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HISTOLOGICAL STUDY OF SKIN AND HAIR IN MALABAR GIANT SQUIRREL (*Ratufa indica*)

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ABSTRACT

Malabar giant squirrel (*Ratufa indica*) is an arboreal squirrel species endemic in India. The species is categorized under family *Ratufinae* and order *Rodentia*. The specimens were collected from two dead adult Malabar giant squirrels from the forests of Central Kerala. The samples were fixed, processed and sectioned as per routine paraffin embedding and sectioning procedures. The sections were stained by routine and special histological stains. The guard hairs were processed for studying medulla and cuticle morphology. The skin had an outer epidermis made by keratinized stratified squamous epithelium and an inner dermis. Dermis enclosed hair follicles, sebaceous glands, smooth muscle and blood vessels. Guard hair follicles and groups of primary hair follicle clusters were present in the dermis. The scales of cuticle had "transversal" position, "smooth" scale margins, "regular wave" scale pattern and scales were placed "close". Medulla characteristics included composition as "multicellular in rows", structure as "multiserial ladder" and the margins were "scalloped". The guard hair shaft had average medullary index values at proximal half and distal half as 0.547 ± 0.05 and 0.435 ± 0.08 respectively. Medullary index along with other hair parameters and follicle pattern in skin histology may provide species identification key. The paper describes the salient histomorpological characteristics of skin and hair in Malabar Giant squirrel.

Keywords: Malabar Giant squirrel, Skin, Hair

INTRODUCTION

Malabar giant squirrel (*Ratufa indica*) is one of the large squirrels and is endemic to Indian forests. The arboreal giant squirrel species is placed in the family *Ratufinae* (Scott and Hamm, 2006) and order *Rodentia.* The animal habitat consists of tall canopy trees and contiguous forests (Srinivas *et al*., 2008). Therefore, an augmented deforestation, intensified

monoculture plantation, and extensive hunting are the main threats to its population. The subspecies found in Kerala is *R. indica maxima* (Prachi *et al*., 2012). Its fur coat is black on the dorsum, but it has light shade on under parts and brown colour on head. International Union for Conservation of Nature and Natural resources categorized the animal as least concern. Kamalkannan (2017) described hair characteristics in giant flying squirrels. However, studies on skin and hair in Malabar giant squirrel are scanty. Hence, the present study was undertaken to elucidate the histomorphological features of skin and hair in Malabar giant squirrel. The study may provide reference data for taxonomy and wildlife forensics.

MATERIALS AND METHODS

The specimen for the study was collected from two Malabar giant squirrels died due to natural causes at forest ranges of Central Kerala. The skin samples were obtained from the mid dorsal part of body. Permission for the conduct of the study was obtained from Chief Wildlife Warden, Kerala as per order no KFDHQ-915/2019-CWW/WL10 dated 11/03/2019. The samples were fixed in neutral buffered formalin for minimum 48 hours and processed for routine paraffin embedding. Sections of 5 µm thicknesses were taken, stained by Haematoxylin and Eosin and Gomori's one step trichrome (Singh and Sulochana, 1996). The histological features and hair follicle pattern in the sections were observed under optical microscope (OlympusCX21i) fitted with a digital camera. Guard hairs were collected, washed with soap water and rinsed in distilled water. Negative cast of hair for studying cuticle morphology and whole mount of hair for studying medullary features were done as per the methods described by Charjan *et al*. (2019) and Sahajpal *et al.* (2009) respectively. The cuticle and medulla pattern were studied as per the descriptions of De Marinis and Asprea (2006). The width of medulla and hair was measured using Magvision software and the medullary index of the hair was calculated as the ratio of the medulla width to hair width (Sahajpal *et al.*, 2009).

RESULTS AND DISCUSSION

The skin consisted of an outer epidermis and an inner dermis (Fig. 1, 2). Keratinized stratified squamous epithelium made up the epidermis. Four layers *viz*., stratum basale, stratum spinosum, stratum granulosum and stratum corneum were distinct in the epidermis. Stratum basale contained a single layer of cuboidal cells. One or two rows of oval cells and flat cells made up the spinosum and granulosum layers, respectively. Stratum lucidum was lacking. Dead flat superficial cells formed the stratum corneum.

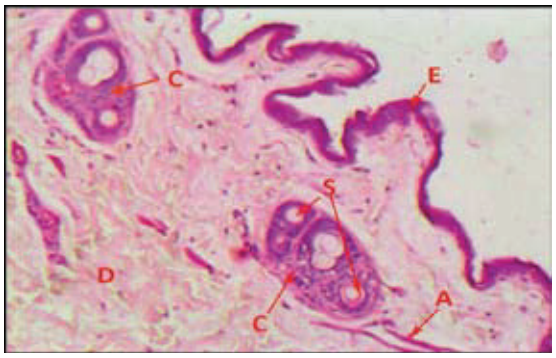

Fig. 1 Photomicrograph of skin in Malabar Giant squirrel (H&E X 50)
E-Epidermis, C-Compound follicle, D-Dermis, A-Arrectores pilorum muscle, S-Fine secondary follicles

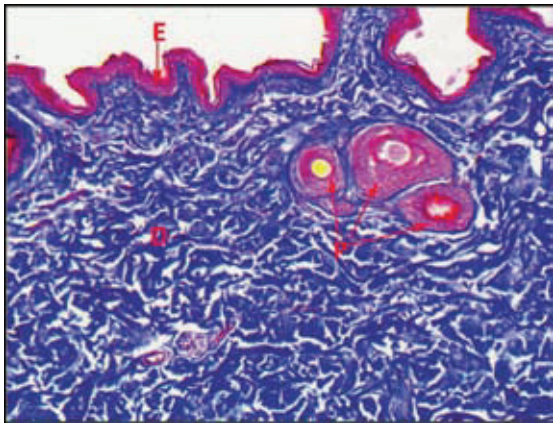

Fig. 2 Photomicrograph of skin in Malabar Giant squirrel (Gomori's one step trichrome X 150)
E-Epidermis, D-Dermis, P-Primary hair follicle cluster

Dense irregular connective tissue enclosing hair follicles, sebaceous glands, smooth muscle and blood vessels constituted the dermis (Fig. 1, 2). The connective tissue was less dense in the superficial papillary layer than in the deep reticular layer. Dense irregular connective tissue separated different hair follicle groups. The follicles within a group were separated by loose connective tissue. Two types of hair follicle pattern were distinct

Fig. 3 Photomicrograph of guard hair in Malabar Giant squirrel showing medullary features (X 150)

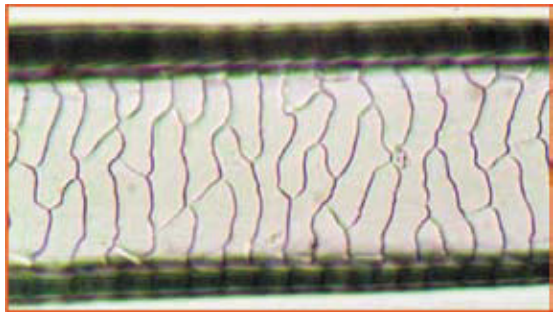

Fig. 4 Photomicrograph of guard hair in Malabar Giant squirrel showing cuticle features (X 500)

in the study: guard hair follicles and groups of primary hair follicles. The guard hair follicles were of compound type, each follicle contained one guard primary hair follicle and three to eight secondary fine hair follicles (Fig. 1). In addition, single primary hair follicles in clusters of three to six were also present (Fig. 2). Our data on hair follicle arrangement vary from the findings in African palm squirrel by Ibe *et al*. (2020) who described only simple hair follicle clusters and did not mention about guard hair follicles. The small, saccular acini of sebaceous glands were present in association with primary and secondary hair follicles. Smooth muscle, arrectores pilorum was present in relation to the glands.

Medullary characteristics of hair *viz*., medulla composition- "multicellular

in rows" structure of medulla- "multiserial ladder" and medullary margin "scalloped" was observed in the present study (Fig. 3). The features of the present study are in accordance with the parameters described by Kamalakannan (2017) in three species of giant flying squirrel. In the cuticle, scales showed "transversal" position, scale margins were "smooth", scale pattern as "regular wave" and the distance between adjacent scale margins was "close" (Fig. 4). Similar features are described by Kamalakannan (2017) in three species of giant flying squirrel. The results of the study and earlier records by Kamalakannan (2017) reveal that the cuticle and medullary pattern may be a reliable identification key to group hair up to genus level. The cuticle and medullary patterns of different squirrel species were consistent so that those features alone may not be reliable key for identification at species level. The calculated average medullary index values of the guard hair shaft at proximal half and distal half were 0.547 ± 0.05 and 0.435 ± 0.08, respectively. Kamalakannan (2017) did not mention about medullary index in giant flying squirrel. Medullary index differs even in closely related species (Sahajpal *et al*., 2009). Therefore, inclusion of medullary index along with other hair parameters and hair follicle pattern of the skin may provide identification key.

SUMMARY

The paper describes the salient histomorpological characteristics of skin and hair in Malabar Giant squirrel. The study revealed that the skin is comprised of an outer epidermis and an inner dermis. Dense irregular connective tissue constituted the dermis and enclosed compound hair follicles. Compound follicles contained guard hair follicles and groups of primary hair follicle clusters. In the guard hairs, the cuticle scales had "transversal" position, "smooth" scale margins and "regular wave" scale pattern. Medulla composition was "multicellular in rows" and its structure was "multiserial ladder". The guard hair shaft had average medullary index values at proximal half and distal half as 0.547 ± 0.05 and 0.435 ± 0.08, respectively.

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