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A Comparative Study of Some Morphological and Microscopic Identifying Features of Genuine Rhino (*Rhinoceros unicornis*) Horns and Fake Horns

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Abstract

Rhino horn is hard, elongated, conical structure composed entirely of keratin but lacking bony core. Illegal trade of rhino horn has high financial rewards. Both genuine and fake horns are confiscated by authorities and sent for identification. Various morphological and microscopic features of 214 genuine horns of one horned Rhino (*Rhinoceros unicornis*) stored at Armed Forest Protection Training Centre, Tikauli and Office of the Chitwan National Park, Kasara, Nepal were studied. The studied morphological parameters were color, surface texture, height, mass, basal circumference, frontal groove, basal elevation, porosity, light penetration capability at the base and density. Similarly, microscopic features like shape, size and frequency of filamentous units, Interfilamentous space, ratio of medulla and filamentous unit and laminae were studied after thin transverse sections made from the tip of 140 genuine horns. Some of these features were compared with that of 65 fake horns which were received in the laboratory and identified as fake samples. The distinctive filamentous units were observed in all studied genuine rhino horns with varied number, shape and sizes. Based on this study, identifying features of genuine rhino horns were properly established. The study also reveals the possibility of approximation of maturity of rhino horns.

Keywords: Rhino horn; Fake horn; Frontal groove; Filamentous units

Introduction

A rhino horn is a tapering cone of solid keratin with a shallow well at the base which covers a bony knob on the skull [1]. Rhino horn lacks a bony core and is anchored to the dermis covering the frontal and nasal bones [2]. The chief non-keratinous components of the rhino horn are calcium and melanin which make the horn more resistant to physical wear and breakdown [2,3]. Rhino horn seemed to be world's most valuable substance and thus its illicit trade has been steadily increasing with different purposes of ritual, medicinal and recreational activities. Nowadays, horns of water buffalo, cattle and yak [4], bones, woods and synthetic materials are frequently being used to imitate rhino horn. A variety of methods, such as microscopy, thin layer chromatography, atomic absorption spectrometry, HPLC, UV spectrometry, mass spectrometry, Infrared spectroscopy and modern genetic techniques are available to characterize a genuine rhino horn and its products [5-11]. Suspected rhino horns are frequently received in this laboratory for identification. It is often difficult task to differentiate the fake and genuine horn visibly due to admirable craftsmanship (Figure 1). In the present study, various morphological and microscopic features of genuine horns of one horned rhinoceros (*Rhinoceros unicornis*) were studied, compared to the fake horn, and the distinctive characteristics of genuine rhino horn were established. This paper emphasizes the morphological and microscopic based identification of rhino horn along with potential estimation of maturity even from the pieces. Despite being conventional, these simple, rapid, non-destructive but reliable testing methods can be extensively applied in fields such as the customs, scientific, law enforcement agencies and so on.

Materials and Methods

Samples

A total of 214 genuine rhino horn samples securely stored at the Armed Forest Protection Training Centre, Tikauli, Chitwan, Nepal and Office of the Chitwan National Park, Kasara, Nepal were used for the study after obtaining necessary permission from relevant authorities. Similarly, 65 suspected horn samples received in this laboratory as case samples and declared fake horns were taken for the comparative study.

Morphological study

The observed morphological parameters in genuine rhino horns were color, surface texture, basal elevation, frontal groove, light penetration capability at the basal margin, straight height (cm), outside curve length (cm), basal circumference (cm), mass (g) and density (g/cm^3). Likewise, the studied parameters in fake horns were mass (g), straight height (cm) and basal circumference (cm). Except straight height (N=210), density (N=206) and frontal curve length (N=204), all parameters were observed in 214 real horn samples. Density of horns was calculated according to Pienaar and Hall Martin [8].

$$\text{Density (g/cm}^3\text{)} = 12\,\pi\, m/C^2 h$$

Where, m=mass of horn (g), C=base circumference of horn (cm), h=straight height of horn (cm)

Microscopic study

Thin transverse sections (T.S) from the apex of 140 genuine horns were taken with the help of a sharp razor blade. The sections were cleansed with xylene, dried, mounted in a clean microscopic slide with DPX and lastly observed under 100X and 200X using light microscope (Olympus TGHM, Japan). Some of the T.S. of horns were also subjected

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Figure 1: Fake (left) and genuine (right) Horn.

in the 85% formic acid and then heated at 105°C (approximately 5 minutes) for the purpose of separation of filamentous units from the inter filamentous material. The studied microscopic parameters included shape and size of filamentous unit, frequency of filaments per square millimeter (mm), and prevalence of filamentous unit shapes, inters filamentous distance and ratio of medulla and filamentous unit area. Inter filamentous unit distance was determined by measuring the distance between the surface of prevalent filament shape and its three adjacent filamentous units and calculating their mean value for each sample. All numerical measurements were taken using ocular micrometer after standard calibration with stage micrometer. Similar approach was applied for microscopic examination of fake horns.

Data analysis

Microsoft® Excel was used for data processing, data analysis and comparison purpose.

Results and Discussion

Morphology of horn

The color of rhino horns varied from brown (52%), black (19%) to light black (19%). According to Shengqing et al. the color of rhinoceros horn, its products and powder are yellow, brown and gray-white respectively.

The surface of most rhino horns (76.17%) was rough and the remaining had smooth surface. Similarly, 69.90% of rhino horns possessed hairy appearance (Figure 2). The 35.58%horns with hairy exterior indicate that this is one of the causes of basal roughness of horn. Shengqing et al. suggested that the most significant difference in appearance between rhinoceros horn or its products and its substitutes is the hair-pattern structure, which can be observed carefully with naked eye or through microscope.

The light transmittance of a rhino horn is good [7]. However in the present study, light penetration capability was observed in merely 37% of rhino horns (Figure 3).

Some of the distinguishing features visible in rhino horn are the occurrence of a frontal groove, basal elevation and nature of the base of horn (Figures 4 and 5). Frontal groove is the perpendicular depression along the frontal curvature of horn, which was prominently present in 78% of the samples. Basal elevation is the horizontal height extending from the frontal margin of the base to the center of the base of rhino horn. The observed frequency of basal elevation in the rhino horn was 83.56%.The bases of all rhino horn samples were porous and concave.

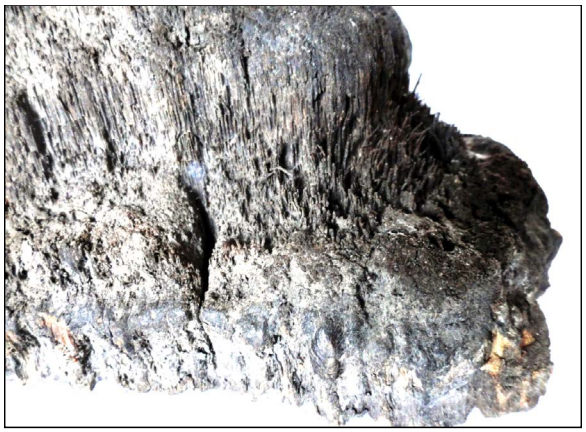

Figure 2: Hairy appearance.

Figure 3: Light penetraibility of genuine horn.

Figure 4: Frontal groove.

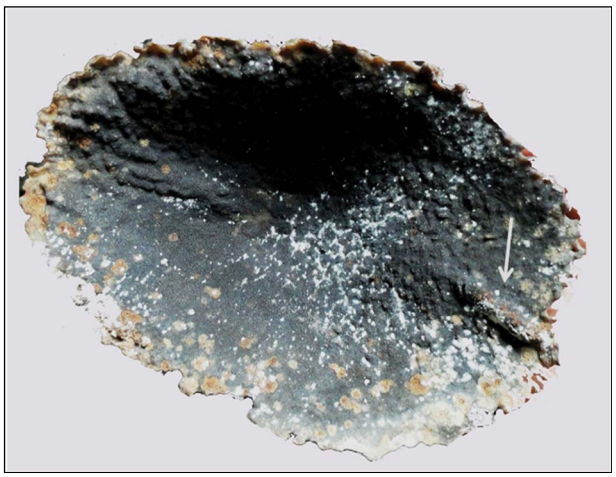

Figure 5: Nature of base : Elevation (arrow marked), concavity and porosity.

The ranges and average measures of mass, straight height, frontal curve length, tip circumference and basal circumference of genuine rhino horn were 8 g to 1690 g (avg. 666.33 g), 1.38 to 31 cm (avg. 16.28 cm), 3 to 40 cm (avg. 21.54 cm), 3.00 to 15.50 cm (avg. 6.98 cm) and 14.5 to 59 cm (avg. 42.62 cm) respectively. Similarly, the ranges of mass, straight height and basal circumference of fake horn were 50.56 to 1780 g (avg. 945 g), 7 to 24 cm (avg. 17.73 cm) and 11.2 to 55 cm (avg. 40.29 cm) respectively. The Figure 6 shows the increase in length and circumference with the increase in mass of genuine horns.

The density of rhino horn ranged from 0.465 to 6.047 g/cm^3 with the average value of 0.794 g/cm^3 ± 0.22. Similarly, the density of fake horn ranged from 0.42 to 2.24 g/cm^3 with the average value of 1.21 g/cm^3 ± 0.34. Most of the rhino horn samples (89%) had a density between 0.5 to 1.0 g/cm^3. But the majority of the fake horns (58%) had a density between 1.0-1.5 g/cm^3. The proportional density of rhino horn and fake horn depicted in the Figure 7 indicate that there is a cluster of rhino horn density with higher frequency compared to disperse density of fake horn. In the present study, the mean value of rhino horn density (0.794 g/cm^3 ± 0.42) was found quite lower than that of African rhino horn (1.26 g/cm^3 ± 0.02) [8].

Microscopy of horn

Rhinoceros horn is an epidermal derivative, composed of closely packed keratinized filamentous units embedded in an amorphous matrix [3,7,11,12]. The matrix is made up of non-crystalline keratinized fusiform interstitial cells [11].The core of each filament has a central structure that resembles the medulla of a hair [12] and is referred as "medullary cavity" [3]. Each filamentous unit grows from a generative layer of epidermis (stratum germinativum) covering a dermal papilla while the matrix is grown from the stratum germinativum of the epidermis between dermal papillae [3].

The filamentous units (Figures 8 and 9) were observed in all studied rhino horns with varied number, shape and sizes. The total number of filamentous units observed per square mm ranged from 2 to 18 with the average of 5.43 ± 2.62. Details illustrated in the Figure 10 showed that most horn samples (69%) possess 4-7 filamentous units per square mm. The filament density (7 mm^{-2}) of rhinoceros horn was also calculated by McKittrick et al. [13].

The geometrical types and individual frequency of filamentous

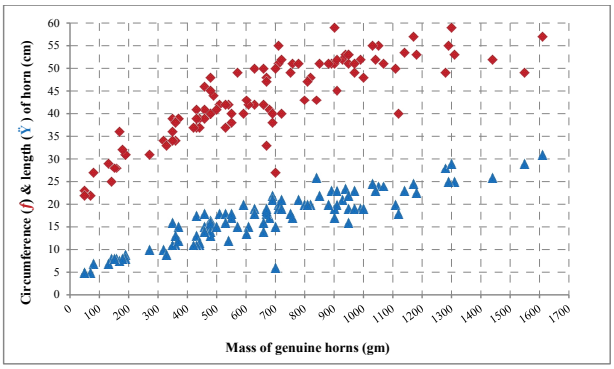

Figure 6: Circumference and length co-relation with mass.

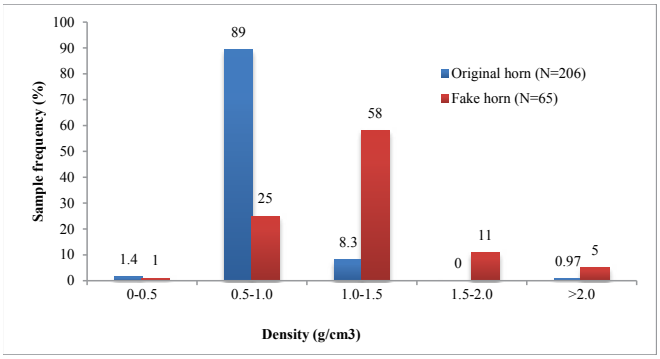

Figure 7: Density of genuine and fake horns.

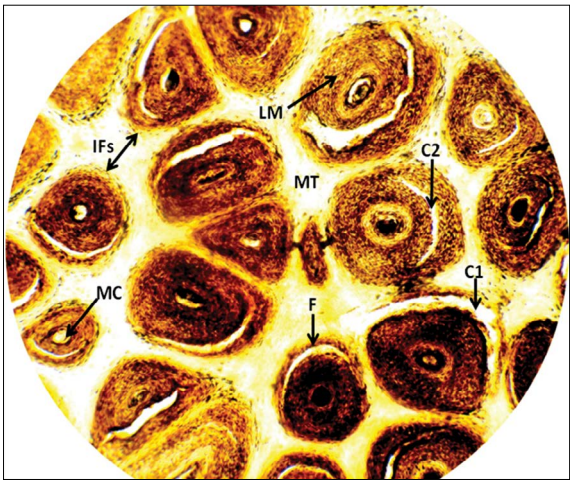

Figure 8: Microscopic features of genuine horn : F: Filamentous unit, MT: Matrix, MC: Medullary cavity, IFs: Inter filamentous space, LM: Laminae, C1: outer crack, C2: inner crack.

units observed in the horn samples were oval (53%), triangular (50%), circular (29%), trapezoid (21%), rectangular (19%), papaya-like (9%), kite-like (8%) followed by square (3.74%), pentagonal (1.87%), bean (3.73%) and bread (1.40%) shaped. The occurrence of filamentous unit type/s in a single horn illustrated in the Figure 11 indicates that the majority of horns (65%) possess 3-4 types of unit.

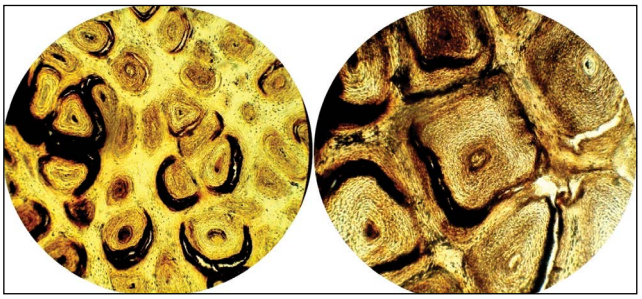

Figure 9: Differences in number, size and Inter filamentous space between immature (left, wt :130 g) and mature (right, wt: 700 gm) rhino horn observed under 100X.

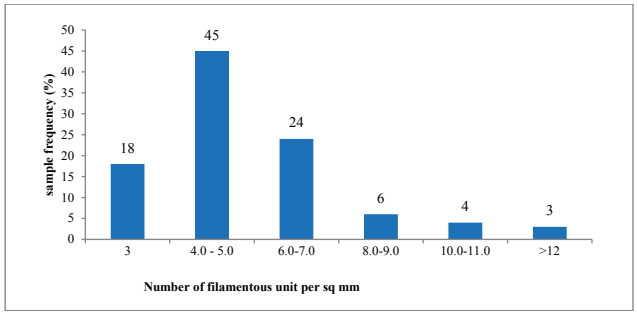

Figure 10: Number of filamentous unit per sq mm in genuine horns.

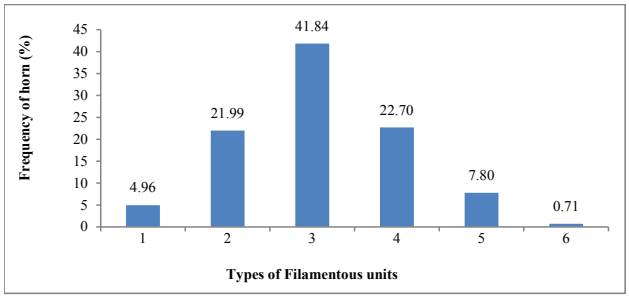

Figure 11: Frequency of type/s of filamentous unit in a horn.

In the present study, the observed diameter of filamentous units in major and minor axis ranged from 120-900 µm (avg. 517.11 µm) and 140-800 µm (avg. 421.83 µm) respectively. Similarly, the recorded diameter of medullary cavity in major and minor axis ranged from 10-250 µm (avg. 68.90 µm) and 10-200 µm (avg. 47.34 µm) respectively. Overall, the calculated average ratio of diameter of medullary cavity and filamentous units in the major and minor axis were 0.136 and 0.114 respectively. Ryder recorded the diameter of filamentous units ranged from 300-500 µm with a medullary cavity of ~20 µm X 60 µm in major and minor axis respectively.

The medullary cavity appears roughly as one-tenth the diameter of their filaments [11]. In the present study, the area wise ratio of medullary cavity and filamentous units was varying among the different types of filamentous unit (Figure 12). Overall, this ratio ranged from 0.002 to 0.146 with the mean value of 0.028.

The details of filamentous unit number per square mm, their shape, sizes with reference to horn mass were described in the Table 1 and Figure 13. The number of filamentous unit per mm^2 roughly decreased with the

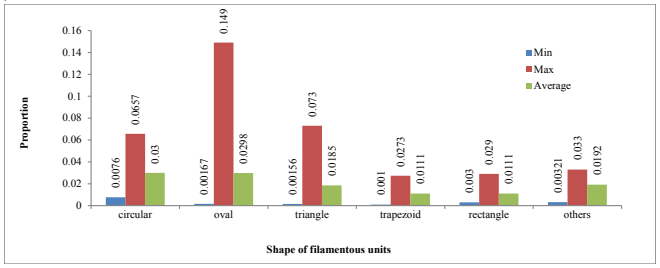

Figure 12: Area wise ratio of medullary cavity and filamentous unit in particular shape.

increase in rhino horn mass. Likewise, lesser the number of filamentous units per mm^2, comparatively larger is the size of filamentous units but narrower inter filamentous space (Figure 9).

According to Ryder, the filaments are composed of about 40 keratin laminae arranged concentrically around the medulla. In the present study, the approximate number of laminae ranged from 13 to 53 with the mean value of 29.82.

In the present study, the cracks were observed within and at outer surface of the filamentous units (Figure 8) by means of frequency of 51% and 28.82% respectively. Altogether 75.57% of horns had cracked filamentous units. The observed average mass (g), density and number of filamentous unit per mm^2 of such horns were 667.90, 0.82 and 5.20 respectively. According to Orden and Joseph the horn filaments are rigid so they can be broken during the mechanical stress but the matrix acts as a flexible resin that inhibits the crack propagation and thereby confining the cracks inside the filaments only [14].

The microscopic characteristics of genuine rhino horns viz. filamentous units were not observed in any fake horn.

Conclusion

The comparative study of morphological and microstructure between genuine rhino horns and the imitated horns showed that the most unique feature of genuine rhino horn is the occurrence of characteristic filamentous unit. Due to this fact, it is practicable to identify the small piece of rhino horn as well. The other studied features like hair, density, light penetrability, frontal groove, basal porosity and elevation provide valuable information for quick and preliminary identification of rhino horn at the field level. Likewise, through the collective study of number, size and inter space of filamentous units, it is feasible to approximate the maturity of rhino horn.

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Mass of Rhino horn (g)				Density			No. of filamentous unit per sq mm			Inter filamentous unit distance (μ)			Prevalent filamentous unit and their size (μ) (Min-Max)		
Range	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Oval	Circular	Triangle
<200	8	190	102	0.55	1.09	0.79	4	18	9.11	40	200	97.25	150 × 350-300 × 400	200 × 250-300 × 350	300 × 600
200-400	200	390	314	0.61	2.61	0.96	3	10	5.41	27	100	66.52	-	300 × 320-320× 350	300 × 450-350 × 480
400-600	400	590	492	0.53	1.16	0.76	3	10	5.48	20	133	65	300 × 400-700 × 750	400 × 400-550× 600	280 × 400-550 × 800
600-800	600	780	683	0.465	6.04	0.90	3	9	5.1	30	133	66.84	120 × 140-400 × 700	-	400 × 450-550×750
800-1000	800	990	906	0.51	0.92	0.69	2	7	4.16	20	110	57.77	380 × 430-550 × 700	-	350 × 350-420 ×700
1000-1200	1000	1190	1096	0.52	1.46	0.75	3	9	4.40	26	156	74.35	450 × 550-550 × 700	-	400 × 400-610 ×790
1200-1700	1200	1690	1382	0.48	0.94	0.74	3	6	4.46	10	106	43.33	450 × 550-500 × 600	-	300 × 400-600 ×650

Table 1: Morphological and microscopic details of genuine horns.

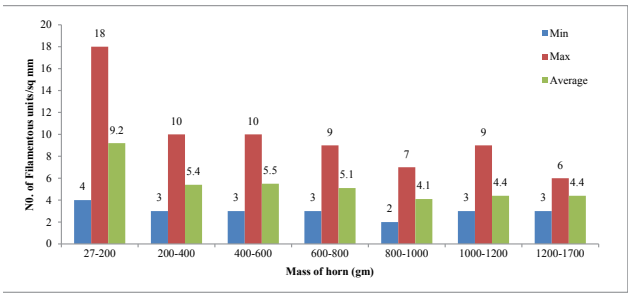

Figure 13: Correlation of rhino horn mass and number of filamentous units per sq mm.

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