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Full Paper

Tiger hair morphology and its variations for wildlife forensic investigation

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Abstract: Tiger population has dramatically decreased due to illegal consumption and commercialisation of their body parts. Frequently, hair samples are the only evidence found in the crime scene. Thus, they play an important role in species identification for wildlife forensic investigation. In this study, we provide the first in-depth report on a variety of qualitative and quantitative characteristics of tiger guard hairs (24 hairs per individual from four individuals). The proposed method could reduce subjectivity of expert opinions on species identification based on hair morphology. Variations in 23 hair morphological characteristics were quantified at three levels: hair section, body region, and intra-species. The results indicate statistically significant variations in most morphological characteristics in all levels. Intra-species variations of four variables, namely hair length, hair index, scale separation and scale pattern, were low. Therefore, identification of tiger hairs using these multiple features in combination with other characteristics with high inter-species variations (e.g. medulla type) should bring about objective and accurate tiger hair identification. The method used should serve as a guideline and be further applied to other species to establish a wildlife hair morphology database. Statistical models could then be constructed to distinguish species and provide evidential values in terms of likelihood ratios.

Keywords: wildlife forensic science, tiger, *Panthera tigris*, hair morphology, tiger hairs, animal hairs

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INTRODUCTION

Tigers (*Panthera tigris*) are critically endangered; only approximately 3.2% of the population size estimated in 1990 remains in the wild [1-5]. This is mainly due to extreme poaching of tigers for their skins and body parts. A number of organisations such as the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and the wildlife trade monitoring network (TRAFFIC) have taken an action to enforce tiger trade regulation in order to protect and manage wild tiger populations at a sustainable level [6]. National legislations are enacted in response to these international controls. Forensic science aids law enforcement by determining if seized materials contain tiger body parts.

For species identification, two reliable and court-accepted methods commonly employed by most forensic laboratories are molecular testing and microscopic examination [7, 8]. The advantage of the molecular approach is its high accuracy and sensitivity. However, it is time-consuming, expensive and destructive. Tiger hairs rather than other tissues are commonly found in a crime scene and are sometimes the only evidence found, hence the importance of the non-destructive method. The traditional hair morphological examination is a viable alternative for tiger species identification.

In an actual wildlife crime scene, it is common to find hairs from many species including domestic animals such as cat and dog. Species identification based on hair morphology has been reported in many animal species [9-15]. A wide range of hair morphological features has also been reported in these different species. However, most studies cannot be reliably used in the forensic context, as the critical process of validation is lacking. An important aspect of validation is a large enough sample size and a reporting of scientific findings with statistics such as likelihood ratios. The conventional hair morphology-based method is subjective, relying heavily on expert opinions. Comparisons can only be made when reference samples are readily available. Also, variations in hair morphology, which are biologically meaningful [10], have been overlooked in previous studies.

To our knowledge, trustworthy data on hair morphological features of tiger and quantifiable, statistics-based method for the identification of this species have never been reported. Therefore, in this study, we aim to establish a tiger hair morphological characteristic reference as well as quantify variations due to difference between individuals, body regions and hair sections. Using this information, we hope to identify the characteristics that are suitable for tiger identification. We hope this study should serve as a guideline for how hair morphology in animals should be reported in order to establish a wildlife hair morphology database to assist reporting hair evidence with probability statements.

MATERIALS AND METHODS

Sample Collection and Hair Specimen Preparation

Hair samples were obtained from Songkhla Zoo and Chiang Mai Zoo (Thailand). Hairs were collected from four mature, healthy individuals of *Panthera tigris* with no familial relationship. For each individual, six guard hairs were taken from each of the four body regions: head, dorsum, ventrum and extremities (n = 24 for each individual). These hairs were degreased using an ethanol (95%)-ether mixture (1:1) and then dried prior to further analysis.

From each hair, three types of specimen, i.e. whole mount, scale cast and cross section, were prepared for examination under a light microscope. Whole mount and cross-section specimens were prepared following the methods described by Brunner and Coman [16]. Scale casts were obtained with the clear nail polish method [13].

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Morphological Examination

Qualitative characteristics

The qualitative hair characteristics that were recorded for each hair consisted of one whole-mount characteristic and eight hair-section characteristics of the proximal, middle and distal parts. The whole mount characteristic was medulla type. The eight hair-section characteristics were hair colour, cortex colour, scale margin, scale separation, scale pattern, cross-section colour, cross-section shape and cross-section medulla size. The classification of these characteristics was based on Brunner and Coman [16].

Quantitative characteristics

For whole mount specimens, six numerical features were obtained as follows:

1. The length (mm) was directly measured using a metric ruler.
2. The proximal width (µm) was the hair width measured at the middle of the proximal position of hair using a calibrated micrometre in the eyepiece.
3. The maximum width (µm) was the hair width measured at the widest point of hair using a calibrated micrometre in the eyepiece.
4. The medulla width (µm) was measured at three positions of hair (proximal, middle and distal) using a calibrated micrometre in the eyepiece.
5. Hair width index was calculated as follows [15]:

$$\text{Hair width index} = \frac{\text{Hair width at the proximal one third of hair shaft}}{\text{Maximum width along the hair shaft}} \times 100$$

6. Medulla index was calculated as follows [15]:

$$\text{Medulla index} = \frac{\text{Medulla width at the maximum width part along the hair shaft}}{\text{Hair width at the maximum width part along the hair shaft}} \times 100$$

For scale-cast specimens, two numerical features were obtained at three hair sections (proximal, middle and distal) as follows:

1. Scale width (µm) was measured from three cuticle scales randomly selected using a calibrated micrometre in the eyepiece.
2. Scale height (µm) measured from three cuticle scales randomly selected using a calibrated micrometre in the eyepiece.

For cross-section specimens, six numerical features were obtained at three hair sections (proximal, middle and distal) as follows:

1. Cuticle width (µm) was measured using a calibrated micrometre in the eyepiece.
2. Minimum diameter (µm) was measured at the minor axis at the widest point of the hair using a calibrated micrometre in the eyepiece.
3. Maximum diameter (µm) was measured at the major axis at the widest point of the hair using a calibrated micrometre in the eyepiece.
4. Medullary fraction was calculated as follows:

$$\text{Medullary fraction} = \frac{\text{Medulla diameter}}{\text{Hair diameter}} \times 100$$

5. Hair index was calculated as follows [15]:

$$\text{Hair index} = \frac{\text{Minimum diameter of cross section}}{\text{Maximum diameter of cross section}} \times 100$$

6. Cuticle index was calculated as follows [15]:

$$\text{Cuticle index} = \frac{\text{Cuticular thickness of cross section}}{\text{Maximum diameter of cross section}} \times 100$$

Statistical Analysis

The multivariate analysis of variance (MANOVA) was used to determine whether there were statistically significant differences between the different hair sections, body regions and individuals. If MANOVA results were significant, a univariate analysis of variance (ANOVA) was further carried out to determine which morphological characteristics were different. A p-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Tiger Hair Morphological Characteristics

Ninety-six hairs from four tigers were examined using whole mounts, cross-sections and scale casts. The descriptive macroscopic and microscopic morphological characteristics are shown in Table 1 and Table 2. A total of 23 morphological characteristics were investigated on three hair sections: proximal, middle and distal. Of these, 9 characteristics are qualitative data which are reported in the form of percentages (Table 1) and 14 characteristics are quantitative data, of which the means and standard deviations are calculated (Table 2). The overall row indicates an average percentage for each qualitative feature calculated over the three hair sections.

Whole mount

Under the whole mount examination, tiger hairs are presented in four colours: white, black, yellow and brown (Table 1). The proximal and distal hair parts are dominantly white and black, whereas black, white and yellow are almost equally found in the middle part. The existence of such colour patterns is common in felid species such as bobcat and cougar [17] while domestic animal hairs are usually unbanded [13].

The tiger hair length in this study is 14.4 mm on average (range of 5.2-45.0 mm) (Table 2). The range is broader than 17.9-20.3 mm reported by Soni *et al.* [18], which is the only previous study of tiger hair characteristics but with no intra-species variations reported. The disparity in range is probably due to the difference in sample size. A very small sample size (ten hairs) was used in the previous study, resulting in a considerably narrow range of tiger hair length compared to ours. The average hair length of tiger (14.4 mm) is shorter than those of domestic cat (25.4 mm) and domestic dog (31.9 mm) [15]. The ranges of tiger hair proximal and maximum widths are found to be 15.0-97.5 µm and 22.5- 120.0 µm respectively, giving the hair width index of 68.3±13.3. Both maximal width and hair width index are quite similar to those of domestic cat (35.3-102.9 µm and 68.6±13.4) but smaller than those of domestic dog (50.0-165.0 µm and 75.0±13.6) [15].

Tiger hair medulla is narrow, resulting in a small medulla index (35.2±11.3) when compared with that of domestic cat (67.8±7.4), domestic dog (57.5±9.4) [15], mongoose (59.6±0.4 to 79.2±0.5) [11] and wild ungulates [9, 19, 20]. On the other hand, tiger hair medulla index is larger than that of four Indian bear species (4±0.3 to 36±0.9) [12].

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Table 1. Qualitative characteristics of tiger hairs. All qualitative variables are shown in percentages.

Feature	**Hair colour**				**Cortex colour**				**Medulla type**			
	Black	Brown	White	Yellow	Black	Brown	White	Yellow	Simple	Uniserial ladder	Absent	Mixed
Proximal	28.1	9.4	56.3	6.3	0.0	36.5	55.2	8.3	64.6	4.2	24.0	7.2
Middle	34.4	2.1	38.5	25.0	5.2	42.7	28.1	24.0				
Distal	47.9	9.4	21.9	20.8	21.9	37.5	15.6	25.0				
Overall	36.8	6.9	38.9	17.4	9.0	38.9	33.0	19.1				

Feature	**Scale margin**				**Scale separation**				**Scale pattern**				
	Crenate	Rippled	Smooth	Mixed	Close	Distant	Near	Mixed	Regular wave	Single chevron	Irregular wave	Streaked	Mixed
Proximal	16.7	1.0	76.0	6.3	0.0	19.8	79.2	1.0	100.0	0.0	0.0	0.0	0.0
Middle	49.0	20.8	27.1	3.1	13.5	0.0	85.4	1.0	39.6	44.4	10.8	4.2	1.0
Distal	27.1	67.7	0.0	5.2	93.8	0.0	6.3	0.0	9.5	78.3	4.9	4.2	3.1
Overall	30.9	29.9	34.4	4.9	35.8	6.6	56.9	0.7	49.7	40.9	5.2	2.8	1.4

Feature	**Cross-section colour**						**Cross-section shape**				**Cross-section medulla size**	
	Black	Brown	Red	White	Yellow	Mixed	Circular	Concavo-convex	Oval	Absent	Medium	Small
Proximal	39.6	0.0	1.0	22.9	20.8	15.6	50.0	29.2	20.8	2.1	81.3	16.7
Middle	40.6	1.0	1.0	15.6	28.1	13.5	41.7	27.1	31.3	2.1	84.4	13.5
Distal	45.8	0.0	2.1	16.7	20.8	14.6	55.2	27.1	17.7	11.5	79.2	9.4
Overall	42.0	0.3	1.4	18.4	23.3	14.6	49.0	27.8	23.3	5.2	81.6	13.2

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Table 2. Quantitative characteristics of tiger hairs. Quantitative variables are given as means ± standard deviation and range ([]). Length, proximal width, maximum width, hair width index, and medulla index are whole hair features.

Feat.	Length (mm)	Proximal width (µm)	Maximum width (µm)	Medulla width (µm)	Scale width (µm)	Scale height (µm)	Cuticle width (µm)	Minimum diameter (µm)	Maximum diameter (µm)	Medullary fraction
Prox.	14.4±7.9 [5.2-45.0]	50±19.1 [15.0-97.5]	73.1±21.1 [22.5-120.0]	16.2±11.8 [2.5-62.5]	36.4±9.4 [14.2-74.2]	8.6±2.1 [2.5-18.3]	1.4±0.7 [0.6-5.0]	53.8±20.3 [20-162.5]	61.2±23.0 [21-192.5]	34.9±8.9 [4.0-59.5]
Midd.				26.3±13.9 [2.5-85.0]	46±12.6 [12.5-72.5]	7.3±1.7 [2.5-11.3]	1.8±1.1 [0.5-7.5]	67.4±19.8 [17-105.0]	77.6±23.0 [23-187.5]	42.8±8.7 [21.1-59.1]
Dist.				27±10.3 [5.0-52.5]	34.4±13.6 [10.0-72.7]	5.1±1.7 [1.5-10]	1.9±1.2 [0.5-6.3]	55.5±21.1 [7-157.5]	62.5±23.4 [10-197.5]	40.7±8.0 [11.5-55.6]

Feat.	Hair width index	Medulla index	Hair index	Cuticle index
Prox.	68.3±13.3 [46.2-100]	35.2±11.3 [12.9-73.9]	87.9±8.7 [64.0-100]	2.4±1.6 [0.7-13.3]
Midd.			86.9±9.8 [53.1-100]	2.4±1.6 [0.6-10.3]
Dist.			89.1±11.4 [8.75-100]	3.4±2.0 [0.7-11.9]

The medulla of tiger hairs consists of three types, viz. simple, uniserial ladder and a mixture of both (Figure 1). The medulla type of each hair does not change along the length of the hair but the width becomes narrow towards the distal end. This characteristic is quite unique for tiger and can be used to distinguish it from other species such as ungulates (filled lattice medulla), mongoose (wide medulla with cortical intrusions and vacuoles), and bear (amorphous medulla) [9, 11, 12].

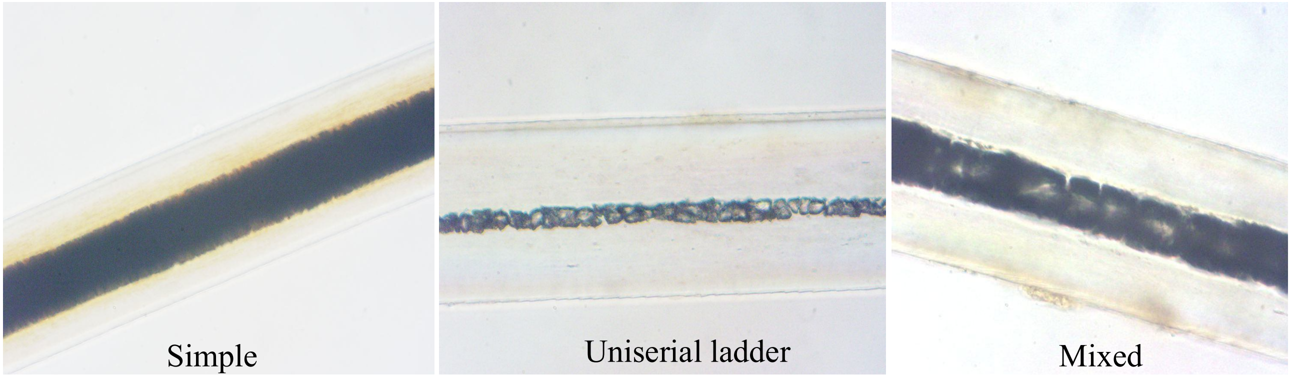

Figure 1. Three types of medulla observed in tiger hairs

Scale cast

Five types of scale pattern, i.e. regular wave, single chevron, irregular wave, streaked and a mixture of these, are observed in tiger hairs (Figure 2). It is worth noting that the scale pattern, scale margin and scale separation are distinctively different among hair parts (Table 1). The scale pattern present in the proximal part is all regular wave with a smooth margin and near separation (Figure 2). The major pattern found in the middle and distal parts is single chevron. The scale margin in the middle part is mostly crenate and shows near separation while rippled scale margin and close scale separation is the majority in the distal part. Scale margins and separations in tiger are similar to those in Indian bear, deer family and mongoose [11-13]. While tiger hairs rarely have distant scale separation, the majority of ungulates have it [9]. Single chevron found in almost half of tiger hairs in this study is quite uncommon among other species and thus can be useful for identifying tigers in wildlife crimes [9].

Tiger hair scale width and height also shows hair section variations (Table 2). The scale width in the middle part shows the highest value (46±12.6 μm) compared to other parts (proximal, 36.4±9.4 μm; distal, 34.4±13.6 μm). Scale height shows a decreasing trend towards the distal end.

Cross section

The cross-section shape of tiger hairs is found in three forms: circular, concavo-convex and oval, with mostly medium-sized medulla (Table 1). While in general the cross-section shapes are similar to Asiatic lion, Indian bear and mongoose, tiger hairs lack the oblong and dumbbell shapes found in ungulates [19]. They also lack the ellipsoidal shape found in roe deer [20], and the cigar shape found in leopard [18].

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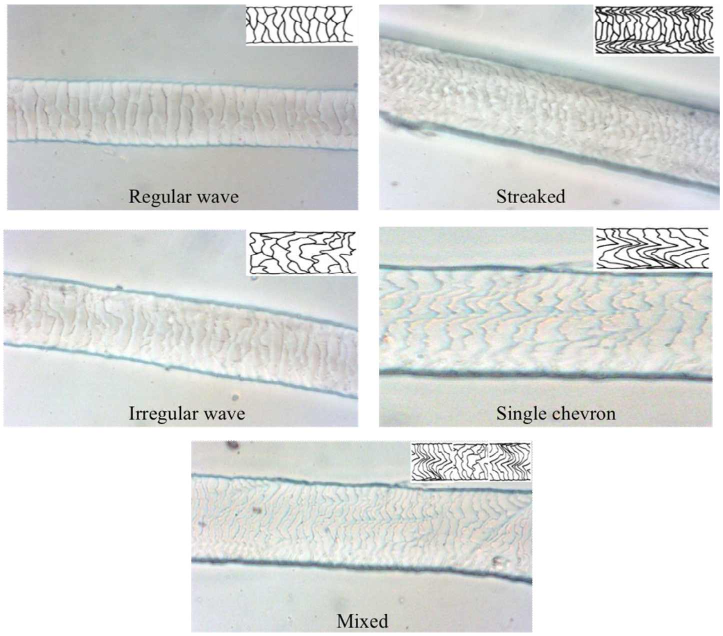

Figure 2. Five types of scale pattern observed in tiger hairs

The cross-section diameter of tiger hairs in our study ranges between 7.0-197.5 µm (Table 2), which overall is similar to that of domestic cat (min: 49.4±12.4 µm, max: 59.3±15.6 µm) but shorter than that of domestic dog (min: 76.4±20.7 µm, max: 95.3±23.7 µm) [15]. As for hair index, all three species are very similar (tiger: 86.9±9.8; domestic cat: 84.5±10.8; domestic dog: 80.1±8.9).

Variations in Tiger Hair Morphology

Three types of hair morphological variation are of interest, viz. variations due to hair section, body region and individual animal. Results of MANOVA are all highly significant ($p < 0.001$), meaning that there are significant differences between the means of the groups being compared.

Variation due to hair section

Of the 17 observed variables measured for all three hair sections, only cross-section colour, cross-section shape, and hair index are not significantly different ($p > 0.05$). All the other 14 variables are significantly different ($p < 0.001$), indicating that these characteristics are different in each hair section (Table 3). It is apparent that if measurements of scale pattern were only made in the proximal part, information regarding the variations in this characteristic would have been lost, possibly leading to misidentification of species. Therefore, when hair evidence are encountered at crime scenes, investigators must report not only the characteristics of the hairs, but also from which hair part the measurements are taken from, in order to achieve an accurate identification.

Variation due to body region

Ten of 23 observed characteristics are not significantly different between the four body regions studied. These are scale separation, cross-section shape, proximal width, maximum width, medulla width, scale width, minimum diameter, hair width index, hair index, and cuticle index. This means that these features are consistent among tiger body areas. The other 13 characteristics measured show body region variation (Table 3). Obviously, it is expected that some variables such as length would differ significantly between the four anatomical regions, as it is normal for animals to have longer hairs in the ventral area compared with the extremities (e.g. 8.6-45.0 mm for the ventral region and 5.2-16.0 mm for the extremities in our tigers). We advise that, as performed by van den Broeck *et al.* [10], any future study that reports on hair morphological characteristics include hairs from many anatomical regions to quantify these variations as well.

Table 3. P-values of variations of tiger hairs within hair section, body region and individual for each morphological characteristic. NC = not compared. Bolded values indicate statistical significance ($p < 0.05$).

Features	Hair section	Body region	Individual
Hair colour	**<0.001**	**<0.001**	**<0.001**
Cortex colour	**<0.001**	**<0.001**	**<0.001**
Medulla type	NC	**<0.001**	**<0.001**
Scale margin	**<0.001**	**0.044**	**<0.001**
Scale separation	**<0.001**	0.062	0.150
Scale pattern	**<0.001**	**0.006**	0.876
Cross-section colour	0.849	**<0.001**	**<0.001**
Cross-section shape	0.205	0.383	**<0.001**
Cross-section medulla size	**0.022**	**<0.001**	**<0.001**
Length	NC	**<0.001**	0.598
Proximal width	NC	0.831	**<0.001**
Maximum width	NC	0.606	**<0.001**
Medulla width	**<0.001**	0.053	**<0.001**
Scale width	**<0.001**	0.245	**<0.001**
Scale height	**<0.001**	**<0.001**	**<0.001**
Cuticle width	**0.001**	**0.023**	**<0.001**
Minimum diameter	**<0.001**	0.073	**<0.001**
Maximum diameter	**<0.001**	**0.042**	**<0.001**
Medulla fraction	**<0.001**	**0.019**	**0.021**
Hair width index	NC	0.253	**<0.001**
Medulla index	NC	**0.027**	**<0.001**
Hair index	0.317	0.107	0.066
Cuticle index	**<0.001**	0.094	**<0.001**

Variation due to individual

Of the 23 observed characteristics, 19 are significantly different among the four tiger individuals. Only scale separation, scale pattern, hair length, and hair index are not significantly different between the four individuals (Table 3), indicating a low intra-species variation of these hair characteristics in tigers. Three of these four characteristics, namely scale separation, scale pattern, and hair length are also found to have high inter-species variation [10, 13, 15]. In order to distinguish between different species, a characteristic that has low intra-species variation and high inter-species variation should be used. Hence, these three characteristics should be the main variables for differentiating tiger hairs from other species. The results correspond to the FBI study on animal hairs [13], in which scale casts are recommended for separation of deer family. Other characteristics that can help include scale count index, medulla type and medulla index, which have been reported as the most reliable characteristics in other studies [11, 12, 17]. Scale count index is not reported in this study, however, and although medulla index and medulla type are different to a certain degree from some other wildlife species and domestic animals, the high intra-species variation observed here suggests that they are of limited value in tigers. Colour patterns have also been recommended [11, 17].

Therefore, to obtain a more reliable identification of tiger hairs, a combination of the main characteristics showing low intra-species variations and other characteristics showing high inter-species variations is recommended. The establishment of a database that contains hair morphological characteristics and their variations of commonly traded or endangered species would be ideal. This database should form the basis for probability calculations (as per the DNA gold standard [21]). Species identification can then be performed using statistical procedures, such as discriminant analysis and cluster analysis, to assign weights to different hypotheses. All these will lead to increased confidence in using hair morphology in species identification and will be beneficial for wildlife forensic investigation.

CONCLUSIONS

In this study, 23 tiger hair morphological characteristics were quantified and the key features useful for tiger identification were noted. It is hoped that the output reported in this study will serve as a guideline for tiger hair identification and raise caution about using data from studies that overlook internal variations of hair morphology. We have shown that there are some variations due to hair section, body region and individual animal in most tiger hair characteristics. We expect to perform a similar study in other species to construct a database of hair morphological characteristics. With such a database, it will be possible to use statistical methods to achieve classifications based on a combination of both quantitative and qualitative hair characteristics, which should help to assign probabilities using likelihood ratios in reporting forensic species identification.

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