Taxonomical applications of guard hair microstructures in tiger (*Panthera tigris*) and leopard (*Panthera pardus*): A scanning electron microscopic study

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Abstract
Taxonomical importance of guard hair has been reported for many animals, but the same has not been studied in detail for several species and sub species of the genus Panthera. Further, although light microscopical studies in this regard are available, there is a lacuna in our knowledge in micro structural features revealed through electron microscopy. The aim of the present study was to have a detailed knowledge on guard hair micro structures with reference to taxonomical applications in two species of the genus Panthera. Distinctness in surface micro structures and internal organisation of cuticle, cortex and medulla of guard hair in *Panthera tigris* and *Panthera pardus* as revealed in the current study through scanning electron microscopy suggests the taxonomical significance of guard hair micro structure in these animals.

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1. Introduction

1.1 Background
One of the earliest applications of scanning electron microscopy (SEM) was in Dermatology, particularly the skin (horny layer), hair and nails. These structures are the ideal objects for SEM because they are easily accessible and need no or very little preparation prior to electron microscopic observations. Despite this, the number of published papers on SEM applications in Dermatology is relatively less (Forslind, 1984). Further, SEM study is lacking in some important areas of Biology such as Wildlife. As for example, reports of SEM studies on human hair in relation to physiological and pathological states of the subjects exit in literature, but the same is not available for wild animals.

1.2 Hair analysis in Physiological and Pathological states
The important observations made on the effect of nutrition and different pathological states including genetic defects, psychomotor development, immunological status, intestinal resorption, disturbed amino acid metabolism etc. on morphology of hair and scale pattern (Jelliffe and Welbourn, 1963, Bradfield, 1967, Braun-Falco et al., 1981, Forslind, 1984) in human and experimental animals suggest that SEM of hair may be applied to studies concerning Wildlife Biology and Conservation in relation to the aforementioned factors. In this context, it is worth mentioning that Dey et al. (1999) detected toxicity and deficiency of certain elements in some wild animal species of North-East India with the help of SEM and Atomic Absorption Spectroscopy (AAS) of hair and relevant behavioural studies.
1.3 Hair structure in animal identification

The surface features of guard hair of some animals appear to be different from others (Montagna and Parakkal, 1974) and may be used as additional means for animal identification, besides other parameters. It is known that guard hair is affected by genetic, hormonal and environmental factors (Montagna and Parakkal, 1974), but this information was not utilized in Wildlife Biology, conservation and management. As far as the role of SEM in guard hair identification is concerned, classification based on micro- morphology of medulla in the major mammalian orders has been documented by some authors (Clement et. al., 1981). However, classification at family, genus and species level are yet to be done in a systematic manner.

1.4 Hair fine structure, behaviour and stress

Studies on fine structural features of hair from different body parts of wild animals and their possible relation with behavioural physiology have not been carried out in detail. Besides this, it appears that the first step in correlating the effects of stress conditions on hair morphology is to have detail knowledge on the micro structural features of normal hair.

2. Review of Literature

2.1 Hair types

Five types of hairs are frequently observed in mammalian species. These include Vibrissae, bristles, over-hairs, under-hairs and guard hairs. Of these, only guard hairs appear to be significant for identification purposes, although over-hairs and under-hairs may provide additional data (Teerink, 1991).

2.1.1 Guard hair

The largest or coarsest of the animal hairs are the guard hairs, which form the main pelage and include a type, often described as shield hairs. In shield hairs the distal part is noticeably wider and flattened, forming a shield. Guard hairs lacking shields are of uniform diameter along most of their length, tapering only towards the tip. The largest of the guard hairs, termed as the primary guard hairs are of paramount importance in hair identification since they exhibit the most diagnostically useful features.

2.2 Hair composition

The hair is composed of the cuticle externally, the cortex or inner sheath, the medulla or central core and pigment granules dispersed in the core and cortex (Meyer et al., 2002).

2.3 Hair structure and its applications

Scale structure is used to determine species. Microscopic structures within the cortex, such as pigment granules and fusi are used to compare one hair with another (Saferstein, 2004; Kubic and Patraco, 2003). The shape of the medulla as well as the pattern it exhibits can be used to determine species (Lane, 1992; Saferstein, 2004). Species identification based on microscopic hair characteristics have been used widely for studying food habits, prey-predator relationship and micro habitats (Mathew, 2001; Mayer, 1952). Descriptive guides on microscopic hair characteristics for some important mammalian species of particular regions have been presented by Brunner and Coman (1974). The hair structure may significantly differ in phylogenetically close species, sub-species and breeds as well as at different developmental stages of the same individual (Noback, 1951). Further, hair is extremely adaptable to environmental conditions. Hair structure of quite a number of mammalian species of India as well as of other countries has been worked out (Hausman, 1930; Brunner and Coman, 1974; Teerink, 1991; Walls, 1993; De and Chakraborty, 2002; Chakraborty et al., 1996, 1999; De et al., 1998).

2.4 Hair analysis with reference to tiger and leopard

For tiger and leopard, the hair analyses are usually carried out from the dorsal body region. However, some workers have studied differences in hair structure in relation to differences in body regions, sexes, age and seasons (Mayer, 1952; Dreyer, 1966). In India, tiger is included in the Schedule I, part I of the wild life (protection) act, 1972 and globally in the appendix I of CITES (Convention on International Trade on Endangered Species of Wild Flora and Fauna). Tiger (Panthera tigris) is the largest felid species. There are eight sub species of tiger, namely, Royal Bengal tiger (Panthera tigris tigris), Amur or Siberian tiger (Panthera tigris altaica), Amoy or South China tiger (Panthera tigris amoyensis), Sumatran tiger (Panthera tigris sumatrae), Indochinese tiger (Panthera tigris corbetti), Bali tiger (Panthera tigris balica), Caspian tiger (Panthera tigris virgata) and Javan tiger (Panthera tigris sondaica).

2.5 Scenario in the current study area

In Assam, a North-eastern state of India, located in the Asian continent, tiger is found in the Brahmaputra valley. Although some studies have been made on the animal from this region, a habitat specific study and variation in guard hair is still awaited. The present study has undertaken analyses of the guard hair structure (length, width, general morphology, cross section, medulla, cuticle pattern etc.) with reference to body regions, sex and seasons in various protective areas of Assam.
2.6 Objectives of the present study
The objectives of the present study are to explore the possibilities of using SEM data of hair for identification of animals and to assess their physiological and pathological status.

2.7 Summary of work plans
The work plan was to provide a detail SEM description of guard hair of tiger and leopard for taxonomical applications and also for assessing the abnormalities related to pathological states and different types of Environmental stress.

3. Materials and Methods

3.1 Materials
The guard hair samples were obtained from the shed off hairs of tigers and leopards from the State Zoo of Assam, a north-east Indian state in Asian continent and from confiscated trophies during the years 2010-2012.

3.2 Methods
3.2.1 Scanning electron microscopy
The guard hair sample for surface studies through Scanning electron microscopy was cleaned thoroughly by rinsing them with acetone repeatedly. The clean samples were secured to brass stubs (10 mm diameter x 30 mm high) with double adhesive tape via a patch of silver paint to ensure charge conduction. For studying the internal organisation of cuticle, cortex and medulla etc. sagittal section of guard hair was taken before adhering them to stubs. Approximately 20-25 guard hair samples, each from head, dorsal region, ventral region and tail of Panthera tigris and Panthera pardus were prepared. Care was taken to avoid trapped air bubbles inside the adhesive tapes. A conductive coating was applied to the samples using a JFC 1100 (Jeol) ion sputter coater. A relatively low vacuum (10-3 torr) was established in the sputtering chamber, and the target material used was gold. Observations were made with a Scanning electron microscope JSM-35 CF (Jeol) using the secondary electron emission mode. The accelerating voltage applied was 20Kv. The working distance (WD) selector was set at 15mm, and the tilt control was fixed at zero degree for setting the specimen stage in a horizontal position.

3.2.2 Statistical Analysis
Statistical analyses (ANOVA, Students’t-test) based on mean values of length & breadth of hair from head, tail and other body regions, dimension of scales, thickness of cuticle, cortex & medulla, width of fusi, inter cellular space etc. per individual were carried out. Differences between body regions and between groups were based on Post-Hoc tests. Guard hair samples from four tigers and four leopards were used in the study.

4. Results and Discussion
4.1 Surface features
The guard hair from different locations of Panthera tigris and Panthera pardus show some differences in the surface pattern, arrangement of scales, and width of the hair in head and tail regions. (Figs.1-4). However, there is no difference between the guard hair of tail and those of the dorsal and ventral side.

4.1.1 Panthera tigris
4.1.1.1 Guard hair from head
The guard hair from head of Panthera tigris was found to be thin with a width of around 12 µm. The cuticle cell is of coronal type since it completely surrounds the hair (Montagna and Parakkal, 1974). Scales are in general, acuminate type but in the edges some elongate scales are present. In an average, the exposed portion of elongate scales is about 5.5 µm long and 1.1 µm broad. The exposed portion of the acuminate scales in major part of the hair cuticle is about 5 µm long and about 10 µm broad. The free edges of the scales are slightly dentate (Fig. 1, table1).

4.1.1.2 Guard hair from tail, dorsal & ventral side
The surface features of hair and the organization of scales are identical in tail, dorsal and ventral side. Hence, the surface features of tail is presented and described which represents the identical features of guard hairs from other body parts i.e. dorsal and ventral regions (Fig. 2, table1). Guard hair representing tail, dorsal or ventral region was found to be larger in width as compared to that of head (~46 µm). The cuticle cell of tail guard hair, like that of the cephalic hair is coronal type in major portion of the hair. However, the scales in the edges of the hair are elongate type. The exposed portion of elongate scales is around 14 µm in length and ~9 µm in width. The exposed portion of acuminate scales in the major portion of the hair cuticle is about 12 µm long and 12.5 µm wide. The free edges of the scales are more or less smooth. The adjacent scales are fused with each other (Fig.2, table1).

4.1.2 Panthera pardus
4.1.2.1 Guard hair from head: The guard hair from head of leopard (Panthera pardus) is found to be larger in average width (23 µm) as compared to that of tiger (Panthera tigris). The cuticle is coronal type. The scales are acuminate type in the major portions of the hair but elongate at the edges. The exposed portion of acuminate scales are found to be around 10 µm long and 1.4 µm broad, while the...
Graphical Abstract

Scanning electron microscopy of guard hair from Panthera tigris and Panthera pardus reveals that surface micro structures and internal organisation of cuticle, cortex and medulla have distinct characters that can have taxonomical value.

Exposed portions of elongate scales are 10 µm in length and 1.1 µm in breadth (Fig. 3, table1). The acuminate scales at the major portion of the hair are found to be rough. The sloughing off of scales at places and deposition of some scale materials are also evident.

Figure 1: Scanning Electron Micrograph of guard hair from head of Panthera tigris (bar=10 µ).

Figure 2: Scanning Electron Micrograph of guard hair from tail of Panthera tigris (bar=10 µ).

Figure 3: Scanning Electron Micrograph of guard hair from head of Panthera pardus (bar=10 µ).

Figure 4: Scanning Electron Micrograph of guard hair from tail of Panthera pardus (bar=10 µ).

Figure 5: Saggital section of guard hair from head of Panthera tigris (bar=10 µ).

4.1.2.2 Guard hair from tail, dorsal & ventral side
The Scanning electron micrograph of guard hair of tail is shown in Fig.4. The width of the guard hair is around 46 µm. The cuticle cell is of coronal type. The scales are acuminate type in major portion of the hair but elongate at the edges. The length of the exposed portion of acuminate scales is around 29 µm while the breadth ranges from 4-9 µm.
4.2 Internal Organization

4.2.1 Guard hair from head

In *Panthera pardus* and *Panthera tigris* the cuticle is about 3-6 µm and 2 µm thick respectively, the cortex is 2.5 and 3.5 µm respectively, medulla is about 62 µm and 14 µm thick respectively. The medulla is characterised by a large number of intercellular spaces and a lumen. The intercellular spaces in *P. pardus* and *P. tigris* are around 0.4-3.5 µm and 8.5-14 µm respectively in diameter. Their distribution is in a definite pattern surrounding the lumen. The fusiform interspersed among the keratinized cells of the cortex in *P. pardus* and *P. tigris* is about 0.2 µm and 0.3-0.7 µm respectively (Figs. 5-7, Table 1).

4.2.2 Guard hair from tail, dorsal & ventral side

In *Panthera pardus* cuticle is about 8 µm thick, whereas in *P. tigris* it is about 3 µm thick. The cortex in *P. pardus* is around 26 µm in contrast to 10 µm in *P. tigris*. The intercellular spaces in the medulla are generally around 0.8-5 µm in *P. pardus* while they are about 2.5 µm in *P. tigris* (Table 1).

In *Panthera tigris* and *Panthera pardus*, remarkable differences are observed in the internal organization between the two species. The sagittal section of guard hair reveals that the cuticle is about 8 µm thick in *P. pardus*, whereas it is about 3 µm in *P. tigris*. The cuticle is followed by a cortex around 26 µm thick in *P. pardus* but only 10 µm thick in *P. tigris*. The average thickness of medulla in *P. pardus* is about 56 µm, while it is about 46 µm in *P. tigris*. The intercellular spaces in the medulla in *Panthera pardus* is about 0.8-5 µm, while it is about 2.5 µm in *P. tigris*. (Table 1).

4.3 SEM of animal hair

A wealth of SEM studies have been published on normal and pathological hair of humans and some laboratory animals (Forslind, 1984), but the same is not available for many wild animal species, distributed in different parts of the world. Despite the fact that North-eastern part of India is a hot spot of Bio-Diversity, very few attempts on basic studies on wild animals involving modern sophisticated techniques have been carried out (Bhattacharjee et al., 1992; Dey, 1992; Dey et al., 1993; Bhattacharjee et al., 1998). It is obvious that besides behavioural and ecological studies, basic studies are important in addressing physiological, pathological and environmental aspects of wildlife biology. Due to the difficulties in availability of tissue samples of wild animals, hair has assumed a great significance in analysing these parameters as far as the wild animals are concerned. Most of the studies on animal hair in different parts of the world however, are concerned with trace-element (Wang et al., 1995) and some biochemical analysis (Ding, 1992) with very little emphasis on other techniques such as SEM. Early literature showed that SEM can resolve the different patterns of cuticle structures of hair in some animal species (Montagna and Parakkal, 1974), thereby helping in identification of animal hair. Clement et al., (1981) made a detailed study on ultrastructure of medulla of several mammalian hair and reported characteristic features in Marsupials, Rodents, Lagomorphs, Insectivores, Primates, Perissodactyla, Artiodactyls, Felidae and Canidae.

4.4 Significance of the current study

In the present study, the type of medullary cells with perforated walls, characteristics of the guard hair of carnivorous mammals (Clement et al., 1981) were observed in both *Panthera tigris* and *Panthera pardus*. Similarly, the cytoplasmic remnants separating the air vesicles were found to be riddled with vacuoles, which gave a sponge-like aspect to the medulla in tiger and leopard. These features were reported to be characteristics of hair.
of Felidae. The differences in width of guard hair in tail, dorsal, ventral portions, the dentition pattern in the free edges of the scale, thickness of the cuticle and cortex, presence of a lumen in the medulla of *Panthera tigris* and its absence in *Panthera pardus* suggest that surface pattern of guard hair and internal organization of medulla, cuticle and cortex have some characteristic features at the species level.

### 4.5. Unique observation in the present study

The present study shows for the first time that SEM of guard hair surface and internal organization of the cuticle, cortex and medulla can be useful in hair identification at genus or species level of animals.

#### Table 1: Comparative data on structural features of guard hair in *Panthera tigris* and *Panthera pardus*.

<table>
<thead>
<tr>
<th>Structural Features</th>
<th><em>Panthera tigris</em></th>
<th><em>Panthera pardus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. External features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Average width</td>
<td>12 ±1.5 µ</td>
<td>23 ±2 µ</td>
</tr>
<tr>
<td>(ii) Type of cuticle</td>
<td>Coronal</td>
<td>Coronal</td>
</tr>
<tr>
<td>(iii) Type of scale</td>
<td>Generalsurface-Accuminate</td>
<td>Generalsurface-Accuminate</td>
</tr>
<tr>
<td>(iv) Average length of exposed portion of elongate scales</td>
<td>5.5 ±0.5 µ</td>
<td>10 ±0.8 µ</td>
</tr>
<tr>
<td>(v) Average breadth of exposed portion of elongate scales</td>
<td>1.1±0.4 µ</td>
<td>3.±0.1 µ</td>
</tr>
<tr>
<td>(vi) Average length of exposed portion of elongate scales</td>
<td>10 ±1 µ</td>
<td>4.±0.1 µ</td>
</tr>
<tr>
<td>(vii) Average breadth of exposed portion of acuminate scales</td>
<td>10 ±1 µ</td>
<td>4.±0.1 µ</td>
</tr>
<tr>
<td><strong>B. Hair from other body parts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Width</td>
<td>46±2 µ</td>
<td>46±3 µ</td>
</tr>
<tr>
<td>(ii) Accuminate scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Length</td>
<td>12±1.1 µ</td>
<td>10±0.6 µ</td>
</tr>
<tr>
<td>(b) Breadth</td>
<td>12.5±0.8 µ</td>
<td>14±0.6 µ</td>
</tr>
<tr>
<td>(iii) Elongate scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Length</td>
<td>14±1 µ</td>
<td>29±2.3 µ</td>
</tr>
<tr>
<td>(b) Breadth</td>
<td>9±0.4 µ</td>
<td>4-9±0.1 µ</td>
</tr>
<tr>
<td><strong>2. Internal Organization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Average cuticular thickness</td>
<td>2±0.2 µ</td>
<td>3±0.3 µ</td>
</tr>
<tr>
<td>(ii) Average cortex thickness</td>
<td>3.5±0.2 µ</td>
<td>2.±0.1 µ</td>
</tr>
<tr>
<td>(iii) Average medullary thickness</td>
<td>14±1 µ</td>
<td>62±3 µ</td>
</tr>
<tr>
<td>(iv) Intercellular spaces in medulla</td>
<td>11.2±0.8 µ</td>
<td>1.9±0.2 µ</td>
</tr>
<tr>
<td>(v) Fusi</td>
<td>0.5 µ±0.02</td>
<td>0.2±0.01 µ</td>
</tr>
</tbody>
</table>

Differences in values of guard hair components between *P. tigris* and *P. pardus* are significant at 0.05.

However, detailed studies on several species of the same genus are required before coming to any conclusion. The present scanning electron microscopic observation and those reported by Hausman, (1930) with optical microscopy showed similar essential anatomical features related to medullary micro structure. However, the current study involving SEM could distinguish guard hairs without medulla from hair in which medulla are discontinuous, intermediate, continuous or fragmental. The advantage of the use of SEM in the present study is obvious due to its high resolving power in revealing the finer details. The present study supports some of the pioneering works (Clement et al., 1980 a, b; 1981) in using SEM data for hair identification. However, the current study appears to be the first attempt towards the possible use of SEM in understanding internal arrangement of medulla in relation to characteristic features at species level. Although morphological data on mammalian hair is available in literature (Waldaver and Grimm, 1884, Smith, 1933, Noback, 1951), no one has ever demonstrated or even proposed the use of the data on hair in drawing a relationship between hair morphology and the taxonomy of mammals until Clement et al. (1980 a, b; 1981) proposed the same in their pioneering work.

### 4.6 SEM of hair and pathology of animals

As far as the use of SEM of hair in pathological manifestation of certain diseases is concerned, several studies have been carried out in some experimental mammalian models (Caputo and Ceccarelli, 1969; Dawber and Comaish, 1970; Porter and Lobitz, 1970; Brown et al., 1971; Mann and Straile, 1972; Demertzis, 1972). However, very
few reports in this regard are available on wild animals. Our study on detailed morphology of guard hair from different body positions of two wild animals will certainly act as reference for detecting any pathological condition resulting from environmental or other causes.

4.7 Hair structure relevant to behaviour
The roughness of guard hair and sloughing and deposition of scale materials on the hair surface in *Panthera tigris* and smoothness of hair from identical region in *Panthera pardus* may be related to their characteristic behaviour. Differences in structural organization in medulla between guard hair from head and other body parts in *Panthera pardus* is not clear, at present, but it also may have some behavioural significance.

**Conclusion**

Scanning electron microscopy of guard hair from two species of the genus Panthera showed a number of similarities as well as differences in surface microstructures and internal organization. The observation made in the present study suggests that micro structures of guard hair may serve as important criteria for identification of animals at genus and species level. The similarities observed in guard hair microstructures in *Panthera tigris* and *Panthera pardus* suggest that these similar features may serve as generic characters. The differences in the micro structure of guard hair in the two animals on the other hand, indicate the possibilities of using these features as species characteristics. However, more studies involving different genus and different species of the same genus are needed for general acceptance of SEM analysis in hair identification of animals.

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