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# Scanning electron microscopy study of guard hair of three Indian pteropodid bats

**Abstract:** A comparative study on the hair microstructure of pteropodid bats *Pteropus giganteus*, *Rousettus leschenaulti*, and *Cynopterus sphinx* was performed using scanning electron microscope. Hair samples were taken from the dorsal, ventral, and neck regions. Among the three pteropodids examined, an imbricate and tightly appressed hair type was observed in *P. giganteus*, while a coronal type of cuticle with margins diverging from the shaft was observed in *R. leschenaulti* and *C. sphinx*. The coronal cuticles of *R. leschenaulti* and *C. sphinx* had relatively thinner scale widths than the imbricate cuticles of *P. giganteus*. There was a significant difference in hair length, scale lengths and scale widths, and scale indices among the three species of fruit bats. However, there was no significant difference in hair length and scale index of male and female *R. leschenaulti* as well as in hair length and scale indices of male and female *C. sphinx*. The presence of bell-shaped coronal cuticle and wide angular scale margin in *R. leschenaulti* and *C. sphinx* reveal their role as active pollinators. The hair patterns, hair length, scale length and width confirm the presence of species-specific characteristics of pteropodid bats and can be used for species identification.

**Keywords:** Chiroptera; fruit bats; hair morphology; pollinators; Pteropodidae; taxonomy.

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## Introduction

Mammals adapt to various environments by changing their pelage. Many mammalian coats, except for those of humans and sheep, are composed of guard hair and underfur. In Chiroptera, the hair structure is essentially uniform over the entire body with the exception of specialized areas such as glands (Benedict 1957). Williams (1938) found that the scale structure was useful for distinguishing the pelage of bat species from that of other insectivores and rodents, and reported that the structure of the hair cuticles varied at different positions along the hair shaft.

Hair morphology can be used for species identification (Kondo 2000). Identification of the hair of mammalian species has practical applications in forensic medicine, taxonomy, paleontology, zooarchaeology, anthropology, and ecology (De Marinis and Asprea 2006, Sahajpal et al. 2009). The value of hair as physical evidence is well appreciated in crime investigation.

Some early studies on hair morphology suggested that hair structure is of rather limited taxonomic value in bats (Nason 1948, Miles 1965). However, many other reports suggest that hair morphology does have taxonomic value (Mayer 1952, Benedict 1957, Wei et al. 1998, Amman et al. 2002), even using hair structure to construct keys to various taxa. For example, Moore and Braun (1983) developed a key to assess the taxonomy of 13 species of Tennessee bats. Dove and Peurach (2001) utilized the microscopic evaluation of hair structure to determine the identity of bat species involved in aircraft strikes.

Hair structure may be related to adaptive features. There are three basic scale structures, namely, coronal (crown-like), spinous (petal-like), and imbricate (flattened) found in bats. Combinations and variations of these types are possible. Imbricate scales are overlapping and encircling the shaft, without divergence from the shaft (Nason 1948). Coronal scales form a complete or cleft cylinder around the shaft with successive scales nested inside each other like a stack of paper cups. Coronal scales from different species differ in the degree to which the distal edge diverges from the shaft, from little or no divergence (appressed) as in a coffee mug, to moderately flaring (divergent) as in many tumblers, to extreme separation (divaricate) reminiscent of a goblet in side view (Schaetz et al. 2009). The term “alternate” describes

coronal scales in which one side is significantly taller than the other, with each scale positioned so that its enlarged half is opposite that of adjacent scales. Coronal scales are commonly found in small mammals (rodents and bats). Howell and Hodgkin (1976) reported that megachiropteran and microchiropteran flower-feeding bats show a divaricate scale structure, which aids in the collection of a heavy coating of pollen. Several authors have noted that these bats pick up such a load of pollen as to appear bright yellow while foraging on flowering trees and when caught by mist netting (Baker and Harris 1959, Nathan et al. 2005). It was noted that the hairs of the neck region do not lie directionally as do the hairs of most mammals but rather stand out like bristles on a bottle brush (Howell and Hodgkin 1976).

The Indian flying fox, *Pteropus giganteus* (Brunnich, 1782), is the largest fruit bat in India where it is widespread. It feeds on fruits and nectar of a wide range of trees. The fulvous fruit bat, *Rousettus leschenaulti* (Desmarest, 1820), is a medium-sized fruit bat distributed throughout India, which feeds predominantly on fruits. The short-nosed fruit bat, *Cynopterus sphinx* (Vahl, 1797), is also a medium-sized fruit bat distributed throughout India. Smaller than *R. leschenaulti*, it feeds on fruits, floral nectar, and leaves. The present study aimed to examine the ultrastructure of hair morphology of these Old World fruit bats using scanning electron microscopy. The significance of hair morphology will be discussed with reference to pollination and species identification.

## Materials and methods

The hair samples from the dorsal, ventral, and neck regions were collected carefully by plucking a few hairs with fine forceps so as to ensure the inclusion of the base of each hair. Broken or worn hairs were not used for analysis. Samples were collected from both sexes of each species as far as possible. Hair samples of *Pteropus giganteus* were collected from two dead male individuals at Mohanlalganj (26°68′N, 80°98′E) and Lucknow (26°46′N, 80°58′E), thus, female sample was not available. Hair samples of *Rousettus leschenaulti* were collected from eight individuals (three males and five females) at Jaunpur (25°75′N, 82°68′E), Faizabad (26°78′N, 82°14′E), Ayodhya (26°79′N, 82°19′E), and Barabanki (26°55′N, 81°11′E). Hair samples of *Cynopterus sphinx* were collected from four individuals (two males and two females) at Lucknow (26°84′N, 80°94′E), Ayodhya (26°79′N, 82°19′E), Sisendy house – Lucknow (26°60′N, 80°94′E) and Unnao (26°45′N, 80°78′E). Hair samples were cleaned and fixed with 2.5% glutaraldehyde for 2–4 h and washed thrice using 0.1 M phosphate buffer. Thereafter, the hair samples were fixed with osmium tetroxide as postfixative for 2 h. The fixed samples were dehydrated using 30%, 50%, 70%, 80%, 90%, 95%, and 100% acetone. The dehydrated hair samples were individually mounted on double-sided carbon tape, which was attached to metal stubs. These stubs were coated with gold-palladium in a sputter coater (JFC 1600; JEOL, Tokyo, Japan) at 20 mA and viewed in a scanning electron microscope (JSM 6490 LV; JEOL, Tokyo, Japan) at different working distances and accelerating voltages. Each specimen was studied extensively and photographed at various magnifications (Figure 1). The hair morphology of the fruit bats was assessed based on scale type, degree of scale divergence from the hair shaft, and the shape of the distal scale margin.

Hair length, scale length (from the free distal edge of one scale to that of the next one), and scale width were measured using the measuring tools of the JEOL software. Benedict (1957) reported that several authors had used the mid-region of a hair for analysis; accordingly, in the present study, the mid-region was used to compare the hair shaft among species and among different body regions. The scale index was calculated by dividing the maximum diameter of the hair by the maximum exposed, proximal-distal length of a scale. The width index was calculated by dividing the maximum diameter of the scale by the minimal diameter of the scale. The angle of divergence of scale from the shaft of neck hair was also measured. The terminology used to describe the scales in this study was adapted from previous studies on chiropteran hair morphology (Brown 1942, Nason 1948, Benedict 1957). One-way ANOVA was used to determine the difference in the hair length, scale length, scale width, scale index, and angle of divergence from the shaft of the three species of bats. The hair lengths of male and female were compared using unpaired "*t*" test.

## Results

The hair morphology of *Pteropus giganteus* was characterized by tight-fitting imbricate cuticles, while *Rousettus leschenaulti* and *Cynopterus sphinx* were characterized by bell-shaped coronal scales. The imbricate cuticles of *P. giganteus* were flattened (Figure 1A) and tightly packed around the hair shaft. The margin of the cuticles was uneven and rough. The coronal scales of *R. leschenaulti* (Figure 1B) and *C. sphinx* (Figure 1C) surrounded the entire

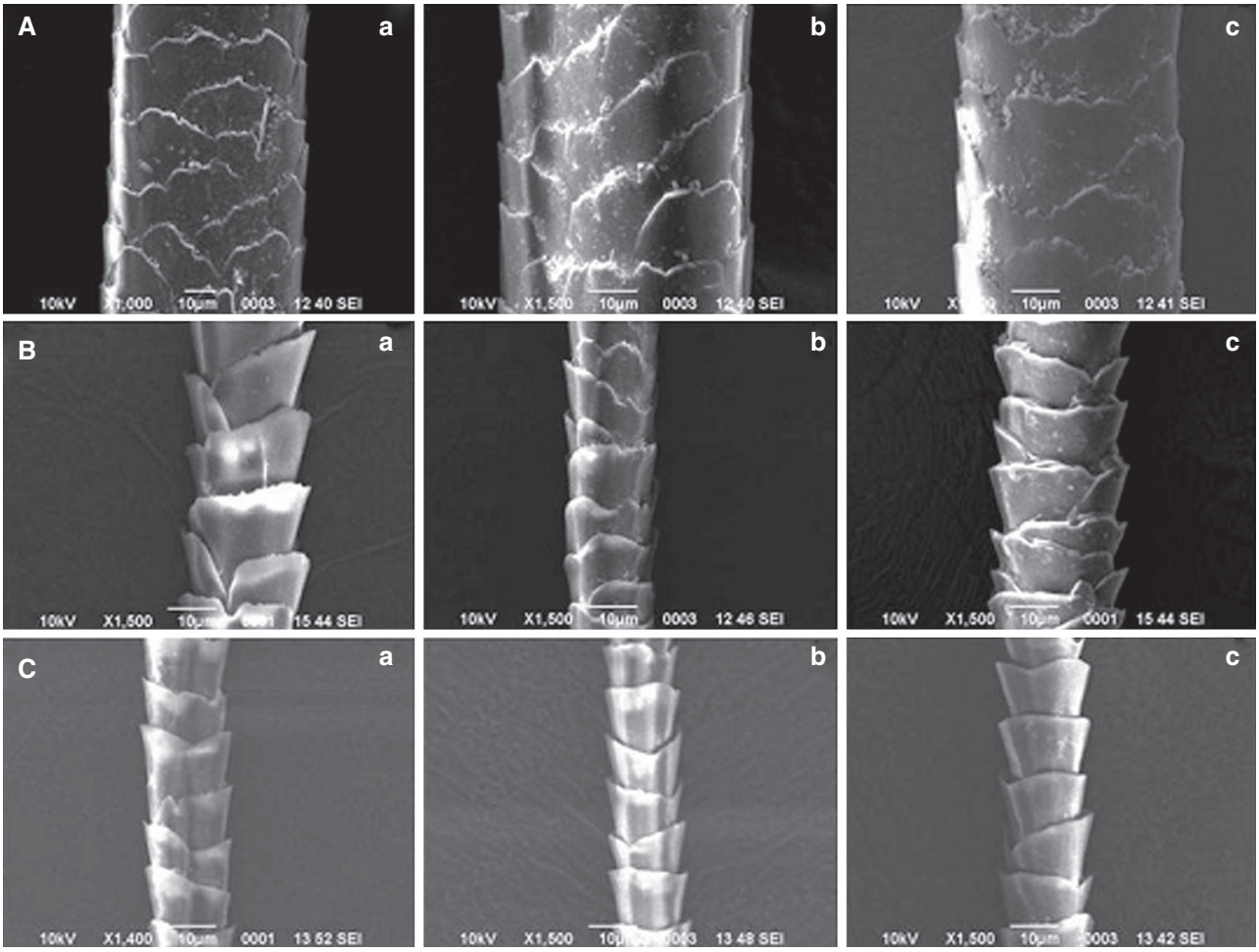


**Figure 1** Electron micrograph depicting the middle region of guard hairs in the (a) dorsal, (b) ventral, and (c) neck regions of the pteropodid bats (A) *Pteropus giganteus*, (B) *Rousettus leschenaulti*, and (C) *Cynopterus sphinx*.

hair shaft while, in *P. giganteus* two or more overlapping scales encircled the shaft. A divergent type of distal scale margin was observed in the dorsal, ventral, and neck regions of *R. leschenaulti* (Figure 1B, a–c) and *C. sphinx* (Figure 1C, a–c).

The nature of the distal scale margin varied somewhat between the studied pteropodids. The distal ends of the hairs of *Rousettus leschenaulti* and *Cynopterus sphinx* were thin, and the scales showed no apical dentition. The hair shaft near the follicle did not show any dentition in all three species of bats. The cuticular scale was appressed along the shaft in the dorsal, ventral, and neck regions of *Pteropus giganteus* (Figure 1A, a–c). The shape of the distal scale margin of *P. giganteus* differed from that of *R. leschenaulti* and *C. sphinx*. A crenate type of hair scale was observed in the dorsal, ventral, and neck regions of *P. giganteus*. The cleft type of hair scale was observed in the dorsal, ventral, and neck hairs of both *R. leschenaulti* and *C. sphinx* (Table 1).

The length of hair (Table 2) varied significantly among the three species of bats ($F_{2, 78}$=32.239, p<0.01), the highest hair length was observed in *Pteropus giganteus*, and there was no significant difference in the hair lengths of male and female *R. leschenaulti* (t=1.783, p>0.05) and *C. sphinx* (t=0.163, p>0.05). Consistently, the scale length and width of *P. giganteus* were relatively higher than those of *R. leschenaulti* and *C. sphinx* (Table 2). The one-way ANOVA showed a significant difference among both the scale lengths ($F_{2, 162}$=162, p<0.001) and scale widths ($F_{2, 118}$=412.99, p<0.001). The scale index of *P. giganteus*, *R. leschenaulti*, and *C. sphinx* also showed a significant difference ($F_{2, 51}$=35.487, p<0.01). Consistently to hair length, the scale indices of male and female did not show significant difference in *R. leschenaulti* (t=1.659, p>0.05) and *C. sphinx* (t=1.542, p>0.05). At last, the mean angle of divergence of scale from the shaft was significantly higher in *C. sphinx* than in *R. leschenaulti* (64.0±2.42 μm vs. 57.2±1.62 μm, t=2.58, df=74, p<0.05).

**Table 1** Summary of hair scale characteristics of dorsal, ventral, and neck regions of *Pteropus giganteus*, *Rousettus leschenaulti*, and *Cynopterus sphinx*.

| Species | Dorsal region | | | Ventral region | | | Neck region | | |
|---|---|---|---|---|---|---|---|---|---|
| | Scale type | Divergence from shaft | Degree of hastateness | Scale type | Divergence from shaft | Degree of hastateness | Scale type | Divergence from shaft | Degree of hastateness |
| *P. giganteus* | Imbricate | Appressed | Crenate | Imbricate | Appressed | Crenate | Imbricate | Appressed | Crenate |
| *R. leschenaulti* | Coronal | Divergent | Cleft | Coronal | Divergent | Cleft | Coronal | Divergent | Cleft |
| *C. sphinx* | Coronal | Divergent | Cleft | Coronal | Divergent | Cleft | Coronal | Divergent | Cleft |

**Table 2** Hair length, scale length, scale width, scale index, width index, and angle of divergence from shaft of dorsal, ventral, and neck regions of *Pteropus giganteus*, *Rousettus leschenaulti*, and *Cynopterus sphinx*.

| Hair characteristics | | *P. giganteus*[1] | *R. leschenaulti* | | *C. sphinx* | |
|---|---|---|---|---|---|---|
| | | M (n=2) | M (n=5) | F (n=3) | M (n=2) | F (n=2) |
| Hair length (mm) | Dorsal | 10.93±5.31 | 7.15±1.94 | 4.71±1.09 | 8.38±1.37 | 6.50±1.47 |
| | Ventral | 9.93±3.48 | 5.02±1.48 | 4.06±1.40 | 8.03±1.30 | 7.29±1.35 |
| | Neck | 10.16±1.54 | 5.27±2.32 | 5.63±1.35 | 5.38±0.47 | 7.76±1.56 |
| Scale length (μm) | Dorsal | 15.19±2.86 | 10.57±2.70 | 12.14±3.56 | 10.28±2.16 | 10.44±2.19 |
| | Ventral | 14.14±2.29 | 8.23±2.21 | 10.21±2.17 | 11.01±1.59 | 8.90±2.37 |
| | Neck | 14.52±3.54 | 10.04±2.13 | 11.35±2.70 | 11.34±1.69 | 11.04±2.37 |
| Scale width (μm) | Dorsal | 54.25±7.78 | 20.47±4.32 | 26.30±1.91 | 18.74±1.14 | 16.86±1.92 |
| | Ventral | 62.76±12.18 | 26.40±3.98 | 24.09±4.78 | 21.20±4.56 | 29.57±0.51 |
| | Neck | 47.13±0.65 | 23.54±3.98 | 20.26±2.76 | 18.81±0.81 | 18.85±0.90 |
| Scale index (μm) | Dorsal | 3.16±0.74 | 1.97±0.17 | 1.36±0.48 | 1.44±0.09 | 1.47±0.08 |
| | Ventral | 4.44±2.10 | 2.14±0.50 | 1.97±0.37 | 1.86±0.54 | 2.74±0.66 |
| | Neck | 2.20±0.94 | 1.85±0.45 | 1.47±0.33 | 1.35±0.17 | 1.59±0.37 |
| Width index (μm) | Dorsal | 1.07±0.02 | 1.19±0.12 | 1.11±0.03 | 1.19±0.03 | 1.20±0.10 |
| | Ventral | 1.03±0.02 | 1.06±0.03 | 1.11±0.07 | 1.12±0.02 | 1.07±0.00 |
| | Neck | 1.04±0.02 | 1.29±0.26 | 1.21±0.11 | 1.13±0.01 | 1.19±0.04 |
| Angle of divergence | Dorsal | Nd | 63.60±2.88 | 61.02±2.35 | 56.45±3.82 | 57.77±3.52 |
| | Ventral | Nd | 54.77±2.99 | 52.70±3.65 | 49.69±1.57 | 48.29±1.43 |
| | Neck | Nd | 57.64±3.40 | 55.35±2.42 | 76.41±4.20 | 67.44±3.94 |

[1]Hair samples collected from dead *P. giganteus*. M, male; F, female; Nd, no divergence.

## Discussion

The study on hair morphology of three pteropodid bats revealed interesting features in the shape and the characteristics of the cuticle. Earlier studies on the hair morphology of fruit-eating bats showed that the coronal cuticle is of more ancient origin compared to the imbricate cuticle (Chernova 2002). The present study shows that both types of cuticles (imbricate and coronal) occur in the family Pteropodidae. The rod-shaped, imbricate pattern with a smooth profile and a small scale index are characteristic features of the genus *Pteropus*. The imbricate cuticle pattern in *Pteropus giganteus* was shown to be related to the underdeveloped medulla and the durability of the cuticle (Chernova 2002). In the present study, the bell-shaped coronal cuticles of *Rousettus leschenaulti* and *Cynopterus sphinx* had a relatively smaller scale width than the imbricate cuticles of *P. giganteus*. The thick cuticle of fruit-eating bats is durable, smooth, and has open edges. This is related to the biology of fruit-eating bats, which do not need fast and maneuverable flight for getting food.

Howell and Hodgkin (1976) reported that flower-visiting bats possess a divergent or divaricate scale type, which is suitable for gathering a large load of pollen from chiropterophilous plants, while bats not associated with flowers show hairs with tightly appressed scales. In accordance with this earlier study, the flower-visiting bats *Rousettus leschenaulti* and *Cynopterus sphinx* are equipped with the divergent cuticle type. The bell-shaped coronal cuticle is suitable for passive pollen transfer (Chernova 2002). The observations on the angle of divergence of the scale margin of *C. sphinx* and *R. leschenaulti* clearly show that

they are active pollinators. Although *Pteropus giganteus* regularly visits the flowers of many chiropterophilous plants (Nathan et al. 2005), its hair morphology seems to be unsuitable for pollen gathering. Thus, the imbricate type of hair cuticle of *P. giganteus* at the neck, dorsal, and ventral regions indicates that this species may not be an active pollinator.

Mean scale length at the middle portion of the hairs varied among the three bat species and may therefore be used for identification purpose, e.g., to identify hair samples taken from archaeological sites or particular roosts or to analyze bat hairs found in carnivore scats and study predator-prey interactions. The scale shape of *Rousettus leschenaulti* and *Cynopterus sphinx* exhibits strong divergence. Although the hair patterns of *R. leschenaulti* and *C. sphinx* look similar, hair length and scale index differed. Each species exhibited distinct qualitative traits in hair morphology that allow their identification. Thus, we propose that hair structure can be a useful taxonomic character to distinguish between pteropodid species.

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