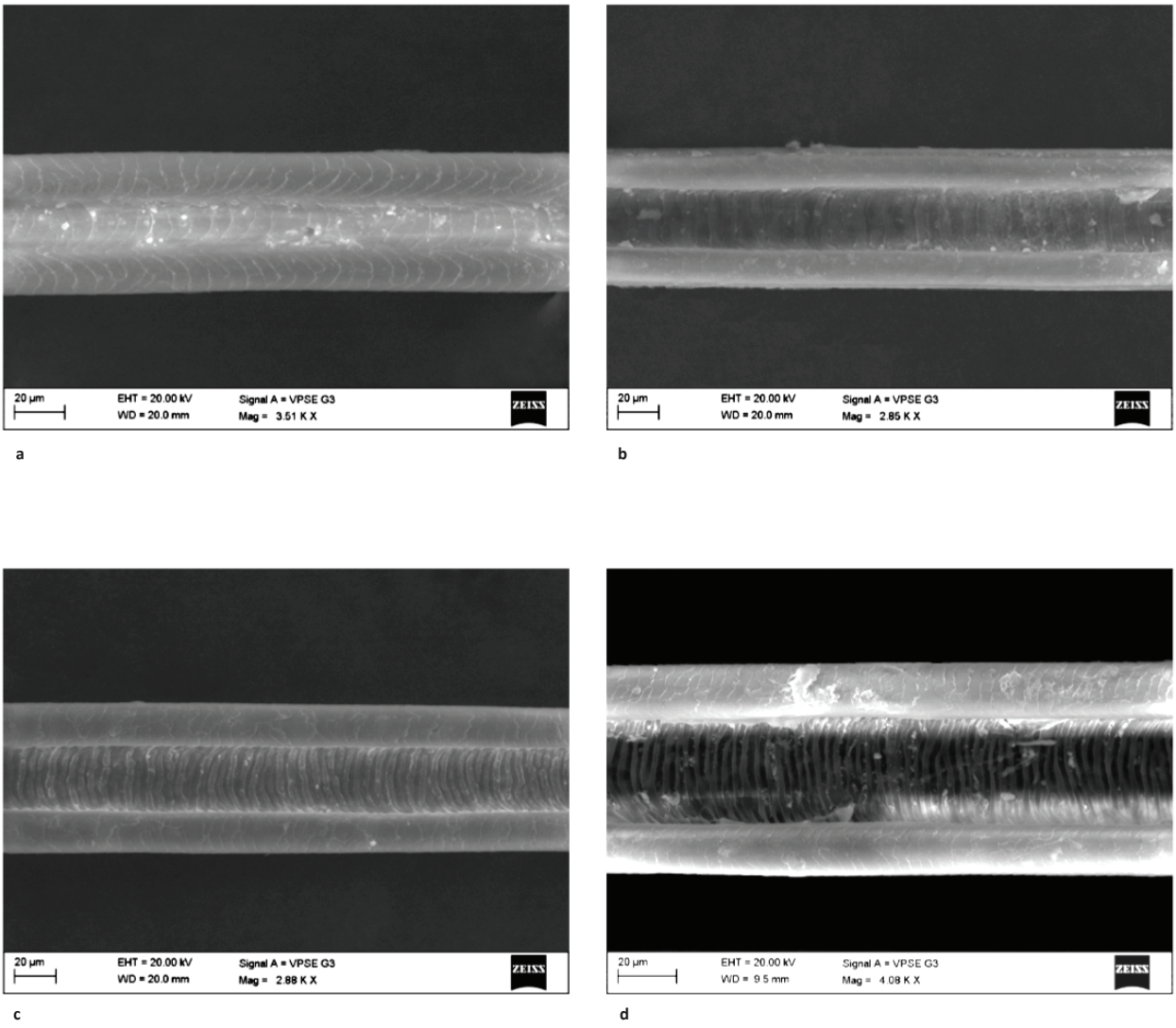
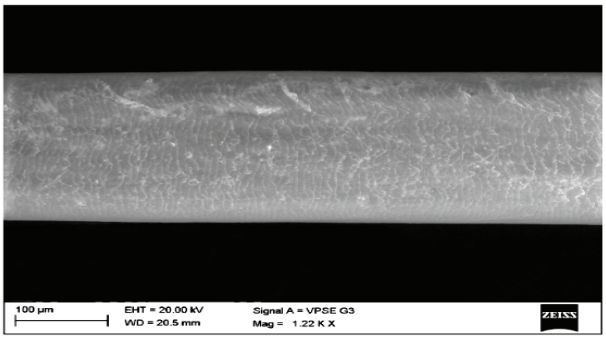


Image 7. Scanning electron micrographs of cuticula (400 X) of dorsal guard hair: a—*O. ladacensis* | b—*O. macrotis* | c—*O. roylei* | d—*O. thibetana*.



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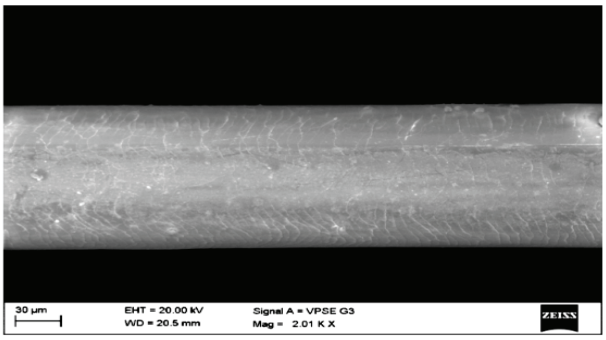

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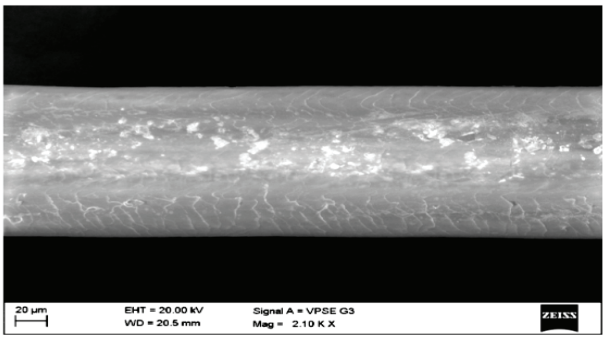

c

Image 8. Scanning electron micrographs of cuticula (400 X) of dorsal guard hair: a—*C. hispidus* | b—*L. nigricollis* | c—*L. oiostolus*.

scale width of hair (range: 7.4–15.1 µm) was recorded in *L. oiostolus* (14.3±0.8 µm) and *C. hispidus* (9.3±2.2 µm), respectively, where *L. nigricollis* was 12.2±1.3 µm (Table 2).

The medullary characteristics of the hair of three species (Image 5) had shown similar characters between the species and were observed as the composition of medulla- 'multicellular in rows', the structure of medulla- 'multiserial ladder' and 'medulla margins-scalloped'. The mean width of medulla was observed to be the highest as 77.1±1.6 µm in *C. hispidus* and lowest as 65.9±1.2 µm in *L. nigricollis*, whereas *L. oiostolus* was 65.9±1.2, µm (Table 3).

The cross-section of hair of the species (Image 6) showed similar shapes in the family Leporidae and was observed as a dumb-bell shape in all the three leporid species (Table 3).

DISCUSSION

Family Ochotonidae

The pikas can be distinguished as the family of the order Lagomorpha by their specific cuticular scale pattern and unique medullary structure such as the different cuticular patterns. The multicellular composition of medulla and isolated structure of medulla of hair differentiates it from the other groups which is confirmed by comparing the previous study of Koppiker & Sabins (1976), Teerink (1991), Chakraborty & De (2010), Dharaiya & Soni (2012), Kamalakannan (2018, 2019). The hair characters, however, are similar between the four species studied. The hair characteristics of pikas of Wyoming, United States by Moore et al. (1974) reviewed that the identification hairs of pika up to the species level is difficult, as the microscopic characters of hairs are similar and the present study also supports the same.

Family Leporidae

The hare of the family Leporidae is one of the easiest to distinguish because of its specific cuticular scale position and pattern, and unique medulla structure and the dumb-bell shape of the cross-section. The transverse cuticular and multiserial ladder medulla patterns of hair differentiates it from the other groups of mammals (Chakraborty & De 2010; Sarkar 2011; Kamalakannan 2018, 2019). The above-mentioned characters are similar in all the three species. The present study shows that the result is consistent with the findings of hares that occur in Wyoming, Unites States by Moore et al. (1974) and western Europe by Teerink (1991).

According to Hoffmann & Smith (2005), the difference between the order Lagomorpha and Rodentia had been discussed first by Simpson (1945). Later, many morphological and molecular phylogeny studies supported the differences between the order Lagomorpha and Rodentia (Huchton et al. 1999). As mentioned earlier, the hares are often differentiated by external morphology from the pikas by the medium-sized body, and length of their tails and ears. The hares have a highly arched skull, pikas have a less arched skull; the hares have an upright posture of the head, strong hindlimbs and pelvic girdle, which the pikas lack (Vaughn et al. 2000; Sokolov et al. 2009). The dental formula (incisors, canines,

premolars and molars of the upper and lower jaw) also varies between these two groups as 2.0.3.3/1.0.2.3x2 = 28 and 2.0.3.2/1.0.2.3x2 = 26 in the hares and pikas, respectively (Sokolov et al. 2009). The present tricho-taxonomic study also shows the difference between the families Ochotonidae (pikas) and Leporidae (hares) under the order Lagomorpha by highlighting the unique characters of cuticula, medulla and cross-section.

Identification up to species level of the order Lagomorpha was difficult through tricho-taxonomic study, as all the four ochotonid species and three leporid species have similar microscopic characters between the species (Moore et al. 1974; Teerink 1991). The macroscopic characters of hair of mammals may also differ due to age, sex, season, climate, geographical variations, etc., especially since the pikas change pelage colour seasonally (Grange 1932; Nowak 1999; Vaughn et al. 2000; Grzimek 2003; Smith 2008). The macroscopic and microscopic characters (Table 1–3) and the microscopic photographs (Images 1–8) of dorsal guard hairs of lagomorphs would be helpful in the identification of species under the families Ochotonidae and Leporidae of the order Lagomorpha by considering the combination of all the characters of hairs.

CONCLUSION

It should be noted that very meagre information is available in the literature on tricho-taxonomic studies of species under the order Lagomorpha particularly as there is no tricho-taxonomic study in India. Thus, this study may be regarded as the first attempt from India.

Hare species are highly trafficked due to the local bush-meat consumption (Menon & Kumar 1999). They are the chief prey of small and large carnivores, similarly, pikas are also chief prey of small carnivores. Hence, the identification keys (provided here) would be useful in animal forensic science as well as in food habit analysis of carnivores.

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