

# Trunk and paw pad skin morphology of the African giant pouched rat (*Cricetomys gambianus*, Waterhouse-1840)

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## SUMMARY

The gross morphology and histology of the skin of the trunk and paw pads in the African giant pouched rat were investigated to evaluate their role in the adaptation of the rodent to its subterranean environment. Samples were stained with haematoxylin and eosin, Masson's trichrome, Alcian blue, Verhoeff's haematoxylin counterstained with Van Gieson, and Weigert's haematoxylin counterstained with Picro-Ponceau stains. Dorsally, fur covering the skin was loosely folded. Fur covered the entire trunk to the level of the radio-carpal and talocrural joints of the forelimb and hindlimbs, respectively. Skin of the dorsum was paler than its grey-coloured fur, while skin of the ventrum was dirty white. There were more hair follicles dorsally than ventrally. The manus and pes had five and six paw pads, respectively. Keratinocytes in the epidermis of the paw pads decreased in number and lost their cellular contents as they migrated towards the *stratum lucidum*. Metatarsal pads had a significantly ( $P < 0.001$ ) thicker *stratum corneum* than metacarpal pads. Elastic fibres were observed in the metatarsal pads. Other results and additional information from the literature were integrated to propose the effect of the structures on the adaptation of the African giant pouched rat to its subterranean environment and tropical climate.

**Key words:** Gross morphology – Histology – Skin of trunk – Paw pads – African giant pouched rat

## INTRODUCTION

The African giant pouched rat (*Cricetomys gambianus*) is a remarkable rodent in many respects. It is one of the largest nocturnal rodents of the *Muridae* family. Its habitat is distinct from other members of the family: it lives in terrestrial burrows constructed using its teeth and manus to loosen substrate and its pes to push excavated soil away (Ewer, 1967). Although Rosevear (1969) and Ajayi (1975) suggested that the pes maybe well adapted for digging, no detailed anatomical study has been done to verify the suggestion.

The African giant pouched rat has been used to detect land mines (Mott, 2004) and diagnose tuberculosis (Maggie, 2003) by their high acuity of odour perception. It is sequel to this importance that the potential of the rat as an experimental model for biomedical research is currently explored. Documentation of the normal features of its integumentary system will serve as a lead for further physiologic and behavioural studies. The adaptive morphology of the mystacial vibrissae have been reported (Ibe et al., 2010).

The numeric and regional distribution of paw pads differ among rodents. They provide a cushioning effect to the underlying skeletal structures, thus protecting them from dislocation (Brown and Yalden, 1973). In some animals, paw pads contain

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eccrine glands whose secretions confer a degree of friction between the pad and adjoining environment (Haffner, 1998). Animals of the same genera can be differentiated by paw pad morphology; Anderson (1999) noted significant morphometric difference in the manus paw pads of the thick-tailed galago (*Otolemur crassicaudatus*) and the Garnett's galago (*Otolemur garnettii*). Anatomy of paw pads has been reported in different rodents (Kimura et al., 1994; Krystufek and Macholan, 1998; Musser and Durden, 2002; Wong et al., 2006; Kimura et al., 2008) and used to explain adaptation to a specific mode of life.

Animal fur is indicated in body insulation and protection from the immediate environment. Sweat glands play pivotal role in cooling mechanism. They have been reported to be sparsely distributed in the naked mole-rat (*Heterocephalus glaber*) as a heat conservation mechanism, to compensate for the absence of fur (Daly and Buffenstein, 1998). Furthermore, skin colour differs in animals and has been associated with thermoregulation (Scharf, 2008).

The present study will be of value in future dermatoglyphic studies in the African giant pouched rat. Since the rats are currently domesticated in Nigeria as supplementary protein source (Olayemi et al., 2001) and in Europe as exotic pets (Cooper, 2008), there is a need to have a good knowledge of their adaptive morphology. This is a prerequisite for an effective breeding and domestication programme. Therefore, the aim of the study was to identify morphologic features of the skin of the trunk and paw pads in the African giant pouched rat that are related to its tropical climate and subterranean environment and compare the results with similar findings in other rodents.

## MATERIALS AND METHODS

Seven healthy adult African giant pouched rats weighing between 1.3 kg and 1.5 kg were captured from the wild in Kaduna state, Nigeria. They were given elephant grass (*Penicetum puperum*), groundnut cake, potatoes and drinking water *ad libitum*. Gross features were observed and recorded via digital photography. Report of Brown and Yalden (1973) was used as guide to describe external features of the paw. Paw length and width were defined using the method of Ade and Ziekur (1999). Following humane sacrifice, the skin of the trunk, forelimbs, hindlimbs and paw pads was removed.

Skin samples of 5 mm<sup>2</sup> each from shaved portions of the dorsal trunk, at the level of the thoracic spine, and the ventral trunk, at the level of the umbilicus, were measured with a vernier calliper (MG6001DC, General Tools and Instruments Company, New York; sensitivity: 0.01mm) and cut out with a scalpel blade. 5 mm<sup>2</sup> each, of the digital skin of digit 3 of the manus and pes, the 2<sup>nd</sup> inter-

digital pads of the manus and pes, the metacarpal and the metatarsal pads were measured and samples taken. The skin samples were cut in such a way that the epidermis, dermis, subcutis, deep fascia and underlying muscles were intact. Tissues were washed with saline and fixed in 10 % phosphate-buffered formalin for 1 week. After fixation, the samples were washed in tap water and kept therein for 24 hours. Finally, specimens were dehydrated in a graded series of alcohol (70 %, 80 %, 90 %, 95 % and 100 %). Tissues were cleared in xylene, paraffin embedded and labelled.

Skin samples from the trunk were sectioned transversely at 5 µm using a Jung rotary microtome (Model 42339, Berlin, Germany). Representative sections were mounted on glass slides, dried, deparaffinized, stained, and coverslipped using DPX as the mountant. Alternate sections were stained with haematoxylin and eosin (H/E), Masson's trichrome (MT) and Alcian blue (AB) stains. A paraffin section of the skin of the dorsal trunk was treated with the Masson-Fontana reducing method to demonstrate melanin, using the method of Bancroft and Stevens (2008) by placing the section in 10 % ammoniacal silver nitrate overnight at room temperature in a dark room. The following day, it was washed in several changes of distilled water and treated with 5 % sodium thiosulphate. Following a tap water flush, it was counter stained with 1 % aqueous neutral red, dehydrated through a graded series of alcohols to xylene and mounted in DPX.

Skin samples from the paw pads were sectioned transversely at 7 µm and processed as described for the trunk skin samples. Alternate sections were stained with H/E, Verhoeff's haematoxylin counterstained with Van Gieson's (VVG) and Weigert's haematoxylin counterstained with Picro-Ponceau (WHP).

H/E was used as the conventional stain to study the general tissue architecture. VVG was used to demonstrate elastic fibres which stained black. WHP is very effective for visualising structures of the dermis and epidermis as well as for providing enhanced cellular detail (Hamrick, 1998). MT was indicated to demonstrate collagen fibres which stained blue against a black (cell nuclei) and red (cytoplasm, muscles or erythrocytes) background. AB was used to demonstrate acid mucosubstance which stained blue against a pink background. The Massons-Fontana staining displayed melanin as black colour against a red background.

The epidermis was divided into two broad layers for clarity. These were the *stratum corneum* and the non-cornified layer. The *stratum lucidum*, *stratum spinosum*, *stratum granulosum* and *stratum basale* were grouped as the non-cornified layer. The thickness of the *stratum corneum* and non-cornified layer of the metacarpal and metatarsal pads were obtained using a calibrated ocular micrometer (Leitz Wetzlar, Germany) following ap-

appropriate calibration of a light microscope (OLYMPUS® - XSZ107BN, Hamburg, Germany) with a stage micrometer (Graticules Ltd., London). For each pad, 15 points were randomly chosen and the mean thickness of the *stratum corneum* and non-cornified layer was utilized. The result was subjected to statistical analysis using student's t-test to determine the difference in thickness of the two epidermal layers. Images of histological sections were photographed with a digital camera and eyepiece (Scopetek® DCM500, Resolution: 5M pixels) attached to the light microscope.

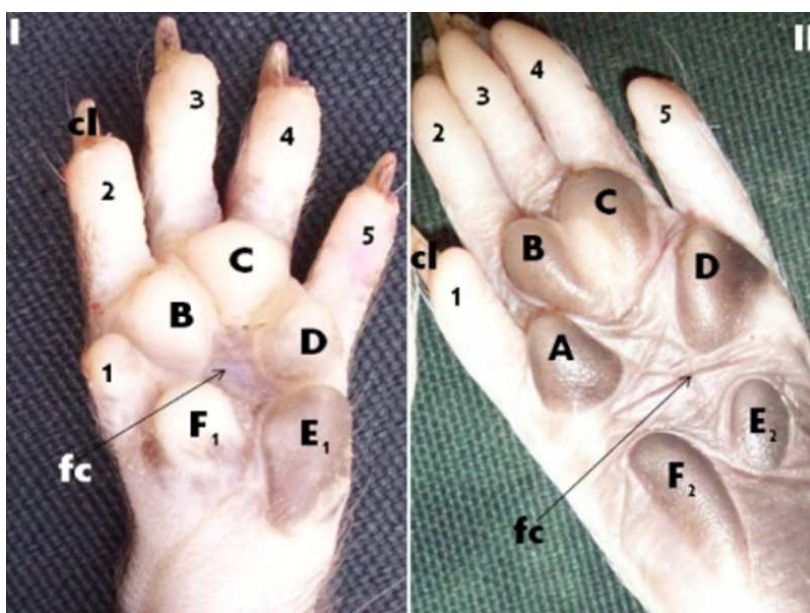
## RESULTS

### Gross morphology

**Skin of the trunk:** The dorsal skin of the African giant pouched rat was loosely folded. The dorsal



**Fig. 1.** Dorsal (a) and ventral (b) fur of the African Giant pouched rat



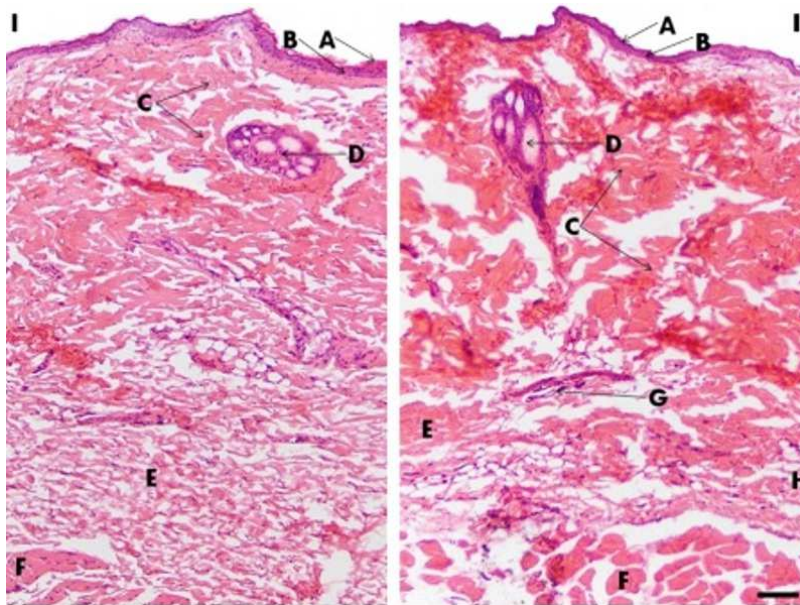
**Fig 2.** Palmer surface (I) and plantar surface (II) of the left foot of the African Giant pouched rat, illustrating 1<sub>a</sub>: pallus. 1<sub>b</sub>: hallux 2-5: 2<sup>nd</sup> to 5<sup>th</sup> digits. A: 1<sup>st</sup> interdigital pad. B: 2<sup>nd</sup> interdigital pad. C: 3<sup>rd</sup> interdigital pad. D: 4<sup>th</sup> interdigital pad. E: hypothenar pad. F: thenar pad. cl: claw. fc: flexion crease. sp: sweat pore.

fur was grey in colour and covered the entire trunk (Fig. 1a). It extended to the level of the radiocarpal and talocrural joints of the forelimbs and hindlimbs, respectively. The ventral fur was greyish white and thinner than the dorsal fur (Fig. 1b). The ventral fur terminated on the medial femoral skin from where the grey-coloured fur continued to the talocrural joint. The extent of the ventral fur in the forelimb was the same as the dorsal fur (to the radiocarpal joint). Shaved skin of the dorsum was light grey in colour while shaved skin of the ventrum was dirty white. The dorsal skin of the fore and hind limbs had hair follicles that terminated on the lateral surface of the paws. The ventral surfaces of the manus and pes were devoid of noticeable hair follicles.

**Paw Pads of the Manus:** Five paw pads were observed on each manus. They included 3 interdigital pads (Fig. 2I: B, C and D), and 2 metacarpal pads (Fig. 2I: E and F). The 1<sup>st</sup> interdigital pad and digital pads were not observed in the manus. There was no fusion of any pads. The thenar pad (Fig. 2I: F<sub>1</sub>) lay proximal to the 2<sup>nd</sup> interdigital pad (Fig. 2I: B) on its axis. The hypothenar pad (Fig. 2I: E<sub>1</sub>) lay proximal to the 4<sup>th</sup> interdigital pad (Fig. 2I: D) on their axis. The interdigital and metacarpal pads lay in a circular pattern. The surfaces of the pads were smooth with indistinct sweat pores. Flexion creases were evident between adjacent pads (Fig. 2I: fc). The digits were not fused and they possessed sickle-shaped claws. Digit 1 (Fig 2I: 1) was the shortest digit while digits 3 was the longest. Skin of the digits was of the same white colour as the paw pads. The mean length and width of the manus were  $1.37 \pm 0.04$  cm and  $1.32 \pm 0.08$  cm, respectively.

**Paw Pads of the Pes:** Six paw pads were present on each pes of the African giant pouched rat. They included 4 interdigital pads (Fig. 2II: A, B, C and D) and 2 metatarsal pads (Fig. 2II: E<sub>2</sub> and F<sub>2</sub>). There were no digital pads on the pes. The 2<sup>nd</sup> and 3<sup>rd</sup> interdigital pads (Fig. 2II: B and C) were fused at their base and were more rostrally placed than the 1<sup>st</sup> and 4<sup>th</sup> interdigital pads (Fig. 2II: A and D). The thenar pad (Fig. 2II: F<sub>2</sub>) lay proximal to the 1<sup>st</sup> interdigital pad (Fig.





**Fig. 3.** Transverse sections of the ventral (I) and dorsal (II) skin of the trunk of the African Giant pouched rat, illustrating A: Stratum corneum of the epidermis. B: Non-cornified layer of the epidermis: C: Collagen fibres. D: Hair follicles and adjacent sebaceous glands. E: Dense network of irregular connective tissues. F: muscle tissues. G: Blood vessel. H: Adipocytes. H/E. Magnification: x 100.

2II: A) on its axis. The hypothenar pad (Fig. 2II: E<sub>2</sub>) lay proximal to the 4<sup>th</sup> interdigital pad (Fig. 2II: D) on their axis. The 6 paw pads lay in a circular pattern. The surfaces of the pads were either smooth or with weak striations. Numerous sweat pores existed on the surfaces of the paw pads. There were flexion creases (Fig. 2II: fc) on the non-pad areas between adjacent pads. The digits were distinctly separated, long and slender with sickle shaped claws. Digit 1 (Fig. 2II: 1) was the shortest of the digits. Digits 3 was the longest. The skin of the ventral surface of the digits was white in colour while the skin of the pads was grey-coloured. The mean (Standard Error of Mean) length and width of the pes were  $1.87 \pm 0.09$  cm and  $1.67 \pm 0.09$  cm, respectively. The difference in length of manus and pes was very highly significant ( $P < 0.001$ ), and the difference in their width was highly significant ( $P < 0.01$ ).

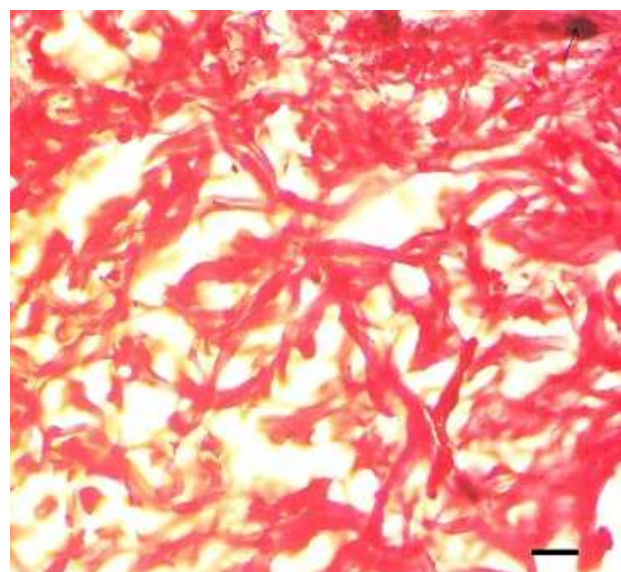
### Histology

*Skin of the trunk:* Histologic sections of the ventral and dorsal skin were similar with slight variations. The epidermis exhibited continuous undulation along its length. It revealed a thin layer of *stratum corneum* and a non-cornified layer (Fig. 3: A and B). In the dermis, bundles of collagen fibres (Fig. 3: C) and dense network of irregular connective tissues (Fig. 3: E) were observed in an acidic mucinous matrix. Numerous hair follicles and adjacent sebaceous glands (Fig. 3: D) were also evident in the dermis. Apparently, there were more hair follicles in the dorsum than in the ventrum,

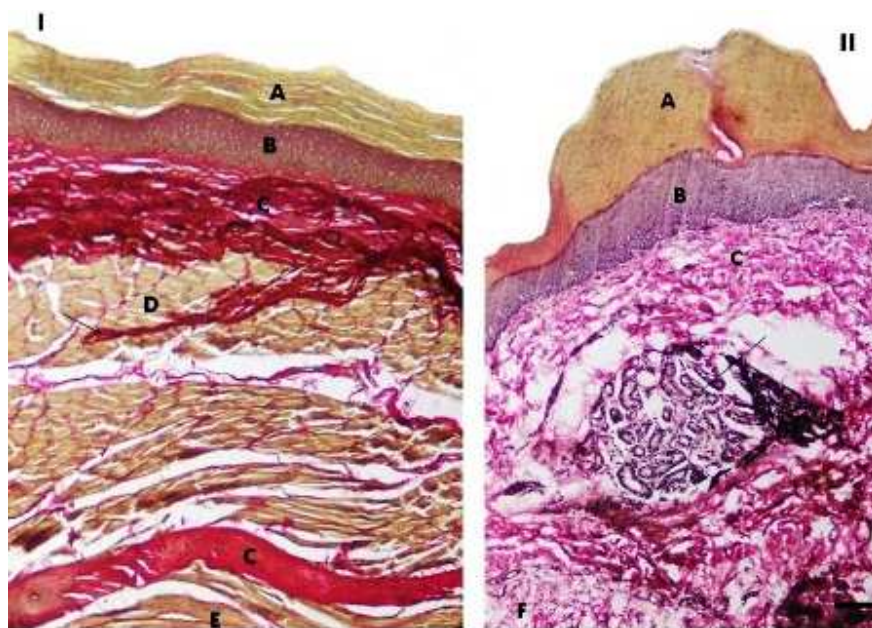
and the dense network of irregular connective tissue was extensive in the ventrum. Blood vessels, adipocytes and muscle tissues were evident in the hypodermis. With the Masson-Fontana stain, melanocytes were observed at the *stratum basale* of the epidermis and around the dermal papillae (Fig. 4).

*Paw pads and digital skin:* Epidermal layers of the metacarpal and metatarsal pads were undulating and varied in thickness (Fig. 5: A and B). The mean thickness of the *stratum corneum* in the metacarpal and metatarsal pads was  $83.267 \pm 4.097$   $\mu$ m and  $124.867 \pm 1.804$   $\mu$ m, respectively. The mean thickness of the non-cornified layer was  $31.467 \pm 2.402$   $\mu$ m and  $33.400 \pm 2.952$   $\mu$ m in the metacarpal and metatarsal pads, respectively. These values and their corresponding P-values were represented in the bar chart (Fig. 6). Beneath the epidermis were bundles of collagen fibres,

running parallel to the overlying epidermal layer (Fig. 5: C). Series of collagen bundles (indicated with an arrow in Fig. 5a) were observed running obliquely, from the bundle of collagen fibres through the underlying sweat glands. This was observed in both metacarpal and metatarsal pads. Sweat glands were scattered among the collagen fibres in the dermis. Also observed in both pads was a second layer of collagen fibres in the hypodermis, separating the sweat glands



**Fig. 4.** Transverse section of the skin of the trunk in the African Giant pouched rat illustrating a melanocyte in the stratum basale with an arrow. Masson-Fontana X 400.



**Fig. 5.** Transverse sections of the palmer (I) and planter (II) thenar pads in the African Giant pouched rat, illustrating A: Stratum corneum of the epidermis. B: Non-cornified layer of the epidermis: C: Collagen fibres. D: Sweat glands. E: Muscle fibres. F: Adipocytes. Arrow in (I) indicates collagen bundles. Arrow in (II) indicates elastic fibres. Figure 5 (I): WHP Magnifications: X 100. Figure 5 (II): VVG X 100.

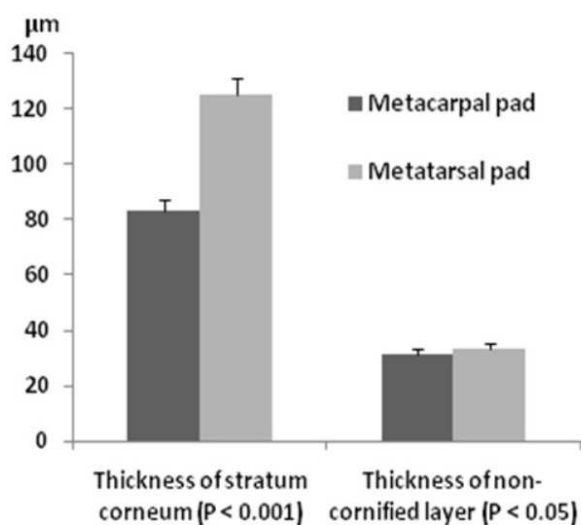
from the underlying thick bundle of muscle fibres. There were more elastic fibres in the metatarsal pads (indicated with an arrow in Fig. 5b). Blood vessels were seen in the hypodermis. There were no hair follicles in the dermis of both paw pads.

The digital skin of the manus and pes were basically similar to the paw pads but for the epidermal layer of the digital skin, which was more undulating than that of the paw pads. The epidermal cells in the non-cornified layers were more numerous in the digital skin than in the paw pads. Dermal papillae were distinct in high-

er magnifications (indicated with arrows in Fig. 7). These sections also clearly differentiated the non-cornified layers of the epidermis into their different strata. The non-cornified layers were composed of cuboidal cells with prominent nuclei. The cells (keratinocytes) were densely packed in the *stratum basale* (Fig. 7: B<sub>1</sub>). They decreased in number and lost their cellular contents as they migrated towards the *stratum lucidum* (Fig. 7: B<sub>4</sub>).

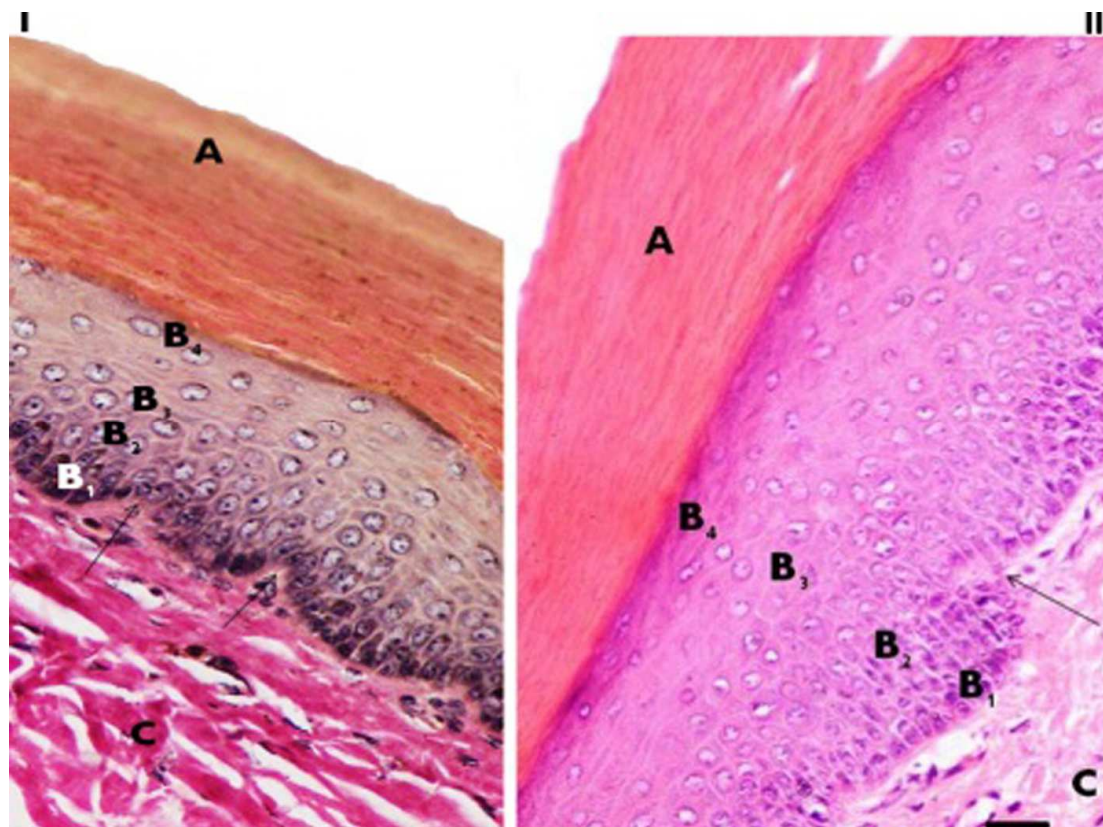
## DISCUSSION

The integument serves as a protective barrier to an animal's external environment. The colour pattern of the skin of the African giant pouched rat may be an adaptive morphology to evade predation in the wild. The grey-coloured dorsum coincides with the environment and thus camouflages, unlike the dirty white ventrum. The pale colour of the skin of the rat may also be an adaptive feature to reduce heat radiation, typical of its tropical climate. The fur is not as dense and as long as the water vole's as reported by Foreman (2005). This may also be indicative for heat dissipation in the African giant pouched rat as against heat conservation in the water vole, due to the contrasting ambient temperature in their habitat. Similarly, the numerous skin folds observed in histologic sections are indicative of a large surface area with resultant increased heat dissipation and cooling effect in the African giant pouched rat. However, the adipocytes observed in the hypodermis of the skin of the trunk function in conferring a degree of insulation to the animal when the need arises.



**Fig. 6.** Bar chart representing the thickness of the epidermis in the Palmer and planter thenar pads.





**Fig. 7.** Transverse sections of the palmer (I) and planter (II) digital skins in the African Giant pouched rat, illustrating A: Stratum corneum of the epidermis. B<sub>1</sub>: Stratum basale of non-cornified layer of the epidermis; B<sub>2</sub>: Stratum spinosum of non-cornified layer of the epidermis. B<sub>3</sub>: Stratum granulosum of non-cornified layer of the epidermis. B<sub>4</sub>: Stratum lucidum of non-cornified layer of the epidermis. C: Collagen fibres. Arrows indicate dermal papillae. Figure 7(I): WHP X 400. Figure 7(II): H/E X 400.

The grey coloured dorsal fur differs from the fawn yellowish to tawny brown dorsal fur of another *Muridae*: the Indian gerbil (*Tatera indica*) as reported by Yigit et al. (2001). Also, the extension of the fur to the radiocarpal and tibiotarsal joints differs from that of another *Muridae*: the Sulawesi rat (*Sommeromys macrorhinos*), in which the fur extends only to the proximal half of the lower forelimb and proximal two-thirds of the lower hindlimb, without reaching the radiocarpal or tibiotarsal joints (Musser and Durden, 2002). Thus, the result of the present study buttresses the fact that there is high gross morphological divergence in skin colour and fur disposition in animals of the same family. This divergence exists even in members of the same genus.

The African giant pouched rat shares similarity with the naked mole-rat both the numeric and regional distribution of the paw pads. Haffner (1998) recorded 6 paw pads on each pes of the naked mole-rat; 2 metatarsal pads and 4 interdigital pads. The interdigital pads of the naked mole-rat build, together with the metatarsal pad, a circle (Haffner, 1998) similar to what we observed in the African giant pouched rat from the present study. Haffner (1998) also recorded 5 paw pads on each manus in the naked mole-rat; 3 interdigital pads, (excluding the 1<sup>st</sup> interdigital pad) and 2 metacar-

pal pads. This was observed in the manus of the African giant pouched rat from this study. The naked mole-rat is not a member of the *Muridae* family, to which the African giant pouched rat belongs, but they share a similar subterranean habitat. It is a member of the *Bathyergidae* family.

The water vole (*Arvicola terrestris*) is a Murid but has 10 paw pads in the pes and 10 paw pads in the manus (Haffner, 1998), unlike the African giant pouched rat. The interdigital pads in the pes of the water vole are arranged in a trapezium (Haffner, 1998) unlike the circular distribution in the African giant pouched rat. The natural habitat of the water vole is the banks of ditches, dykes, slow-moving rivers and streams, and grassland, they have been found on hillsides at altitudes as high as 900 metres (BBC, 2008a). Thus, they are not predominant burrowing rodents, but also very good climbers. The house mouse (*Mus musculus*) and harvest mouse (*Micromys minutus*) are Murids, but differ from the African giant pouched rat in the distribution of their paw pad. Kimura et al. (2008) reported 11 paw pads in their pes; 5 digital pads, 4 interdigital pads and 2 metatarsal pads. Kimura et al. (2008) also reported 10 paw pads in their manus; 5 digital pads, 3 interdigital pads (excluding the 1<sup>st</sup> interdigital pad) and 2 metacarpal pads. The house mouse lives in close association with humans

(Ballenger, 1999) while the harvest mouse inhabits hedgerows, reed beds, and other areas of tall, dense vegetation (BBC, 2008b). They are good climbers (Haffner, 1998). The hazel dormouse (*Muscardinus avellanarius*), which is not a Murid but belongs to the family *Gliridae*, shares the same digital pad profile as the harvest mouse and house mouse. The hazel dormouse does not live in burrow but in woodlands (Büchner, 2008). The only Murid we found in the literature with similar pes paw pad profile to that of the African giant pouched rat is the Sulawesi rat. However, unlike our observation in the African giant pouched rat, the 2<sup>nd</sup> and 3<sup>rd</sup> interdigital pads of the Sulawesi rat are not fused and the two metatarsal pads are of the same size (Musser and Durden, 2002). Another subterranean Murid that has been reported to lack digital pads but to possess sickle-shaped claws like the African giant pouched rat is the *Nannospalax ehrenbergi* (Ade and Ziekur, 1999). Digital claws are larger and more elongated in rodents that spend much of their time digging, but smaller in arboreal species (Aplin et al., 2003). These findings imply that there is high intra-family variability in the regional and numeric distribution of paw pads in rodents. Thus, paw pad profile in the order *Rodentia* may be independent of taxonomy but depends on the predominant habitat. Indeed skin morphology may be a better indicator of habitat and lifestyle than phylogeny (Hildebrand, 1988). For example, the absence of digital pads in the subterranean rodents may be a plus for effective digging, owing to the preference of claws to pads for digging. The combination of the sickle-shaped claws and incisors is more suitable for digging in the African giant pouched rat. Except for some paw pads with weak striations, the smooth nature of the paw pads in the present study depicts typical paw pads seen in terrestrial Murids unlike arboreal Murids with obviously striated pads (Aplin et al., 2003).

The longer and more sickle-shaped claws in the manus than in the pes, and the increased number of paw pads in the pes than the manus observed in the African giant pouched rat in the present study implies that the manus is more adapted for digging while the pes is used to push the excavated soil away with the aid of the paw pads. This supports the initial suggestion by Ewer (1967) that the teeth and manus in the African giant pouched rat may be used to loosen substrate and their pes to push excavated soil away. These claws and paw pads in both manus and pes of the African giant pouched rat may also be indicated in carrying and manipulating food and objects, moving the young, fighting, and other social interactions. However, the significantly thicker epidermal layer in the pes as recorded in the study implies that the pes is more actively utilized than the manus. The significantly longer and wider pes than manus supports

this claim and may also indicate that the pes provides more support than the manus in the African giant pouched rat.

The location of collagen fibres in the upper part of the dermis, just below the *stratum basale* of the epidermis explains its functional property in anchoring the hypodermal and epidermal layers of the skin as well as conferring toughness and tensile strength. Adipose tissues observed in the dermis may serve as mechanical insulators by absorbing concussive forces when the rodent uses the paw pads.

## Conclusion

The present study has provided some explanations to the adaptation of the skin of the trunk and paw pads of the African giant pouched rat to burrows and hot tropical climate. Such idea is essential in the domestication of the rodent as there is a need to provide a near-natural habitat during the domestication, for effective production.

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