COMPARATIVE SKIN MORPHOLOGY AND WOUND HEALING IN KENYAN AFRICAN MOLE RAT (*TACHYORYCTES IBEANUS*) AND NAKED MOLE RAT (*HETEROCEPHALUS GLABER*).

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A thesis submitted in partial fulfillment of the requirements for award of the degree of Master of Science in Veterinary Anatomy, University of Nairobi

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any university.

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I dedicate this thesis to my beloved parents James Kimani Ngugi and Teresia Njeri Kimani for their unwavering support towards my education.

ACKNOWLEDGEMENTS

I greatly appreciate the support, guidance and encouragement accorded to me by my supervisors; Prof. Stephen G. Kiama, Dr. Philemon K. Towett and Prof. Ashley W. Seifert. Financial support provided by the Deans' Committee of the University of Nairobi (Grant 500-655- 640) and laboratory material support by Prof. Stephen G. Kiama , Prof. Ashley Winn Seifert and Dr. Daido Dulu that enabled completion of this project is also highly acknowledged. Special thanks go to the University of Nairobi for awarding me a waiver of fees for Msc studies.

I am grateful for the excellent technical assistance rendered by the following: Erna van Wilpe

EM Unit Manager Department of Anatomy & Physiology, Faculty of Veterinary Science, University of Pretoria, J. Gachoka and J.Mukiri of the Department of Veterinary Pathology, Microbiology & Parasitology, University of Nairobi, and S. Marete, P. Kiguru, G. Kamau, J. Kiai, J. Mugweru, P. Irungu, F. Okumu, A. Mwasela and S. Keya of the Department of Veterinary Anatomy & Physiology, University of Nairobi.

My deep appreciation also goes to my friends, workmates, relatives and parents for their encouragement. Above all, may glory and honour go to our Almighty God for enabling me to carry out this project to the end.

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ABSTRACT.

The skin lines the body surface and therefore is in direct contact with the immediate environment that an animal inhabits. As such, it is prone to injury. Interspecific differences may exist in the content and organization of the dermis and similarly between species' ability to heal injured skin. Furthermore, how the adnexa (hair follicles and associated glands) affects the outcome of the healing process is incompletely understood. In order to examine this question, two mammals, one bearing a thick hair coat (*Tachyoryctes ibeanus)* and the other having very sparsely distributed hairs (*Heterocephalus glaber*), were selected for the study. The initial study sought to establish the structure of the skin in the two species and to determine any differences that may reflect their unique subterranean habitats. Skin samples from different cutaneous sites were obtained from five animals of each species. The skin samples were processed for histology, electron microscopy and morphometric analysis. Morphometric analysis entailed measurement of the thickness of the overall skin, epidermis and stratum corneum. There were notable morphological differences between the skins of these two animal species. *Tachyoryctes ibeanus* had an insulating adipose tissue layer constituting the hypodermis in the dorsum and a dermis containing compound hair follicles associated with the arrector pili muscle. Sweat glands were only located in the pubic region. Conversely, the skin of *Heterocephalus glaber* had adipose tissue consisting mainly of unilocular adipocytes which were sparsely distributed and occurred either as isolated or grouped cells forming a discontinuous layer. The dermis of the naked mole rat contained pigment cells and very sparsely distributed simple tactile hair follicles which were well equipped with longitudinal palisades of lanceolate nerve endings and were lacking an arrector pili muscle. Large multilobular sebaceous glands were observed in labial lobes and pubic regions in *Heterocephalus glaber*. In both animals, the rhinarium (glabrous skin of the nose), palmar and plantar pads had a highly interdigitated dermal-epidermal interface which were

well innervated. The average total skin thickness was significantly greater in *Tachyoryctes ibeanus* than *Heterocephalus glaber*'s neck, thoracic, lumbar and abdominal regions ($p < 0.05$). The skin of *Heterocephalus glaber* was only significantly thicker at labial lobes ($p < 0.05$). In contrast, the average thickness of epidermis and stratum corneum in thoracic, lumbar and sternal regions was significantly greater in *Heterocephalus glaber* than *Tachyoryctes ibeanus* ($p < 0.05$). These observations may be a reflection of the selective pressures exerted by mechanical, temperature, and sensory aspects of their unique subterranean environments.

To examine wound healing in these mammals, two full thickness excisional wounds on halothane anaesthetized animal, were made on the lumbal dorsum using a 4mm biopsy punch. Progression of wound repair was monitored daily, wound diameter measured and photographs taken upto day 80 post-wounding. The wounded skin was excised at day 3, 7, 14, 30 and 80 post- wounding for histological analysis. In each time-point, five animals of each species were used. Semi-quantitative parameters (wound re-epithelialization; presence of inflammatory cells, fibroblasts, collagen and elastic tissue) were used to evaluate the histological changes during wound healing. In *Tachyoryctes ibeanus*, the scab was shed between days 12-14 post-wounding and wounds healed by forming a prominent scar. In contrast, *Heterocephalus glaber* took more time to shed the scab taking between days 21-24 after wounding with the wound showing less scarring. Fibroblast proliferation and migration, collagen and elastic fibres deposition in the wound area occurred much earlier in *Tachyoryctes ibeanus* compared to *Heterocephalus glaber*. Furthermore, the inflammatory phase was prolonged in *Heterocephalus glaber*, where polyphonuclear cells were noted in the wound area, 7 days post-wounding. These observations indicate a much slower healing process in *Heterocephalus glaber* compared to *Tachyoryctes ibeanus* but with a better wound healing quality. The differences in healing between the two species could be attributed to their markedly different skin structure. Further studies need to be carried out to understand the

molecular mechanisms that may be involved in the wound healing process in *Heterocephalus glaber* and *Tachyoryctes ibeanus*.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 General description, habitat and distribution of *Tachyoryctes ibeanus* and *Heterocephalus glaber*

1.1.1 *Tachyoryctes ibeanus*

Tachyoryctes ibeanus (Kenyan African mole rat) belongs to the order Rodentia, suborder Myomorpha, family Spalacidae, subfamily Rhizomyinae and tribe Tachyoryctini (Fig.1, 2) (Allen, 1939; Ellerman, 1941; Carleton and Musser, 2005; Musser and Carleton, 2005). Musser and Carleton (2005), identified 13 species of the genus Tachyoryctes.

Tachyoryctes ibeanus are solitary, fossorial rodents that have compact stoutly built bodies with short muscular limbs and are readily recognizable by their prominent orange incissors (Nevo, 1979; Kingdon, 1984). Their eyes and ears are small and they have a rat-like tail ranging in length from 50-95 mm (Nevo, 1979; Kingdon, 1984). Their head-body length and body mass ranges are 160-260mm and 160-280g respectively (Kingdon, 1984).The hair coats are very soft and thick, usually some shades of russet in colour, but with a dark grey undercoat showing when the hair is ruffled. Juveniles less than 4 months old are black with brown colouring in adults, extending from the flanks over the whole body (Kingdon, 1984).

The distribution of species in the genus *Tachyoryctes* encompasses a wide range over the uplands of North eastern Africa, from Ethiopia and parts of Somalia as far as Eastern Congo, Rwanda, Burundi, Uganda, Northern Tanzania and Kenya (Kingdon, 1984). In Kenya they occur in mountainous areas and over the uplifted floor of the Rift

Valley (Kingdon, 1984). *Tachyoryctes ibeanus* is endemic to Kenya, being found in southern and central Kenya, near Nairobi and western margins of the Athi plains (Kingdon, 1984; Musser and Carleton, 2005). Habitats of members of the genus *Tachyoryctes* are characterized by deep fertile soils with a reasonable and well distributed rainfall, seldomly being found in areas with less than 500mm annual rainfall (Kingdon, 1984). The mole rats favour open grasslands, thinly treed upland savanna, moorland and cultivations that have replaced forests (Kingdon, 1984). The success of *Tachyoryctes* in a favourable habitat is demonstrated by abundance of their mole hills. *Tachyoryctes* make relatively short tunnels which run 15-30 cm below the surface at the level of the grass roots they feed on (Kingdon, 1984). However, in the dry season, they dig deeper tunnels reaching up to one metre below the surface (Musser and Carleton, 2005).

1.1.2 *Heterocephalus glaber*

Heterocephalus glaber (naked mole rat) occurs as a monotypic genus in the order Rodentia, suborder Hystricomorpha, infraorder Hystricognathi, family Bathyergidae and subfamily Heterocephalinae (Fig.1, 2.) (Allen 1939; Jarvis and Sherman, 2002). *Heterocephalus glaber* are fossorial, eusocial rodents with colonies containing an average of 75-80 animals (Brett, 1991a; Braude, 2000). Their bodies are cylindrical, with their backs arched dorsally over lumbar and sacral regions (Hamilton, 1928). Among the Bathyergids, naked mole rats are the smallest with a head-body length ranging from 103-136mm and body mass range of 30-50g (Jarvis and Sherman, 2002). However, among members of the family *Bathyergidae* naked, mole rats tails are longest ranging from 32-47mm (Jarvis and Sherman, 2002). *Heterocephalus glaber's* eyes are tiny with thickened eyelids and minute eyelashes and the opening to the auditory canal is slightly raised with external pinnae being absent. The naked mole rat's loose, wrinkled skin is brownish pink in color and darker

dorsally than ventrally in young animals with this counter shading disappearing as they age (Braude *et al*., 2001). Unlike *Tachyoryctes ibeanus, Heterocephalus glaber's* skin is not concealed by pelage, having only isolated tactile hairs occurring all over the body (Jarvis and Sherman, 2002).

Heterocephalus glaber live in arid environments, characterized by high temperatures and low irregular rainfall (Brett, 1991a). They are found most frequently in hard, consolidated, lateritic loams, although they can live in fine and pure gypsum (Hill *et al*., 1957; Jarvis, 1985; Brett, 1991b). *Heterocephalus glaber* is endemic to hot, dry regions of Eastern Africa. Distribution encompasses most of Somalia, central Ethiopia, and much of Northern and Eastern Kenya, extending south as far as the Eastern border of Tsavo West National park and town of Voi (Jarvis and Sherman, 2002). Dwellings of these animals are recognized by the presence of clusters of volcano-shaped soil mounds on the surface above burrows. Immediately below the soil mounds is a network of narrow superficial tunnels, where animals move in single file. Superficial tunnels leads to more deeply slanting connecting burrows that are higher so individual animals can pass over one another. Connecting burrows end in wide unbanked burrows which are the colony's main tunnel.

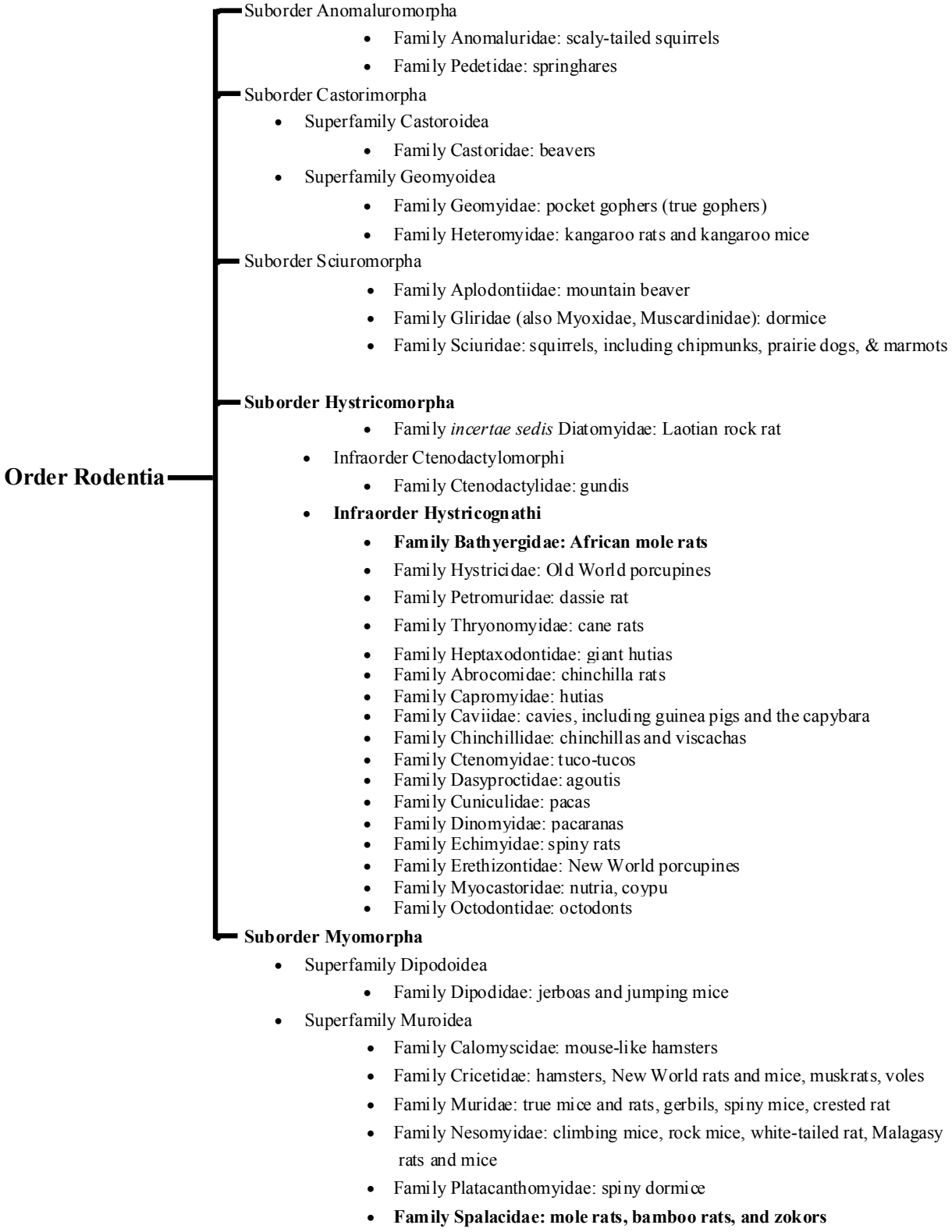


Fig. 1. Classification Scheme showing the position of family Spalacidae and Bathyergidae in the order Rodentia (Carleton and Musser, 2005; Musser and Carleton, 2005).

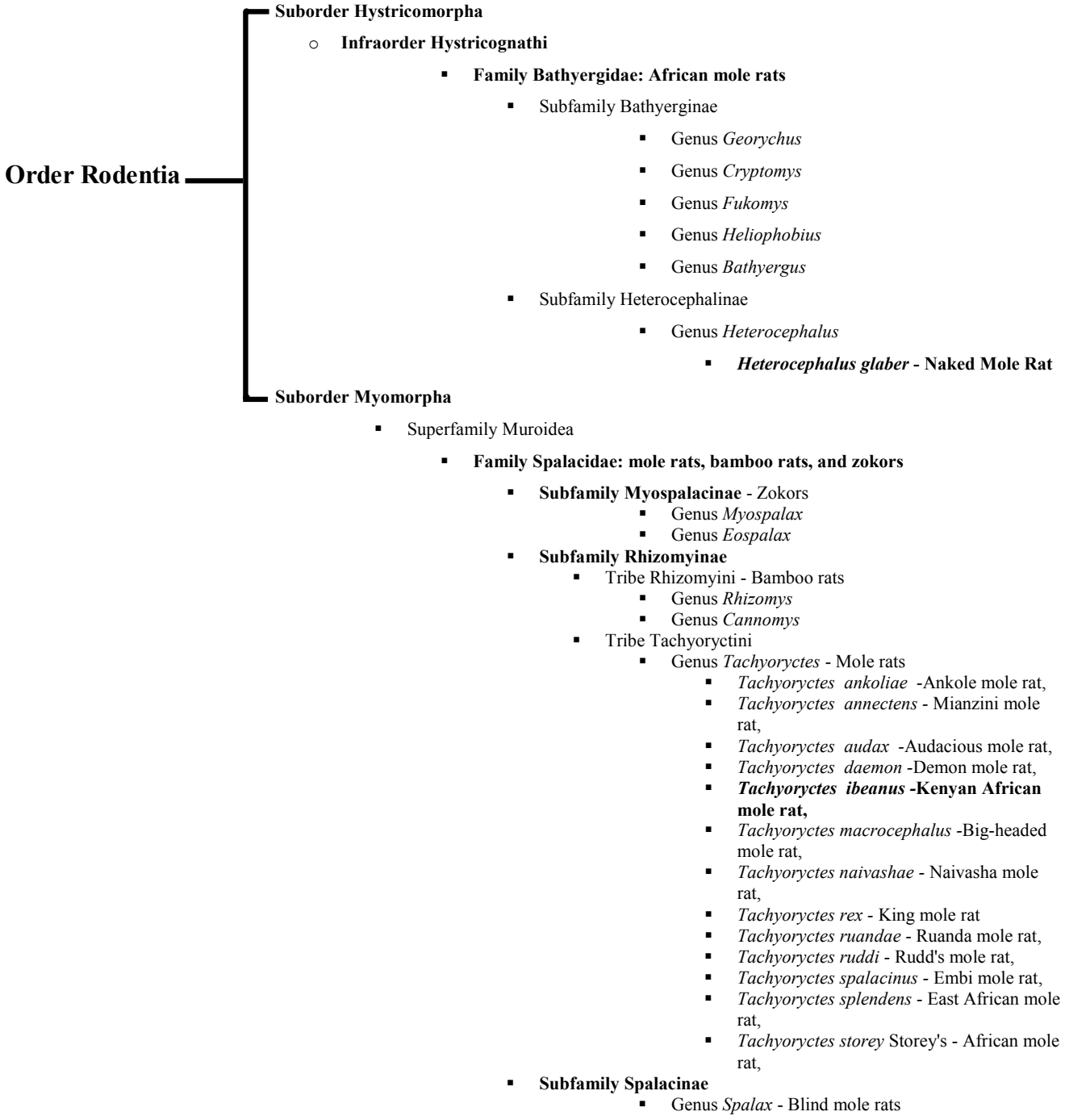


Fig.2. Classification scheme showing the position of *Heterocephalus glaber* and *Tachyoryctes ibeanus* in the order Rodentia (Carleton and Musser, 2005; Musser and Carleton, 2005).

1.2 Mammalian Skin Structure

The skin covers the body and it is comprised of three anatomical layers, the epidermis, dermis and hypodermis (Fig. 3) (Kanitakis, 2002; Kent Van De Graaff, 2009). The epidermis is the outer most layer of the skin where it prevents water loss through evaporation, contains receptor cells, and provides an effective protective barrier for the underlying tissues against chemical, physical and biological insults (Elias and Feingold, 1992; Boulais and Misery, 2008; Brandner *et al*., 2010). The dermis consists of dense irregular connective tissue that provides structural support and nourishment for the epidermis. The dermis serves as blood reserve and participates in sensory reception and temperature regulation (Braverman, 1989; Braverman and Sibley, 1990; Braverman, 2000). The hypodermis lies under the dermis and is a loose connective tissue that contains a pad of adipose cells (fat) that contour the body (Avram *et al*., 2005). It plays an important role in thermoregulation, provision of energy and protection from mechanical injuries. The epidermis may be specialized to form various skin appendages such as hair, sweat glands, sebaceous glands, digital organs and specialized glandular structures (Dellman and Eurell, 1998). In general, skin architecture constituting the epidermis, dermis and hypodermis is similar in all mammals. However, inter and intraspecific differences exist in skin thickness, content, stratification and pigmentation of both the epidermis and dermis (Sokolov, 1982; Dellman and Eurell, 1998).

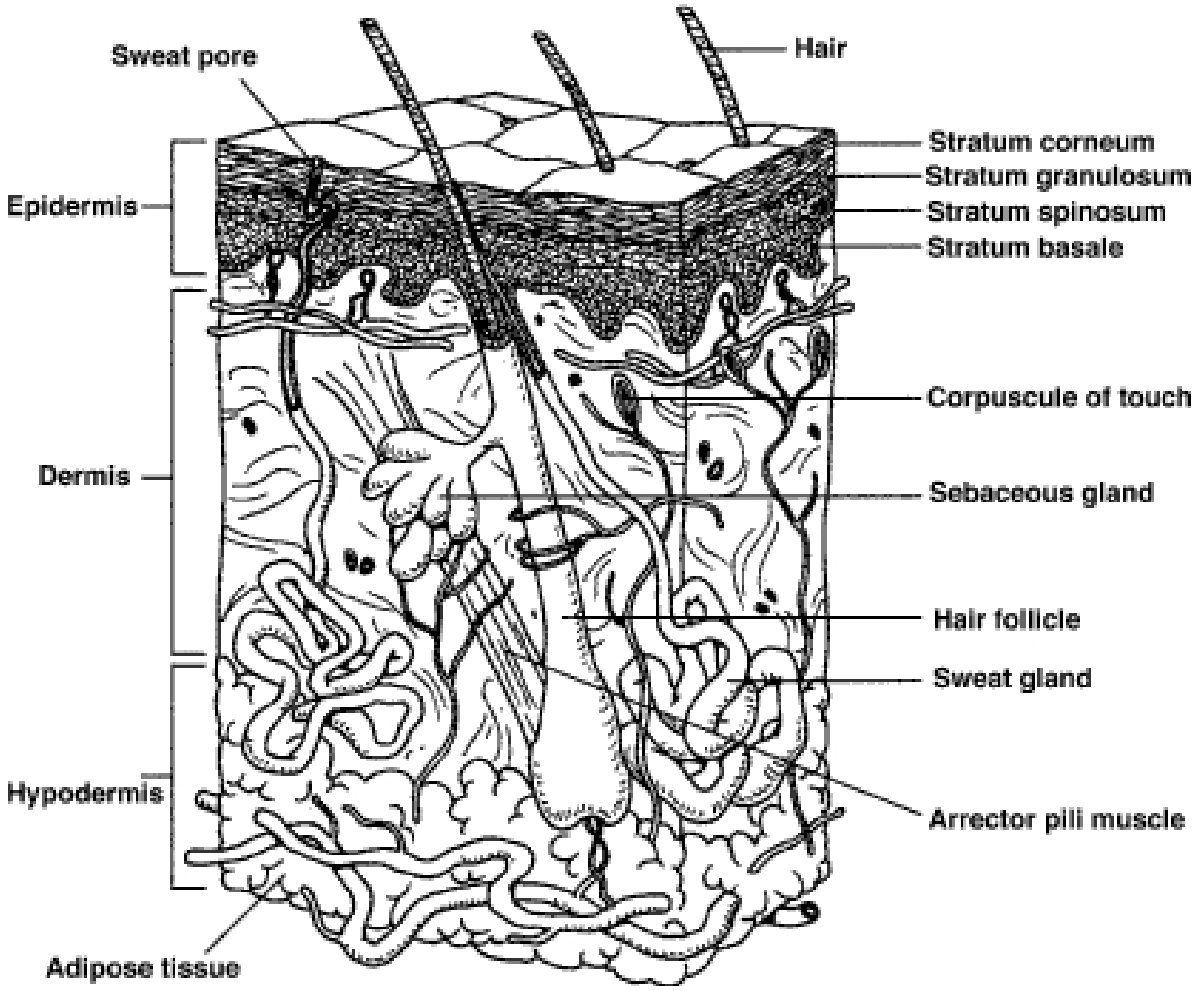


Fig.3. Schematic diagram of the skin showing the three anatomical layers: epidermis, dermis and hypodermis (Adapted from Kent Van De Graaff , 2009).

1.2.1 Epidermis

The epidermis is a keratinized stratified squamous epithelium composed mainly of keratinocytes and a few other cell types namely; melanocytes, langerhans and Merkel cells (Fig. 4) (White and Yager, 1995).

Keratinocytes undergo sequential differentiation from the basal surface of the stratified epithelium to its superficial surface, resulting in the following layers; stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Fig. 4) (Watt, 1989). The stratum basale is the innermost layer of epidermis consisting of a single layer of cuboidal cells that rests on the basal lamina (Dellman and Eurell, 1998). Mitosis occurs in this layer, where it acts as a source of stem cells that have the ability to divide and produce new keratinocytes (Fuchs and Raghavan, 2002; Blanpain and Fuchs, 2006). Basal cells contain numerous mitochondria and the golgi apparatus (Sokolov, 1982). Following basal cells division, some progeny remain in the basal layer as stem cells while others migrate into the upper epidermal layers. Besides keratinocytes, the basal layer also contains melanocytes and merkel cells (Fig. 4) (Fradette *et al.,* 1995; White and Yager, 1995). Melanocytes are derivatives of the neural crest (Clewes *et al*., 2011). Melanocytes can be identified by the presence of several dendritic processes extending between adjacent keratinocytes and the pigmented ovoid granules in the cytoplasm referred to as melanosomes (White and Yager, 1995). Melanocytes produce melanin by a reaction that occurs in a series of steps and involves the enzyme tyrosinase (Slominski *et al*., 2004). After melanogenesis, the melanosomes migrate to the tips of the dendritic processes of the melanocytes (Wu *et al*., 1998; Wasmeier *et al*., 2008). Melanosomes are transferred from the melanocytes to the keratinocytes and are randomly distributed within the cytoplasm (Ernfors, 2010). Their distribution is modified by UV radiation such that

they form a cap - like structure covering the side of the nucleus exposed to sunlight (Brenner and Hearing, 2008; Wasmeier *et al*., 2008; Plonka *et al*., 2009**).** Skin colour is determined by the amount of melanin produced rather than the density of melanocytes (Yoshida-Amano et *al*., 2012). Mammals exist in different colors and patterns, which arise from the unique distribution of pigments throughout the body. Pigmentation of the skin is highly heritable, being regulated by genetic, environmental, and endocrine factors (Costin and Hearing, 2007). These factors modulate the amount, type, and distribution of melanins. Merkel cells are slow adapting mechanoreceptors, derived from a myelinated nerve but as they approach the epidermis, they lose the myelin sheath and terminate as a flat meniscus on the basal aspect of the cell (Halata *et al.,* 2003; Lucarz and Brand, 2007). Their nucleus is lobulated and irregular, and the cytoplasm is clear and lacks tonofilaments (Halata *et al.,* 2003).The cells have a characteristic vacuolated cytoplasm containing spherical electron-dense granules and connected to the adjacent keratinocytes by desmosomes (Lucarz and Brand, 2007).

The succeeding outer layer, the stratum spinosum consists of several layers of irregular polyhedral keratinocytes (Dellman and Eurell, 1998). The cells have undulations on their plasma membranes that interdigitate with those of adjacent cells and are firmly attached to each other by prominent desmosomes (Sokolov, 1982). Langerhans cells are commonly found in the upper layer of the stratum spinosum (Fig. 4) (Romano and Balaguer, 1991; White and Yager, 1995; Dellman and Eurell, 1998). They have an indented nucleus with cytoplasm that contains typical organelles and lacks tonofilaments and desmosomes (Lombardi *et al*., 1993 Jaitley and Saraswathi, 2012). A defining feature of these cells is the rod-shaped birbeck granules in the cytoplasm (White and Yager 1995; Dellman and Eurell, 1998). They have long dendritic processes that traverse the intercellular space up to the granular layer and are functionally related to monocytes-

macrophages series (Romano and Balaguer, 1991).

The third layer is the stratum granulosum, which consists of several layers of flattened cells lying parallel to the epidermal-dermal junction (Kanitakis, 2002). This layer is not present in all species and where present it is not found in all body areas. The cells in this layer are identified by the irregularly shaped, non-membrane bound, electron-dense keratohyalin granules (Lavker and Matoltsy, 1971; Breathnach, 1975). The cytoplasm of cells of the upper, stratum spinosum and stratum granulosum also contains smaller lamellated granules, known as lamellar granules or bodies, membrane-coating granules or Odland bodies (Fig. 4) (Kanitakis, 2002). The number and size of keratohyalin granules increases towards the surface of granular layer. Golgi apparatus occur less frequently and mitochondria are rare. The cells of the granular layer do not divide, The keratin granules become more prominent as the keratinocytes of the granular layer age and migrate outward.

The fourth layer, the stratum lucidum, is a layer found in specific areas of exceptionally thick skin and hairless regions such as plantar and palmar surfaces (Borysenko *et al*., 1979). It is a thin, translucent, homogenous line between the stratum granulosum and stratum corneum (Fig. 4). This layer consists of several layers of fully keratinized, closely compacted dense cells devoid of nuclei and cytoplasmic organelles. The migrating cells terminally differentiate to form the cornified layer of the epidermis (stratum corneum) that consists of several layers of flattened, squamous completely keratinized dead cells, corneocytes (Eckert and Rorket, 1989). Corneocytes are rich in fibrous protein keratin and keratohyalin surrounded by plasma membrane coated by exterior lipid matrix (Kanitakis, 1998). These cells lack the nuclei and the main cytoplasmic organelles. There is continuous peeling off of the outer cornified squamous cells singly or in clusters. The interface between the epidermis and the dermis is the epidermal-dermal junction. This junction varies in

different parts of the body between being relatively smooth in thin skin to highly corrugated with dermal papillae and epidermal ridges that interdigitate into the dermis in thick skin (Briggman and Wheeler, 1975). The undulating zone has been noted in the palm of primates and rhinarial skin of dogs, cats and rats (Macintosh, 1975; Dellman and Eurell, 1998). The fine structure of epidermal-dermal junction reveals four distinct layers, comprising (from top to bottom): the cell membrane of basal cells with their hemidesmosomes, to which cytoskeletal filaments attach, the lamina lucida, an electron-lucent space traversed by anchoring filaments, the osmiophilic lamina densa, and the sub-basal lamina filamentous zone, mainly made of anchoring junctions (Briggman and Wheeler, 1975; Dellman and Eurell, 1998; Kanitakis, 2002).

1.2.2 Dermis

The dermis consists of superficial papillary layer that conforms to the contours of the stratum basale and the deeper reticular layer (Dellman and Eurell, 1998; Kanitakis, 2002). Papillary dermis is made up of collagen fibres arranged in loose bundles and thin elastic fibres stretching perpendicularly to the dermal-epidermal junction. The reticular layer is characterized by an extracellular matrix containing a network of dense coarser collagen bundles tending to lie parallel to the skin surface and thicker elastic fibers. Collagen is the major fibrous protein in the dermal extracellular matrix and gives tensile strength to the skin, while the smaller amount of elastin confers resiliency, allowing skin to be stretched and then assume its original shape (Gelsea *et al*., 2003; Starcher *et al*., 2005). Collagen and elastic fibers in both papillary and reticular layers are organized in a basket weave pattern (Van Zuijlen *et al*., 2003). The major collagen in dermal matrix is type I, while type III collagen occurs in smaller amounts (Gelsea *et al*., 2003).

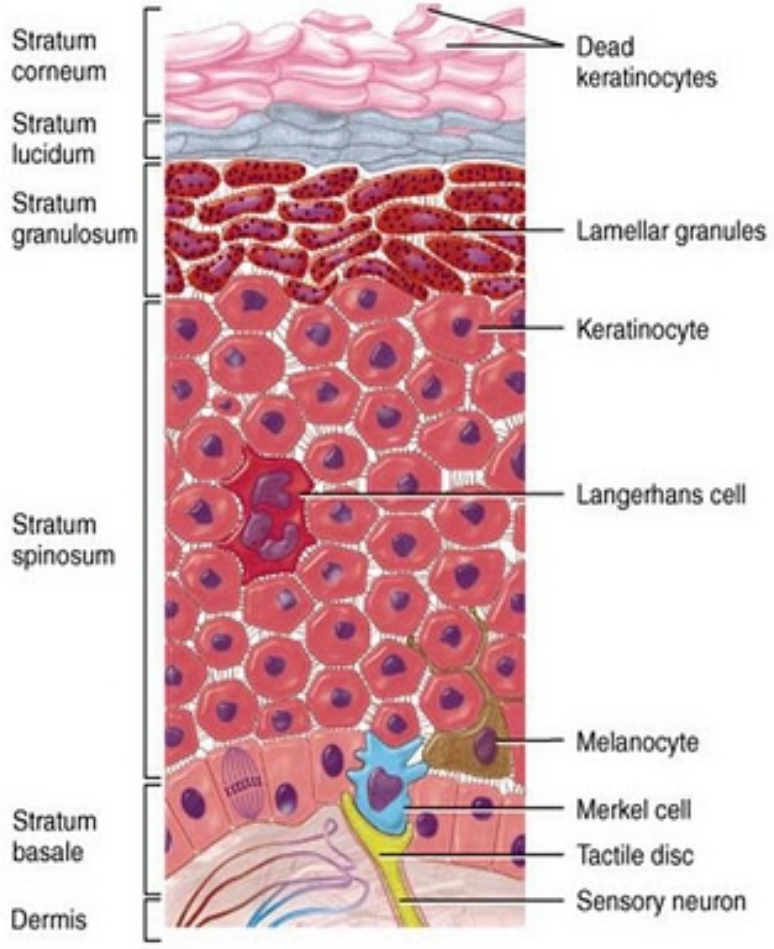


Fig. 4. Schematic diagram showing the layers and various cell types of the epidermis (Adapted from Delmann and Eurel, 1998).

Proteoglycans and adhesive glycoproteins are the other classes of proteins found in the dermal extracellular matrix (Lewandowska *et al*., 1991; Alberts *et al*., 1994; Clark, 1996). The prominent dermal proteoglycans are dermatan sulfate, heparan sulfate and chondroitin sulfate. All of these bind water and give the uninjured ECM (extracellular matrix) its property of resisting comprehensive force while also creating space for cell migration. The predominant dermal adhesive glycoproteins are Fibronectin, Vitronectin and Tenascin-C which through interaction with cell surface receptors, regulate cell functions such as cell adhesions, migration, growth and differentiation (Clark, 1996). Cellular elements in the dermis are generally sparse among mammals, except for the relatively higher numbers reported in the order chiroptera (Quay, 1970; Dellman and Eurell, 1998). The most abundant cell type is the fibroblast, while other cells like mast cells, macrophages, plasma cells and fat cells occur to a lesser extent (Dellman and Eurell, 1998). Fibroblasts are generally spindle shaped, though their shape may also vary with location (Sokolov, 1982). Mast cells are large, oval shaped mononuclear cells of bone marrow origin that are sparsely distributed in the perivascular and periadnexal dermis (Kanitakis, 1998). Their cytoplasm is filled with coarse metachromatic granules that are easily recognized by toluidine blue stain (Kanitakis, 1998; Kanitakis, 2002). Dermal mast cells play a central role in immunomodulation of allergic reactions (Hart *et al*., 1999; Theoharides and Cochrane, 2003). Macrophages are mononuclear phagocytes, that confer skin immunity by engulfing and digesting organic particles intracellularly (Dupasquier *et al*., 2004). Plasma cells are found in only small numbers in the normal skin, but are significantly increased in pathologic conditions (Boehncke *et al*., 1989; Miyagawa-Hayashino *et al*., 2009). Fat cells in the dermis of most mammals occur in isolated regions, however in cetaceans and sirenians, fat cells are found directly beneath the epidermis and the number increases deeper in the dermis before connecting to the subcutaneous fat tissue (Sokolov, 1982; Reeb *et al*., 2007). Additionally, considerable population of pigment

cells have been reported in dermis of primates (Erickson and Montagna, 1975; Sokolov, 1982; Prum and Torres, 2004).

The dermis possesses a rich vascular network consisting of a mixture of arterioles, venules and capillaries (Kanitakis, 2002). This network is involved in nourishment of the skin, thermoregulation, wound healing and immune reactions. The dermis also contains an extensive lymphatic network that is independent of the vascular plexus. Sensory and autonomic nerves within the peripheral nervous system penetrate the dermis with tiny nerve fibers, which may be myelinated or non-myelinated (Chang *et al.,* 2004). These fibres terminate as a range of nerve endings that are variably distributed in different parts of the body resulting in regional variations in sensation. Hairy skin has free nerve endings supplying the hair follicles and rest of the dermis, while non-hairy skin, additionally, contains encapsulated nerve endings and Merkel's discs (McGrath *et al.,* 2010).

1.2.3 Hypodermis

The hypodermis is a transitional layer of loose connective and adipose tissue that anchors the dermis to the underlying aponeurosis and fasciae of the muscles (Kanitakis, 1998). The main cells of the hypodermis are adipocytes; large, rounded cells with a lipid-laden cytoplasm compressing the nucleus against the cell membrane (Avram *et al*., 2005). Adipocytes are arranged in lobules, separated from each other by connective tissue septa containing stromal-vascular cells, including fibroblasts, leukocytes, macrophages, and preadipocytes (Avram *et al*., 2005; Geerlings, 2009). A continuous layer of adipose tissue in the hypodermis constitutes a panniculus adiposus (Sokolov, 1982; Delmann and Eurell, 1998). In many mammals, a thick sheet of skeletal muscle, the panniculus carnosus muscle, lies beneath the hypodermis (Borysenko *et al*., 1979).

1.2.4 Skin appendages

The skin is specialized to form the various skin appendages such as hair, sweat glands, sebaceous glands and specialized glandular structures.

1.2.4.1 Hair

Hair is a flexible structure, consisting of keratinized epidermal cells emanating from a hair follicle, a down growth of the epidermis, embedded in the dermis. Among mammals, hairs may vary in shape, texture, length, thickness, density and colour and these differences also occur within the same animal in different areas of the body (Sokolov, 1982; Scott *et al.,* 1991; Otberg *et al*., 2004; Oznurlu *et al.,* 2009; Davis *et al*., 2010). For instance, elephants possess bristle like hairs, and Tachyglossus species have sparse coarse hairs, while cetaceans lack pelage completely (Lyne and Mcmahon, 1950). The free part of the hair above the surface of the skin is the hair shaft, while the part within the follicle is the hair root which has a terminal hollow knob called the hair bulb, which is attached to the dermal papillae (Fig. 5) (Delmann and Eurell, 1998). The hair shaft is composed of three layers namely; an outermost cuticle formed by a single layer of flattened keratinized cells, followed by a cortical layer of densely packed keratinized cells and an inner medulla which forms the centre of hair and is loosely filled with cuboidal cells (Delmann and Eurell, 1998). Hairs of most mammals are categorized as vibrissae, guard, pile, intermediate and fur (Loo and Halata, 1991; Marotte *et al*., 1992; Sokolov, 1982).

Vibrissae or tactile hairs are found around the head and have long, thick, conical straight or slightly bent shafts, and are highly specialized for tactile sense (Sokolov, 1982; Delmann and Eurell, 1998). The guard, pile, intermediate and fur hairs form the pelage of mammals (Sokolov, 1982). The guard hairs are the longest, but least numerous with thick, spindle shaped shafts. The pile hairs are the second order in size from guard hairs and are lancet-shaped. Intermediate hairs are shorter and thinner than pile hairs, but longer and thicker

than fur. Fur are usually numerous, short and thin compared to the other categories of hairs. Rodents have vibrissae, guard, pile and fur with well developed medulla except in vibrissal hairs (Sokolov, 1982).

The hair follicles can be classified as primary, secondary, simple or compound (Delmann and Eurell 1998; Oznurlu *et al*., 2009). Primary hair follicles have large diameter and are rooted deep in the dermis while secondary hair follicles are smaller in diameter and the root is nearer the surface (Delmann and Eurell, 1998). Simple hair follicles have only one hair emerging to the surface while compound hair follicles are composed of cluster of several hair follicles located in the dermis.

Hair follicles consist of four major components namely; the internal root sheath, external root sheath, dermal papilla and a hair matrix (Fig. 5) (Eroschenko, 2008). The internal root sheath is the innermost, next to the hair root. The internal root sheath is composed of cuticle formed by overlapping keratinized cells, Huxley's layer formed by cells rich in trichohyalin granules and Henle's layer composed of a single layer of keratinized cells (Joshi, 2011). The external root sheath is composed of epidermal cells external to the homogeneous glassy membrane and a connective tissue sheath consisting of collagen and elastic fibers enclosing the entire hair follicle (Delmann and Eurell, 1998). The cells covering the dermal papilla of hair follicle and forming most of the hair bulb are the hair matrix cells. Associated with most hair follicles are bundles of smooth muscle, the arrector pili muscle that attaches to the connective tissues sheath and extends towards the epidermis (Poblet et *al.,* 2002). Arrector pili muscle plays a crucial role in thermoregulation by erecting the hair and also emptying the sebaceous glands (Poblet *et al.,* 2004). Some animals like Pennipids (seals and sea lions), Cetaceans (whale, dolphins and porpoises) and Enhydra lutris (sea otters) lack arrector pili muscle (Sokolov, 1982; Khamas *et al.,* 2012).

Many differences exist in the arrangement of the hair follicles among mammals. For instance, horses and cattle have single hair follicles that are distributed evenly, while pigs have follicles grouped in clusters of two to four (Mowafy and Cassens, 1975; Delmann and Eurell 1998).A diversity of hair types has also been noted in different breeds of a given species. For example, among dogs, German shepherds have a greater number of secondary hairs whereas Rottweilers and Terriers possess a greater number of primary hairs (Muller *et al*., 2001). Among rabbits, Angora rabbits possess compound hair follicles consisting of a single primary follicle and multiples smaller secondary follicles. This differs from New Zealand white rabbits that possess from 2-4 compound hair follicles surrounding a central primary follicle (Oznurlu *et al*., 2009). In rodents, hair grows in tufts or groups, and this acts as an identification feature between different families. For instance hair grows in tufts of, 7-17 in *Aplodon rufa* (Mountain beaver), 5-7 in *Pteromys volans*(flying squirrel), 10- 12 in *Geomys bulbivora* (pocket gopher) whereas hair grows singly or in pairs forming groups in *Nesokia indica* (Short-tailed bandicoot rat), *Rattus rattus*(Black rat) and *Rattus norvegicus* (Brown rat) (Sokolov, 1982).

Vibrissal follicles differ in structure from the typical body hair follicle in that they are very large, characterized by a blood filled sinus between the inner and outer layers of dermal sheath and lack an arrector pili muscle (Fig. 6) (Rice *et al*., 1986). The blood filled sinus is divided into an upper non-trabecular annulus sinus and a lower trabecular cavernous sinus (Rice and Munger, 1986). Skeletal muscles are attached to the outer sheath of the follicle and nerve bundles penetrate through the sheath and ramify in the trabeculae and the inner dermal sheath (Rice *et al*., 1997). A number of differences in structure of vibrissal hair follicles have been reported in different mammalian species. For instance, in horses and ruminants, the annulus sinus is traversed by fibroelastic trabeculae throughout the length whereas in pigs and carnivores have sinus pad formed by the inner layer of the dermal sheath surrounded by

an annulus sinus free of trabeculae. In tammar wallaby (*Macropus eugenii*) vibrissal hairs lacks a ring sinus (Marotte *et al*., 1992; Delmann and Eurell, 1998).

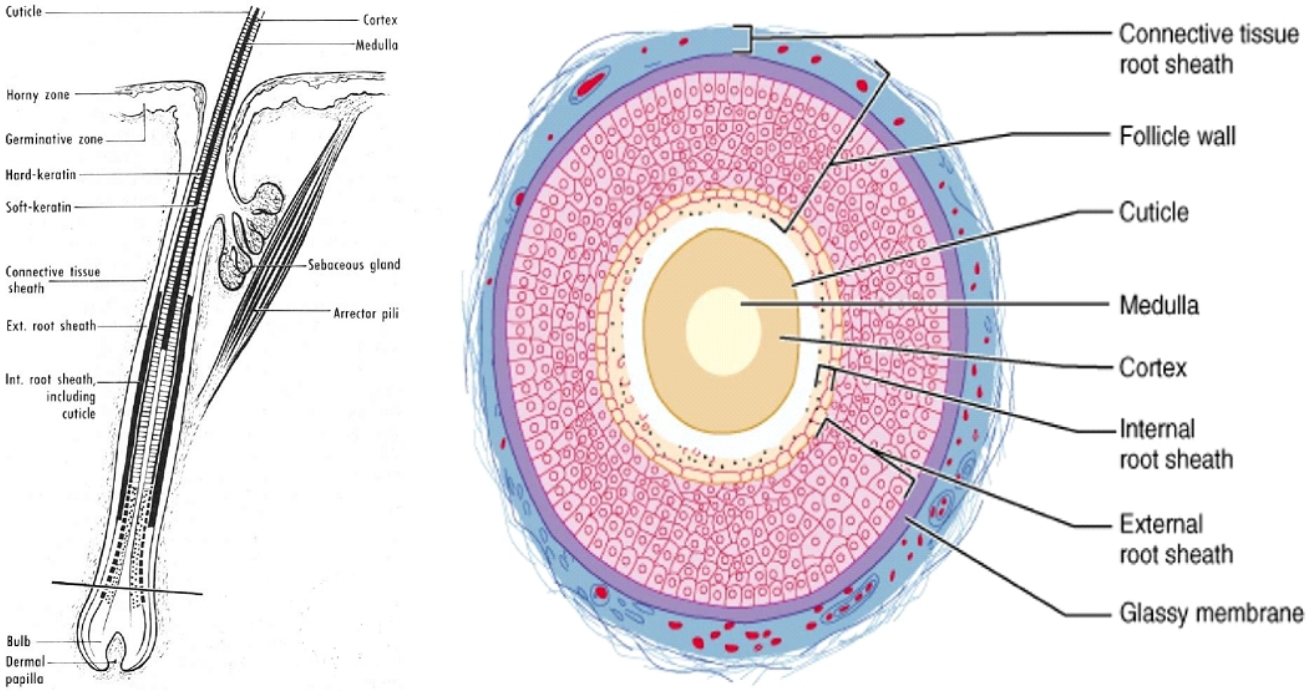


Fig. 5. A diagrammatic representation of the structure of the hair follicle (Adapted from Sokolov, 1982; Delmann and Eurel, 1998).

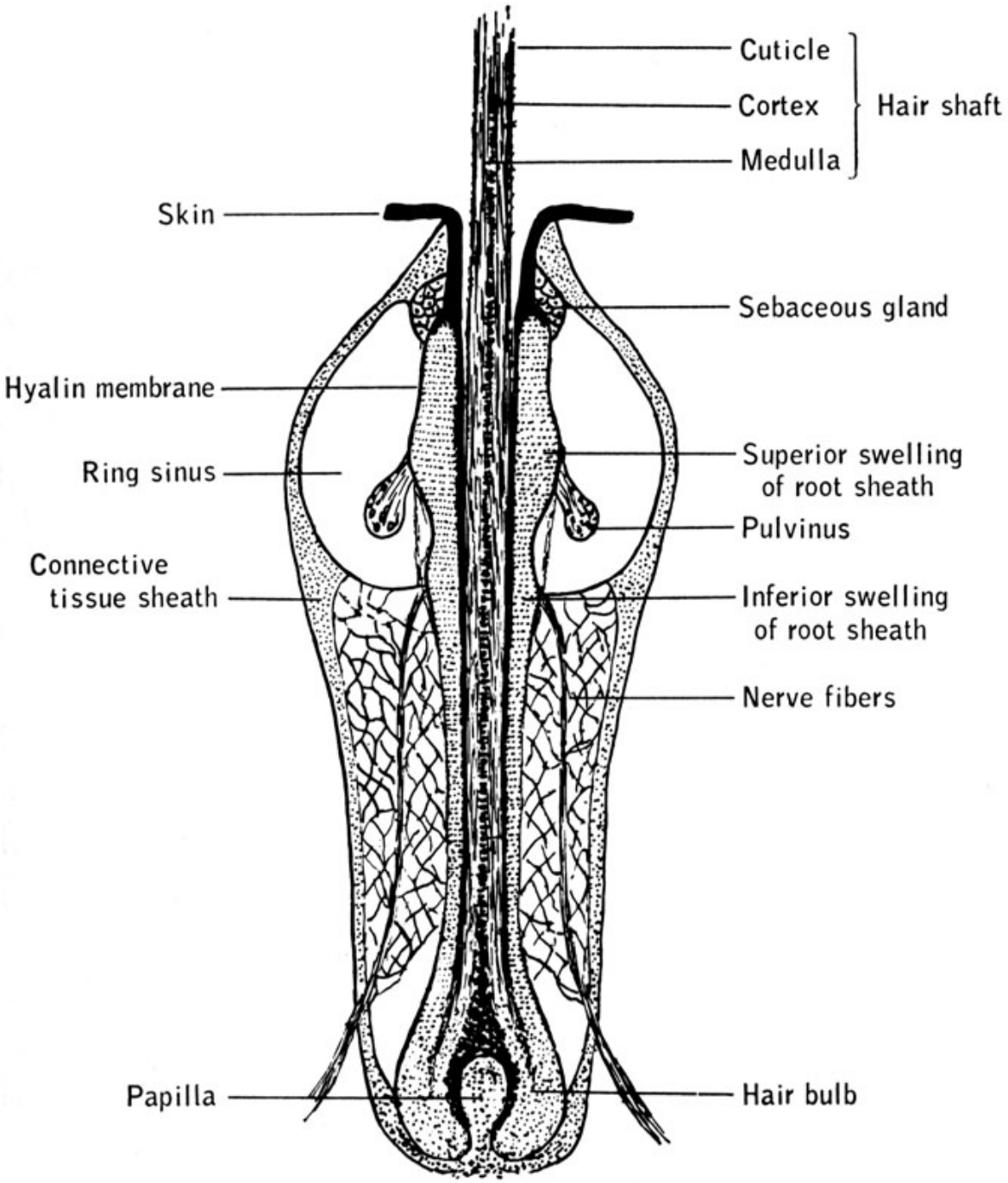


Fig. 6. A diagrammatic representation of the structure of the sinus hair follicle (Adapted from Marotte *et al*., 1992).

1.2.4.2 Sebaceous glands

The sebaceous glands are holocrine glands found distributed throughout the integument of most mammals except on the palmar and plantar surfaces (Borysenko *et al*., 1979). However, sebaceous glands are totally absent in species of the order cetacean, proboscidae and sirenia (Sokolov, 1982). They are most frequently associated with the hair follicle. In certain hairless areas, such as the teat, lips, glans penis and the prepuce of different mammals, sebaceous glands empty directly onto the skin surface through a duct lined with stratified squamous epithelium (Dellman and Eurell, 1998). Sebaceous glands may be simple, branched or compound alveolar glands with secretory unit consisting of a solid mass of epidermal cells, enclosed by a connective tissue sheath (Bohm and Von Davidoff , 2013). At the periphery of the glandular mass, a single layer of cuboidal cells rests on a basal lamina. Most of mitotic activity takes place in this layer, and as the cells move inwards, they enlarge, become polygonal and accumulate numerous lipid droplets (Dellman and Eurell, 1998). Sebum, is secreted by the sebaceous glands, is derived from the disintegration of epithelial cells and it is passed through a duct via hair follicle lumen or directly to the skin surface (Borysenko *et al*., 1979).

1.2.4.3 Sweat glands

Based on the structure and mode of secretion, two types of sweat glands are found in mammals; these are the eccrine and apocrine glands. Sweat glands are however totally abscent in *Tachyglossus* species of order monotremata and cetaceans (Sokolov, 1982).

Eccrine sweat glands are the most numerous and best developed in higher primates, while in other mammals they are only found on the thickened epidermis in areas subject to wear such as the plantar and palmar skin where the watery secretion improves contact with

the ground (Spearman, 1973; Sokolov, 1982; Kennedy *et al.*, 1984). Structurally, eccrine glands are simple coiled tubular glands. The tightly coiled secretory unit may be located in the dermis or hypodermis. The diameter of the duct is smaller than the diameter of the secretory coil (Borysenko *et al*., 1979). Histologically, the duct is composed of a stratified cuboidal epithelium with two cell layers. The secretory portion consists of a single layer of pyramid shaped cells. Between the secretory cells and the basement membrane is the myoepithelial cells (Sokolov, 1982).

The **apocrine sweat glands** are numerous in most mammals and are limited in axillary and circumanal regions in primates (Borysenko *et al*., 1979). Thermoregulatory apocrine sweat glands have mainly been reported in hoofed animals (artiodactyla and perissodactyla) and in marsupials. In rodents, in particular apocrine glands have been reported in *Angulus oris* (Microtine rodents) and in the dorsum of *Crytomys hottentus*, but found missing in many other rodents (Quay, 1962; Spearman, 1973; Sokolov, 1982; Daly and Buffenstein, 1998). They are simple coiled tubular glands associated with the hair follicles and they have a compact and a larger secretory portion than eccrine glands. A duct emerges from secretory portion and runs parallel to and opens into the hair follicle, above the entrance of sebaceous glands.

1.2.4.4. Specialized skin glands.

Apart from sweat glands and sebaceous glands, special type of skin glands that perform other functions other than thermoregulation, form localized accumulations, with size, form and locations varying among mammalian species (Konig and Liebich, 2007). The secretions of these specific skin glands in mammals contain volatile substances that are a source of odorous chemical signals and therefore they are most developed in species with

a strong olfactory sense (Spearman, 1973; Konig and Liebich, 2007). In some mammals the secretions of these glands, functions as territorial markers or as accessory sexual scent glands which enable males and females to locate one another during the breeding season (Spearman, 1973; Konig and Liebich, 2007). Example of these glands include the perianal and circumanal glands in the dog, mental and carpal glands in pig, horn glands in goat and the musth (temporal) glands in elephants (Spearman, 1973; Dellman and Eurell, 1998. Scent glands of the skunk, produce the pungent odour as a defensive mechanism (Spearman, 1973). Rodentia species have also been noted to have numerous special glands in the anal, corners of the mouth and abdominal regions (Sokolov, 1982).

1.2.5 Thickness of the skin

Skin thickness among the different species of mammals show a wide variation. For instance, *Phoecaena phoecaena* (Cetacean) and *Elephant indicus* (Proboscidae) have relatively thick skin with an average dorsal thickness of 22mm and 16.5mm respectively compared to *Manis pentadactyla (*Pholidota*)*- (319µ m) and *Sciurus vulgaris* (Rodentia) (952 µm) (Sokolov, 1982) with much less thickness. Thickened skin of animals assumes more protective role, as demonstrated by great thickness of dermal amour of rhinocerous that is adapted to resist blows from horns that might occur during aggressive behavior (Shardwick *et al*., 1992). The extremely thick skin of whales resists the friction they encounter due to their aquatic way of life (Sokolov, 1982). The thickness of the skin of mammals changes regionally and has been attributed mainly to an increase in depth of the epidermis or through increased depth of collagen-rich tissue in the dermis and the hypodermis (Jarman, 1989). Palmer and plantar surfaces of feet of non-hoofed mammals give a typical example of areas of integument where considerable abrasive actions occurs which leads to a relatively thick epidermis and stratum corneum layer compared to the rest

the body (Borysenko *et al.,* 1979). For instance, the fishing bat's foot pads have an average skin thickness of (349 ± 14 μm), with the stratum corneum being the thickest layer (35 ± 7 μm), the rest of its epidermis (31 ± 2 μm) and the dermis (192 ± 4 μm) thick respectively. In contrast, the average skin thickness of its dorsum is (605 ± 22 μm), with stratum corneum, rest of epidermal layers and dermis measuring (19 ±1 μm), (15 ± 1 μm) and (19 ± 1 μm) respectively (Yin *et al*., 2011). In addition to the relationship between repeated external stimuli and increased epidermal thickness, lack of a thick hair coat in humans and rhinocerous, has been suggested to contribute to their relatively thicker epidermis (Cave and Allbrook, 1958; Pinkus, 1963; Margolena, 1965).

1.2.6 Skin morphology of *Tachyoryctes ibeanus* and *Heterocephalus glaber.*

Tachyoryctes ibeanus and *Heterocephalus glaber* are subterranean rodents, that spend most of their lives in sealed underground burrows. Subterranean mammals have developed structural and functional adaptations for burrowing and living underground (Ellerman, 1956; Pearson, 1960; McNab, 1966). Morphologically, subterranean mammals have cylindrical body shape and exhibit structural reductions of tail, eyes and external ears and hypertrophies of the incisors, forelimbs, claws, tactile sense organs and pineal glands that aid in optimizing the ability to burrow and increase their ability to exploit subterranean environments (Prout, 1964; Wright, 1964).

The integuments of *Tachyoryctes ibeanus* and *Heterocephalus glaber* are directly exposed to external influences caused by burrowing which may lead to unique structural and functional adaptations compared to mammals that live above ground. Previous studies focusing on the skin of the subterranean mammals have concentrated on *Heterocephalus glaber* (Naked mole rat) due to its barely naked body (Thigpen, 1948; Tucker, 1981). These studies documented the loosely folded skin of naked mole rat, the presence of sparsely

distributed tactile hairs, the lack of an insulating layer and the lack of sweat glands. Another study compared the dorsal skin structure of *Heterocephalus glaber* to that of another bathyergid rodent, *Cryptomys hottentotus*, in relation to thermoregulation (Daly and Buffenstein, 1998). Major differences between *Heterocephalus glaber* and *Cryptomys hottentotus* were noted in the degree of skin folding, thickness and dermal infrastructure. *Heterocephalus glaber* showed more folding on the skin, with a thicker epidermis and the dermis had pigment cells but lacked sweat glands. Conversely, *Cryptomys hottentotus* skin showed less folding and the dermis, contained sweat glands but lacked pigment cells. Prior to the current work, there is no literature available on studies of the skin of *Tachyoryctes* species, and the only report that exists for the family spalacidae was done in *Spalax microphthalmus* (the greater mole rat) which was limited to the skin of the withers region (Sokolov, 1982).

This thesis contains a comparative topographical and microscopic study of the skin of *Tachyoryctes ibeanus* (Kenyan African mole rat) and *Heterocephalus glaber* (naked mole rat) demonstrating structural features that aid a subterranean existence. Furthermore, their skin structures may explain how these animals repair skin wounds.

1.3 Cutaneous Wounds

Wounds occur when the normal anatomical structure and function of a living tissue is disrupted (Dieglemann and Evans, 2004). The skin, being in direct contact with external environment, is more amenable to traumatic injury than any other organ in the body. Skin integrity can be jeopardized or compromised by a number of factors including, mechanical, chemical, burns and radiation (Wysocki, 2007).

1.3.1 Mechanism of wound healing

When a tissue or organ is injured, either standard repair mechanism (scar formation), regeneration or a pathological response may occur (Diegelmann and Evans, 2004). Wound repair is an intricate process characterized by four distinct, but overlapping phases: hemostasis, inflammation, proliferation and remodeling. This process leads to the replacement of damaged or lost tissue with scar tissue that ablates the original structure and function of the original tissue (Stadelmann *et al.,* 1998; Birch *et al.,* 2005; Braiman-Wiksman *et al.,* 2007; Wysocki, 2007). Conversely, regeneration occurs when an organism has the capacity of replacing structure in both form and function (Diegelmann and Evans, 2004). Pathologic response to tissue injury can either result in excessive healing as in the case of keloids, hyperplastic scarring and contracture or deficient healing which results in non-healing ulcers (Singer and Clark, 1999). Regeneration is the preferred mechanism of repair since the normal function and appearance are maintained, however mammals can only replace a limited amount of the damaged tissue after injury, and most wounds heal by scar formation (Calvin, 1998; Mast and Schultz 1996; Wilgus, 2007). Many invertebrates and some vertebrates have the ability to regenerate their tissues after injury (Brockes and Kumar, 2005; Roy and Levesque, 2006). Even though, adult mammalian dermal wounds heal by forming a scar, cutaneous fetal wounds have the capacity for scar-free healing (Ferguson and O'kane, 2004).

Mechanism of cutaneous wound healing is dependent upon the tissue layers involved (full skin thickness or partial- thickness) and onset and duration (acute or chronic) of repair tissue (Wysocki, 2007). Full- thickness wounds involve total loss of skin layers (epidermis and dermis) and frequently involve loss of subcutaneous tissue (Souba*, et al*., 2001). Partial – thickness wounds involve only partial loss of skin layers, being confined to epidermal and superficial dermal layers (Breuing *et al*., 1992; Wysocki, 2007). The time frame for repair and

the repair process itself differ significantly for full-thickness and partial thickness wounds (Wysocki, 2007). Acute wounds typically are traumatic or surgical in origin, occur suddenly, rapidly and predictably through the repair process (Monaco and Lawrence, 2003). In contrast, chronic wounds are frequently caused by vascular compromise, chronic inflammation or repetitive insults to the tissues and they either fail to close in a timely manner or fail to result in durable closure (Brisett and Hom 2003; Pradhan *et al*., 2009)

1.3.2 Phases of wound healing

1.3.2.1 Hemostasis and clot formation

The first response to wounding is hemostasis, which occurs rapidly to prevent blood loss and seal off the wound. Hemostasis is accomplished primarily by three mechanisms: formation of platelet clumps, vasoconstriction, and fibrin clot formation (Clark, 1996). Immediately after wounding, blood containing platelets suffuses the injured area (Fig. 7a). Platelets are anucleate fragments of megakaryotes, a blood cell lineage of hematopoietic stem cells in the bone marrow (Machlus and Italiano, 2013). The endothelial cells at the site of injury release adenosine diphosphate (ADP), which causes clumps of platelets to adhere to the injury site (Dintenfass, 1985). The clumped platelets degranulate upon contact with exposed collagen in the walls of the injured vessels, releasing more ADP as well as arachidonic acid, fibrinogen, fibronectin, thrombospondin, and Von Willebrand factor VIII (Stadelmann *et al*., 1998). Fibrinogen, fibronectin, and thrombospondin act as ligands for platelet aggregation (Plow *et al*., 1985). Platelets also express glycoproteins on their cell membranes that allow them to stick to one another and to aggregate (Midwood *et al*., 2004).Von Willebrand factor VIII helps mediate the adhesion of platelet to collagen in the injured blood vessels wall (Plow *et al*., 1985). The net result is a sticky mass of platelets, which plugs the vessel.

The arachidonic acid (AA) released by the platelets is converted to thromboxaneA2, which belongs to classes of eicosanoids formed by a cyclooxygenase-dependent pathway (Smith and Murphy, 2002). Thromboxane A2 and serotonin from injured nerve axons are powerful vasoconstrictors that slow blood flow into the wound by constricting blood vessels (Triplett, 2000). The injured nerves also release substance P, a neuropeptide that causes mast cells in the dermis to degranulate, releasing histamine that increases the permeability of vessel walls, allowing further leakage of plasma into the wound (Kulka *et al*., 2007).

The blood plasma present in the wound space contains coagulation factors that induce clot formation (Clark, 1996). Hagemann factor VII, a plasma protein initiates a cascade of reactions involving 12 clotting factors and requiring Calcium ions as an essential co-factor (Lansdown, 2002). Tissue factor is produced at the end of the cascade and converts prothrombin to the enzyme thrombin (Achneck *et al.,* 2010). Thrombin catalyzes the conversion of plasma fibrinogen to fibrin, the major structural protein of the clot (Yamada and Clark, 1996). Fibrin molecules are organized into fibers that intertwine to form a meshwork that traps erythrocytes, leukocytes and platelets (Fig. 7b) (Stocum, 2006). This meshwork also contain plasma fibronectin and vitronectin as well as thrombospondin from platelets (Yamada and Clark, 1996). Formation of a clot then serves as a temporary shield protecting the denuded wound tissue and provides a provisional matrix over and through which cells can migrate during wound repair (Martin, 1997; Clark 2001). The surface of the clot dehydrates to form a protective scab. Cells of the immune system now invade the deeper part of the clot to begin the inflammatory phase.

1.3.2.2 Inflammatory phase

Chemotactic factors released during clot formation increase the permeability of surrounding capillaries, attracting neutrophils, monocytes and T-lymphocytes (Pierce, 1991; Wahl, 1992). These cells are released through spaces between endothelial cells by diapedesis into the provisional fibrin matrix.

Very early after injury, polymorphonuclear neutrophils (PMNs) arrive at the wound site and become the predominant cells in the wound for the first two days after the injury (Fig. 7c) (Martin, 1997; Stramer *et al*., 2007). Neutrophils are attracted to the wound by growth factors released from degranulating platelets, fibrinopeptides cleaved from fibrinogen by thrombin, and fibrin degradation products produced by action of plasmin, and collagen and elastin fragments (Riches, 1996). Neutrophils kill bacteria and debride the wound and also cleanse the wound by secreting proteases that break down damaged tissue (Clark, 1996; Dovi *et al*., 2003). In response to specific chemoattractants, such as fragments of extracellular matrix proteins, transforming growth factor β (TGF ß), and monocyte chemoattractant protein 1, monocytes infiltrate the wound site and become activated macrophages (Fig. 7d) (Martin, 1997; Singer and Clark, 1999). Neutrophils usually undergo apoptosis once they have completed their tasks and are engulfed and degraded by macrophages (Martin 1997; Singer and Clark, 1999).

Macrophages are essential for effective wound healing, such that if their infiltration is prevented, healing is severely impaired (Leibovich and Ross, 1975). The macrophage's main role is to engulf apoptotic neutrophils and phagocytize bacteria and damaged tissue, (Clark, 1996; Martin 1997; Singer and Clark, 1999) and they also debride damaged tissue by releasing proteases (Laskin *et al.,* 2011). Macrophages engulf the apoptotic cells by recognizing the changes in their cell surfaces by specific integrin, lectin, and phosphatidylserine receptors (Haslett and Henson, 1996). Macrophages also secrete a

number of growth factors, such as platelet-derived growth factor and vascular endothelial growth factor, and other cytokines which attract cells involved in the proliferation stage of healing (i.e cells that re-epithelialize the wound, create granulation tissue, and lay down a new extracellular matrix) (Leibovich and Ross, 1975, Singer and Clark, 1999; Werner and Grose, 2003). Macrophages are stimulated by the low oxygen content of their surroundings to produce factors that induce and speed angiogenesis (Leibovich and Ross, 1975; Rappolee *et al*., 1988). Thus, macrophages appear to have a pivotal role in the transition between inflammation and proliferative phase (Riches, 1996).

T-cell lymphocyte infiltration peaks by the end of first week (Swift *et al*., 2001). T-cells particularly the T_H1 subset of CD4+ cells and CD8+ cells, appear to play a role in regulating macrophage–induced activities (Linares, 1996; Park and Barbul, 2004). As inflammation dies down, fewer inflammatory factors are secreted, existing ones are broken down, and numbers of neutrophils and macrophages are reduced at the wound site (Alam *et al*., 1994; DiPietro, 1995; DiPietro *et al*., 1998).

1.3.2.3 Proliferative phase

The proliferative phase encompasses, re-epithelialization, formation of granulation tissue and wound contraction.

Wound re-epithelization, occurs early in the wound healing process, and is dependent on a number of factors including wound size, wound shape, moisture content of the wound, and species (Soo *et al*., 2000; Gal *et al*., 2008; Seifert *et al.,* 2012). Basal epidermal cells at the edge of the wound undergo marked phenotypic alterations including retraction of intracellular tonofilaments (Paladini *et al*., 1996), dissolution of most inter-cellular desmosomes, and formation of peripheral cytoplasmic actin filaments, and these in turn contribute to promote cell migration (Gabbiani *et al*., 1978 ; Goliger and Paul, 1995).

The dissolution of intercellular desmosomes and hemidesmosomal links between the epidermis and the basement membrane prevent their adherence to each other and therefore ensures lateral movement of epidermal cells (Fig. 7e) (Sivamani *et al.,* 2007). The leading edge keratinocytes express new integrin receptors which allow them to interact with a variety of extracellular-matrix proteins that are interspersed with stromal type I collagen at the margin of the wound and interwoven with the fibrin clot in the wound space (Clark *et al.,* 1996; Clark, 2001). Epidermal cells produce plasminogen activator which activates plasminogen to plasmin, a chief fibrinolytic enzyme (Collen and Lijnen, 1991; Vassalli *et al*., 1991). Epidermal cells also produce matrix metalloproteinases (MMPs) which are involved in degradation of extracellular matrix protein (Biljana *et al*., 2011). Fibrinolysis and degradation of extracellular matrix protein facilitates migration of cells (Chapman, 1997). The migrating epidermal cells cut a path through the wound separating the fibrin clot from the viable tissue. This path is determined by an array of integrins expressed on the cell membrane of the epidermal cells.

Once the denuded wound surface has been covered by a mono-layer of keratinocytes, epidermal migration ceases and a new stratified epidermis with underlying basal lamina is reestablished from the margins of the wound inward (Gipson *et al*., 1988). Suprabasal cells cease to express integrins, instead undergo the standard differentiation program of cells in the outer layers of unwounded epidermis (Larjava *et al*., 1993; 1996). As basal lamina is being re-established, MMP expression is shut off, and new hemidesmosomal adhesions to the basal lamina reassemble (Martin, 1997).

Granulation tissue formation is characterized by replacement of the fibrin clot by a capillary rich-fibroblastic tissue which begins to invade the wound space approximately four days after injury (Fig. 7e) (Singer and Clark, 1999). Macrophages, fibroblasts, and the numerous new capillaries give the new stroma a granular

appearance that constitutes the granulation tissue (Clark, 1996). Platelet derived growth factor and transforming growth factor produced by macrophages stimulate fibroblast migration and proliferation (Singer and Clark, 1999).

The fibrin, fibronectin, and hyaluronic acid in the newly formed provisional matrix, contribute to the formation of granulation tissue by providing a scaffold or conduit for cell migration (Greiling and Clark, 1997). Resident dermal fibroblasts and fibroblasts differentiating from circulating mesenchymal stem cells from the bone marrow enter the wound from the vasculature (Fathke *et al*., 2004). Movement of fibroblasts into the provisional matrix is mediated by their integrin receptors and proteolytic enzymes that cleave the path for cell migration. These enzymes include plasmin derived from plaminogen, metalloproteinases and gelatinase (Vaalamo *et al*., 1997; Li *et al*., 2003). After migrating into wounds, fibroblasts commence the synthesis of extracellular matrix (Welch *et al*., 1990; Clark, 1995). The provisional extracellular matrix is gradually replaced with a collagenous matrix (Welch *et al*., 1990; Clark, 1995). Collagen type III is the major collagen that occurs early in extracellular matrix synthesis, and precedes the appearance of collagen type I (Whitby and Ferguson, 1991). The early appearance of type III collagen is associated with deposition of fibronectin which acts as a template for the deposition of its fibrils (McDonald, 1988).

Regeneration of new blood vessels from the existing vessels termed as angiogenesis is necessary to sustain the newly formed granulation tissue (Singer and Clark, 1999). Blood vessels carry oxygen and nutrients necessary to sustain cell metabolism. Many molecules have been found to have angiogenic activity, including vascular endothelial growth factor, transforming growth factor β, angiogenin, angiotropin, angiopoietin 1, and thrombospondin (Folkman and D'Amore, 1996; Iruela-Arispe and Dvorak, 1997). These molecules appear to induce angiogenesis by stimulating the production of basic fibroblast growth factor and

vascular endothelial growth factor by macrophages and endothelial cells (Singer and Clark, 1999). Low oxygen tension and elevated lactic acid may also stimulate angiogenesis (Detmar *et al.,* 1998). Basic fibroblast growth factor and vascular endothelial growth factor stimulate endothelial cells to release plasminogen activator and procollagenase (Singer and Clark, 1999). Plasminogen activator converts plasminogen to plasmin and procollagenase to active collagenase, and in concert these two proteases digest basement membranes. The fragmentation of the basement membrane allows endothelial cells stimulated by angiogenesis factors to migrate and form new blood vessels at the injured site. Once the wound is filled with new granulation tissue, angiogenesis ceases and many of the new blood vessels disintegrate as a result of apoptosis (Ilan *et al*., 1998). Apoptosis is probably regulated by a variety of matrix molecules, such as thrombospondins 1 and 2 (Guo *et al*., 1996), and antiangiogenic factors, such as angiostatin, endostatin, and angiopoietin 2 (Folkman, 1997).

Dermal wound contraction is a key process in wound healing in loose skinned mammals and accounts for much greater percentage of wound closure in rodents (Yanna, 2001). Dermal contraction is initiated early in the phase of structural repair by fibroblasts that differentiate into myofibroblast under the influence of TGF–β1 and extracellular matrix (Gabbiani, 1998; Lorena *et al*., 2002). Myofibroblast like smooth muscles have contractile apparatus consisting of large bundles of actin microfilaments running along the inner surfaces of the plasma membrane (Welch *et al*., 1990). They also have cell to cell or cell to matrix linkages. Myofibroblasts are attracted by fibronectin and growth factors and they move along fibronectin linked to fibrin in the provisional extracellular matrix in order to reach the wound edges (Deodhar and Rana, 1997). At an adhesion called the fibronexus, actin in the myofibroblast is linked across the cell membrane to fibronectin and collagen in the extracellular matrix at wound edges (Mirastschijski *et al.,* 2004). Myofibroblasts have

many such adhesions, which allow them to pull the extracellular matrix when they contract, reducing the wound size (Hinz, 2006). Fibroblasts lay down collagen to reinforce the wound as myofibroblasts contract (Stadelmann *et al.*, 1998). In full thickness excisional wounds, contraction peaks at 5 to 15 days post wounding (Hinz *et al.,* 2007). Contraction can last for several weeks and continues even after the wound is completely reepithelialized (Tanaka *et al*., 2004). The contraction stage in proliferation ends as myofibroblasts stop contracting and undergoes apoptosis (Hinz, 2006). The breakdown of the provisional matrix leads to a decrease in hyaluronic acid and an increase in chondroitin sulfate, which gradually triggers fibroblasts to stop migrating and proliferating (Melrose *et al.,* 1996).

1.3.2.4 Dermal matrix remodeling

In the final stages of healing, wounds are fully re-epithelialized and the granulation tissue is remodeled into a relatively acellular fibrous scar tissue (Fig. 7f). (Braiman-Wiksman *et al.*, 2007). As the matrix is being remodeled, the wounded skin regains its strength and elasticity, and proceeds through reorganization of the collagen and elastic fibers for final reconstruction of the dermis. During matrix remodeling, type III collagen, which is prevalent during proliferation, is replaced by type I collagen (Guerret *et al*., 2003). Instead of the random basket-weave organization of normal dermis, type I collagen fibers in the scar are degraded by matrix metalloproteinases (MMPs) and cross-linked by enzyme lysyloxidase into thick bundles that are oriented parallel to the surface of the scar (Bailey *et al.,* 1975; Linares, 1996). Matrix metalloproteinases enzymes are secreted by macrophages, epidermal cells, and endothelial cells, as well as fibroblasts (Mignatti *et al*., 1996). As the scar matures, the density of the vascular and neural networks in the granulation tissue returns to normal and there is reduction in the number of fibroblast by apoptosis (Lorena *et al.,* 2002). Wounds gain only about 20 percent of their final strength in the first three

weeks, during which time fibrillar collagen has accumulated relatively rapidly and has been remodeled by contraction of the wound (Singer and Clark, 1999). Thereafter the rate at which wounds gain tensile strength is slow, reflecting a much slower rate of accumulation of collagen and matrix remodeling and an increase in proportion of the degree of cross-linking (Bailey *et al*., 1975). Establishment of a mature, stable scar takes approximately 80 days in rodents, but it does not achieve more than 80 percent of the tensile strength of normal dermal tissue (Levenson *et al*., 1965; Mast, 1992). Histological analysis of the scar always reveals a small section marking of the wounded area, identified by a gap in hair-follicle distribution (Braiman-Wiksman *et al.*, 2007).

1.3.3 Abnormal wound healing.

The phases of wound healing normally progress in a predictable and timely manner, if they do not, healing may progress inappropriately resulting in excessive healing such as keloids, hypertrophic scars and contracture, (Rahban and Garner, 2003) or deficient healing such as non-healing ulcers (Crew *et al*., 2012). In excessive healing there is too much deposition of connective tissue that results in altered structure and may lead to, loss of function (Van Zuijlen *et al*, 2002). Deficient healing occurs when there is insufficient deposition of connective tissue matrix and the tissue is weakened to the point where it can fall apart (Diegelmann and Evans, 2004).

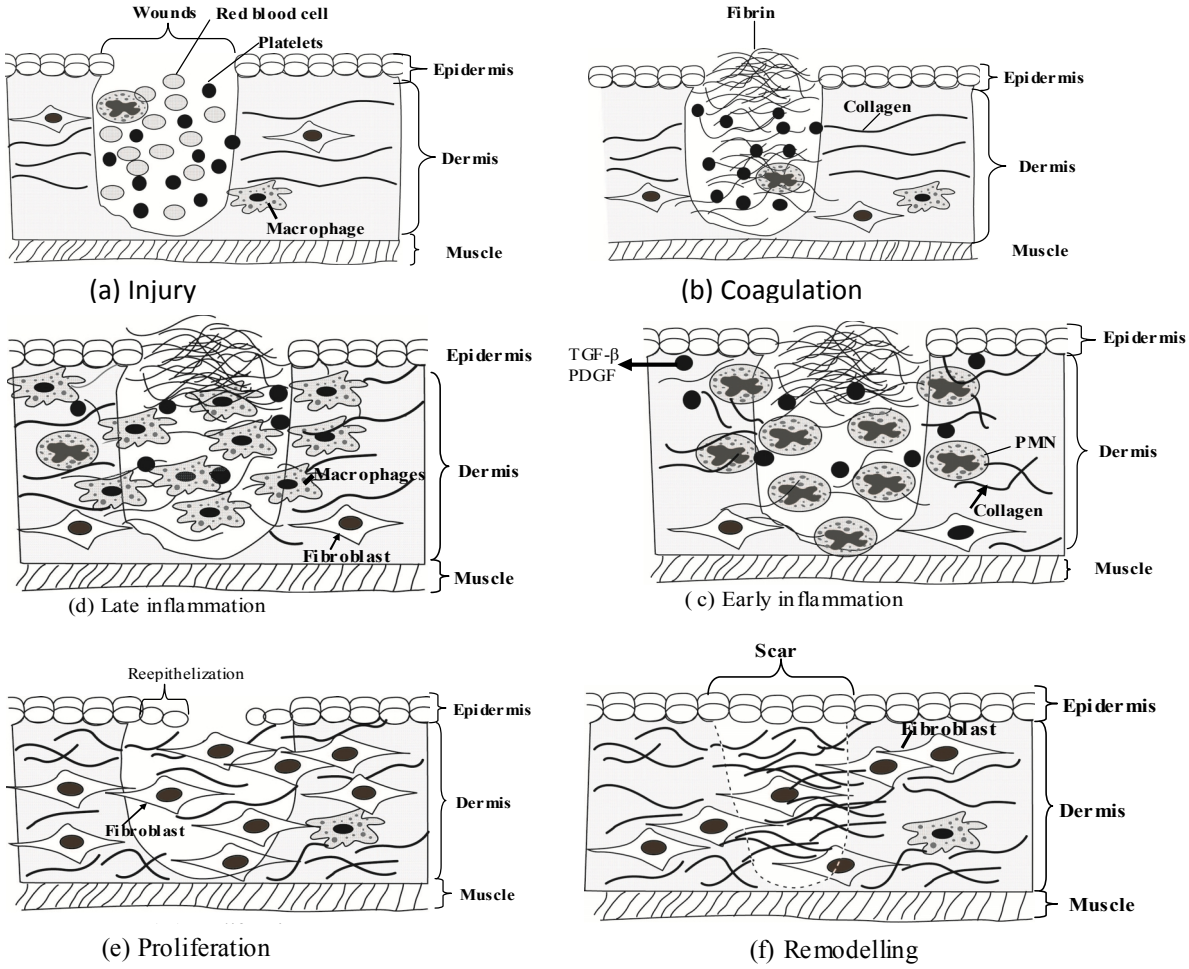


Fig. 7. Phases of excisional cutaneous wound healing. (a) Following cutaneous injury, blood chemotactic factors extravasate from locally damaged blood vessels within the dermis resulting in increased permeability that allows platelets, neutrophils (polymorphonuclear neutrophils [PMN]) and plasma proteins to infiltrate the wound. (b) Coagulation then occurs as platelets aggregate with fibrin, which is deposited in the wound following its conversion from fibrinogen. (c) platelets release several factors, including platelet derived growth factor (PDGF) and transforming growth factor β (TGF – β), which attract PMNS to the wound, signaling the beginning of inflammation. (d) As inflammation advances, macrophages becomes the principal inflammatory cell. PMNs and macrophages remove debris from the wound, release growth factors, and begin to reorganize the extracellular matrix. (e) During the proliferation phase, fibroblasts are recruited to the wound by growth factors released by inflammatory cells and begin to synthesis collagen. (f) Remodeling phase of repair involves collagen cross linking and reorganization that occurs after collagen have been deposited. (Adapted from Beanes *et al*., 2003 with modifications).

1.3.3.1 Hypertrophic scar

Hypertrophic scar is a cutaneous condition characterized by deposits of excessive amount of collagen during healing to give rise to a typically raised, red and sometimes pruritic scar (Wolfram *et al*., 2009).These scars do not exceed the margins of the original wound and usually subsides with time (Murray,1993; Nemeth, 1993; English and Shenefelt, 1999 ; Slemp and Kirschner, 2006). Hypertrophic scars are mainly made up of type III collagen bundles with their fibers arranged in a wavy pattern but predominantly oriented parallel to the epithelial surface (Blackburn and Cosman, 1966; Calnan and Copenhagen, 1967; Linares and Lasson, 1976; Ehrlich *et al*., 1994). Hypertrophic scar is also characterized by increased vasculature, increased numbers of white blood cells and fibroblasts (Atiyeh *et al*., 2005; Wolfram *et al.,* 2009).

1.3.3.2 Keloids

Keloids are lesions that occur as a result of excess deposition of collagen fibers at the site of a healed skin injury (Diegelmann and Evans, 2004). Unlike hypertrophic scars, keloids infiltrate into surrounding normal tissue and continue to evolve over time and rarely regress (Wolfram *et al.,* 2009). Collagen organization contrast that of hypertrophic scar in that the fibers are not organized into bundles and the collagen type I and type III lie in connected loose sheets, randomly oriented to the epithelial surface (Grinnell, 1994; Costa *et al*., 1999).

1.3.3.3 Wound Contracture

Wound contraction is part of the normal process of wound healing but if excessive, it becomes pathologic and is known as a contracture (Nedelec *et al.,* 2000). Connective tissue contracture is a slow, permanent, low energy shortening process which involves matrix, dispersed cells and is dominated by extracellular event such as

matrix remodeling (Glimcher, 1992). In fibrocontractile diseases, collagen type I is replaced to a greater extent by collagen type III which is present in remodeling tissues that are subject to mechanical stress (Gabbiani and Badonnel, 1976). Matrix shortening and increased stiffness indicates that the resident cells have locked a tension into the collagen structure, but in an interstitial and incremental manner (Grinnell and Ho 2002). The ability of myofibroblast to generate and maintain contractile forces is essential for tissue contracture. As the extracellular matrix is being remodeled, the load that is normally carried by the extracellular matrix is now carried by the contracted myofibroblast (Tomasek *et al*., 2002). Once a new shortened matrix is laid down, the myofibroblast is stress-shielded by the load carrying extracellular matrix. Myofibroblast can either disappear by apoptosis or contract again to continue the process of extracellular matrix shortening (Tomasek *et al*., 2002). As this occurs the shortening of the collagen network ceases to depend on the cell-generated forces as the increased stiffness and ability to carry load is built into the matrix material and is present even after the loss of cell contraction (Grinnell and Ho, 2002).

1.3.3.4 Chronic ulcers

Chronic ulcers are disruptions of normal anatomic structure of the skin resulting in loss of its barrier function (Timur-banu *et al*., 2001). Development of chronic non-healing cutaneous ulcers is the consequence of inadequate tissue repair (Steed *et al*., 1996). A variety of etiologies account for these ulcers including arterial insufficiency, chronic venous insufficiency and diabetes mellitus (Timur-banu *et al*., 2001). Excessive infiltration of these ulcers by neutrophils is a significant biological marker (Diegelmann and Evans, 2004). The over-abundant neutrophil infiltration is responsible for the chronic inflammation characteristic of non-healing pressure ulcers. The neutrophils release significant amounts of

enzymes such as collagenase (matrix metalloproteinase-8) that is responsible for destruction of the connective tissue matrix (Nwomeh, 1998, 1999). In addition, the neutrophils release an enzyme called elastase that is capable of destroying important healing factors such as platelet-derived growth factor (PDGF) and TGF-ß (Yager *et al.*, 1996). Another marker of these chronic ulcers is an environment containing excessive reactive oxygen species that further damage the cells and healing tissues (Wenk *et al.*, 2001). These chronic ulcers do not heal until the chronic inflammation is reduced (Diegelmann and Evans, 2004).

1.3.4 Comparative wound healing in mammals

Generally the repair process of adult mammalian wounds, includes four classically defined phases comprising of an initial heamostasis and inflammatory response, a proliferation and migration phase, and a maturation and remodeling phase resulting in scar formation (Martin, 1997). However, fetal mammalian skin repairs without scarring after injury (Olutoye and Cohen, 1996; Ferguson and O'kane, 2004). This has been attributed to a number of differences between the healing of embryonic and adult wounds. For example the fetal, normal basket weave pattern of collagen synthesis and architecture is associated with three differences compared to adult wounds, extracellular matrix synthesis. Firstly, fetal wounds fibroblast synthesizes higher levels of hyaluronic acid and hyaluronic acid receptors (DePalma *et al.,* 1989; Longaker *et al.,* 1989; Alaish *et al*., 1994). Hyaluronic acid is bound by the receptors and remains in the wound. The higher level of hyaluronic acid inhibits fetal fibroblast proliferation, decreasing collagen synthesis and therefore decreased scar formation of the wounds (Mast *et al.,* 1993). Secondly, Sulfated proteoglycan synthesis does not accompany collagen synthesis in fetal wounds (Whitby and Ferguson, 1992). The fetal skin also has a higher ratio of type III compared to type I collagen (Merkel *et al.,* 1988).

There is also a difference in inflammatory response and skin morphogenesis of a developing embryo and an adult mammal. In the embryo the immune system is developing and the response to injury of these primitive immune cells is different from that in the adult (Cowin *et al.,* 1998). As a consequence, embryonic wounds have far fewer inflammatory cells (markedly reduced numbers of neutrophils, lymphocytes, monocytes and macrophages), that are less differentiated and the length of time these cells are present is markedly reduced compared with adult wounds. Additionally, since the embryo is rapidly developing and growing with a considerable expansion of skin volume, normal embryonic skin and embryonic wounds contain high levels of morphogenetic factors involved in skin growth, remodeling and morphogenesis (Cowin *et al.,* 1998). These differences in inflammatory response and skin morphogenesis between adults and fetus involve differences in the growth factor profile in terms of types, amounts and the length of time the growth factors are present in embryonic wound healing compared with an adult wound (Whitby and Ferguson 1991; O'Kane and Ferguson 1997; Shah *et al.,* 2000; Cowin *et al.,* 2001). Thus, for example, there are major differences in the transformation growth factor (TGF β isoforms) present in embryonic and adult wounds. Embryonic wounds express very high levels of TGFβ3, a skin morphogenetic factor predominantly synthesized by keratinocytes and fibroblasts and very low levels of TGFβ1 and TGFβ2. In contrast, adult wounds contain predominantly TGFβ1, which is derived initially from degranulating platelets and subsequently from inflammatory cells such as monocytes and macrophages (Ferguson and O'kane 2004). Adult wounds also contains TGFβ2 and large quantities of platelets derived growth factor (PDGF), which is virtually absent in embryonic wounds (owing to the lack of platelet degranulation), whereas embryonic wounds contain higher levels of endogenous fibroblast growth factors (FGFs) involved in skin morphogenesis (Whitby and Ferguson 1991*b*). But it is TGFβ1 that has been shown to

play a central role in scar formation (Singer and Clark, 1999).

During skin repair in adult mammals, part of the failure to regenerate the skin results from an inability to regenerate epidermal appendages (hair follicle and associated sweat and sebaceous glands) and to correctly replace and reorganize the dermal extracellular matrix (Martin, 1997). Loss of an adult follicle is therefore considered permanent. However, the possibility that hair follicles can develop *de* novo following wounding was raised in earlier studies in rabbits (Breedis, 1954, Billingham and Russel, 1956, mice (Lacassagne and Latarjet, 1946) and humans (Strauss and Kligman, 1956). These observations were generally discounted because definitive evidence for follicular neogenesis was not presented (Straile, 1967). Despite the general believe that adult mammals are considered to lack the regenerative capabilities of fetuses, there are a handful of model systems in which a variation of appendage regeneration has been described. These include follicular neogenesis in genetically normal adult laboratory mice and African spiny mice (Ito *et al*., 2007; Seifert *et al.,* 2012), the closure of excisional tissues in ears following hole punch in rabbits, laboratory mice and African spiny mice (Metcalfe *et al*., 2006; Seifert *et al.,* 2012), the annual regeneration of antlers in deer (Price *et al*., 2005; Kierdorf *et al*., 2007), and the regeneration of amputated digit tips known to occur in humans and rodents (Han *et al*., 2005). The findings from these studies suggests that adult mammals may possess a higher capacity for skin regeneration than previously realized.

There are also some studies that have shown differences in healing processes in particular among the various mammalian species. Some of the reasons that have been advanced for the differences in wound repair are structural and functional properties of the skin. For instance, small mammals (rabbits, guinea pig, rat and mouse) that are mostly used for wound healing studies are loose skinned, have a dense layer of body hairs, thin epidermis and dermis and possesses subcutaneous panniculus carnosus muscles (Dorsett-Martin, 2004). Conversely,

pigs and human are considered to be tight-skinned with a thick epidermis and sparsely distributed hairs (Sullivan *et al*., 2001). The loose skin and presence of panniculus carnosus muscle contribute greatly to healing by wound contraction in small mammals which is lacking in humans and pig (Sullivan *et al*., 2001). Within the order rodentia, African spiny mice (Acomys) with a structurally weak skin can regenerate their dermis and shows high rates of wound re-epithelization and wound contraction compared to other loose skinned rodents (rabbits, rat, *Mus msculus*) (Soo *et al*., 2000; Yanna, 2001; Gal *et al*., 2008; Seifert *et al*., 2012).

Naked mole rat *(Heterocephalus glaber)* and Kenyan African mole rat *(Tachyoryctes ibeanus)* skins differ markedly macroscopically. These differences in skin properties have been necessitated by physiological and behavioural adaptations to exist in an underground environment in which each of these species resides (*Daly* and Buffenstein, 1998; Lacey *et al*., 2000). *Heterocephalus glaber* has few scarcely distributed hairs on pinkish-gray wrinkled skin (Tucker, 1981; Lacey *et al*., 2000). The integument is exceptionally loose, thereby reducing integumentary stresses when the animal is digging and moving in narrow tunnels (Tucker, 1981). In contrast *Tachyoryctes ibeanus* is observed to have a thick hair coat. Several studies have been carried out to establish the participation of hair follicles in wound healing. Both epidermal and follicular keratinocytes have been reported to participate in epidermal repair in response to injury (Levy *et al*., 2007). Additionally, hair follicle dermal cells have been shown to be involved in induction of follicular neogenesis and reformation of the new dermis in manner similar to fibroblast (Jahoda and Reynold, 2001). Histological studies examining the process of wound healing in naked mole rat and Kenyan African mole rats has not been characterized. However, macroscopic study on naked mole rats wounds have been carried out, which indicated a slower rate of wound closure compared to mice which was attributed to lack of neuropeptide substace P

(Ruttencutter *et al*., 2007). Given that naturally *Heterocephalus glaber* has sparsely distributed tactile hair follicles and absence of sweat glands in their dermis (Tucker, 1981; *Daly* and Buffenstein, 1998), there is an expectation that wound repair would be somewhat distinctive from that of hairy mammals such as *Tachyoryctes ibeanus*.

1.4 RESEARCH OBJECTIVES

1.4.1 Overall Objective

To investigate microanatomy of the skin, wound repair and the potential for dermal regeneration in adult naked mole rat (*Heterocephalus glaber)* and adult Kenyan African mole rat (*Tachyoryctes ibeanus*).

1.4.2 Specific Objectives

1. To analyse and compare the microanatomy of the skin in the Kenyan African mole rat *(Tachyoryctes ibeanus*) and naked mole rat (*Heterocephalus glaber)* from different anatomical sites.
2. To characterise the process of wound healing in the Kenyan African mole rat *(Tachyoryctes ibeanus)* and naked mole rat (*Heterocephalus glaber*).

CHAPTER TWO

2.0 MATERIALSAND METHODS

2.1 Experimental animals

2.1.1 *Tachyoryctes ibeanus* (Kenyan African mole rat)

Tachyoryctes ibeanus were trapped in Ngong, Kajiado county after getting permission from Kenya Wildlife Services. Animals were transported using aerated plastic buckets to the laboratory and housed in conditions simulating natural environment conditions: ambient temperature was maintained at 25^0C (room temperature) and animals were exposed to a 12/12-hour dark/light cycle. Animals were kept singly in strong wire mesh cages (30x35x30cm) containing untreated wood sawdust. A number was assigned each cage to identify the animals. Animals were fed on sweet potatoes, carrots, Irish potatoes and green kikuyu grass *ad libitum*. Prior to the start of experiments, both the naked mole rat and Kenyan African mole rat were allowed to acclimatize to the laboratory conditions for at least 14 days and during this time they were handled.

2.1.2 *Heterocephalus glaber* (naked mole rat)

Heterocephalus glaber were trapped in the field at Kathekani in Makueni county of Kenya after getting permission from Kenya Wildlife Services (KWS). Animals were housed in the Department of Veterinary Anatomy and Physiology, at the University of Nairobi. Housing conditions were designed to mimic the animals' natural environmental conditions: ambient temperature range 28-31°C, relative humidity 70%, and 24/0-hour dark/light cycle (constant dark). Naked mole-rats were kept in communal cages measuring (70x50 x20cm). The cages were made of black painted plastic glass, and covered on top to prevent light exposure. Each cage was subdivided into two, with an open tunnel connecting the two compartments. Wood shavings were mixed with sand and used as bedding that was changed twice a week. Cages

were kept in a sound and vibration proof room warmed by electric heaters or infrared (250W) lamps. Animals were fed on fresh carrots, sweet potatoes, carrot tops and freshly cut grass to simulate the roots, bulbs and tubers in the wild. Food was provided *adlibitum* and water was not provided so as to simulate natural conditions where water demands are met through food intake (Urison and Buffenstein, 1994).

2.2 Experimental design

A total of thirty adult male animals of each species weighing 160-280g (*Tachyoryctes ibeanu*s) and 30-50g (*Heterocephalus glaber*) were used for the experiments conducted in accordance to the internationally accepted principles for laboratory animals use and care. These animals were divided into six groups of five animals each before the start of experiments. One group of animals was used to study the skin structure and the other five groups for wound healing experiments, each group representing a time point (days 3, 7, 14, 30 and 80 post-wounding) in wound repair.

2.3 Harvesting and fixing skin samples

Animals of each species (*Heterocephalus glaber* and *Tachyoryctes ibeanus)* were halothane overdosed in a closed chamber and sacrificed by cervical dislocation followed by decapitation. Skin samples were obtained from the following anatomical locations: nose, including rhinarium, Labial lobes, vibrissal fields, cervical region (neck), thoracic dorsum, lumbal dorsum, sternal region, abdominal, pubic region and dorsum, palmar and plantar of the feet. Furred areas of *Tachyoryctes ibeanus* were shaved with electric clippers before the skin was excised from the animal. Tissues were fixed in 10% neutral-buffered formalin for histological analysis and 2.5% glutaraldehyde for transmission electron microscopy (TEM).

2.4 Wounding

Experimental animals were maintained in good health and just before wounding they were examined and found to be free from clinical signs of disease. Prior to, and during wounding, rats were anesthetized with halothane-soaked cotton wool in a closed chamber. *Tachyoryctes ibeanus* were shaved with electric clippers on their dorsum prior to wounding. Two full thickness excisional wounds were made on each experimental animal at the lumbar region, one on either side of midline using a 4mm dermal biopsy punch. Toe amputation was done on the wounded *Heterocephalus glaber* and the digit number recorded for identification. After recovering from anesthesia and stabilizing, *Heterocephalus glaber* were returned to their communal cages. *Tachyoryctes ibeanus* were returned to the cages bearing their identification number

2.4.1 Macroscopic assessment of wounds

The progression of wound repair was monitored daily until 80 days post injury. The wounds were photographed using a Sony digital camera. The wound size was measured by using a calibrated metric ruler.

2.4.2 Harvesting and fixing wound biopses

The entire wound area, including the surrounding skin were harvested using scissors in mole rats anesthetized with halothane, at D3, 7, 14, 30 and 80 days post-wounding. Tissues were fixed in 10% neutral buffered formalin for paraffin embedding and histological analysis. These tissues were used to study the wounded skin structure during progression of wound repair.

2.5 Tissue processing for histology

Both unwound skin samples and wound biopsies were processed the same way. The samples were placed on a glass slide in 10% NBF (neutral buffered formalin, pH =7.0) and allowed to fix flat. These samples were then placed in 2.0ml snap cap eppendorf tubes in 10% NBF and fixed at 4˚Cbetween 16-24hr. Following fixation, samples were rinsed in running tap water overnight then dehydrated in ascending concentrations of ethanols (50%, 70%, 80%, 90%, 95% and 100%). The wounded and unwounded skin samples were then cleared in two changes of methyl benzoate, infiltrated and embedded in molten paraffin wax then left to solidify overnight. Blocking was done by attaching the wax embedded samples onto wooden blocks. Tissue sections 5 to 7 µm thick were then cut from the embedded blocks using a leitz Weitzar ® rotary microtome. The sections were then mounted on glass slides, deparaffinised using xylene, rehydrated through decreasing concentrations of ethanol (100%, 90%, 70% and 50%) and finally the distilled water. The rehydrated sections were then subjected to three staining protocol; Masson's trichrome staining, Gomori elastic tissue staining and Masson’s Fontana reducing method for melanin and melanin precursors. The stained sections were examined and photographed using a Leica ® DM 500 light microscope.

2.5.1 Histological evaluation of skin samples and morphometric analysis

Photographs for morphometric analysis were taken from three fields of view per slide using Leica® DM 500 microscope. Then measurement of the thickness of the overall skin, epidermis and stratum corneum and length of epidermal pegs was done using leica application suite software (Switzerland).

2.5.2 Histological evaluation of wound healing progression and semi quantative analysis.

Coloured images acquired using a light microscope with digital camera (Leica ® DM 500, Switzerland) running under image analysis programme (leica application suite software, Switzerland) were qualitatively assessed for four different parameters related to acute inflammatory response, proliferation and remodeling: presence of inflammatory cells, fibroblasts, collagen deposition and re-epithelialization. The parameters were graded in a scale of 0-4 (0-absent, 1- minimal, 2- mild, 3-moderate, 4-marked) as described by Cynthia *et al*., (2002) and Gal *et al.,* (2008). The average score of the five animals at each time point (D3, D7 and D14 post- wounding) were used to compare the status of the early phases of healing between *Tachyoryctes ibeanus* and *Heterocephalus glaber*. The late stages of matrix remodeling (D30 and D80) were assessed for the presence of elastic fiber deposition in both species based on Braiman –Waksman *et al.,* (2007) using Gomori elastic tissue staining.

2.6 Tissue processing for electron microscopy

Skin tissue blocks fixed in 2.5% glutaraldehyde were washed in two changes of 0.1M sodium cacodylate buffer for 1 hour each, then post-fixed in 2% aqueous osmium tetroxide (2% w/v Osmium tetroxide and 98% w/v de-ionised distilled water) for 2hours.The tissues were then rinsed in two changes of distilled water, for 5 minutes each, before dehydrating in two changes of ascending concentrations of ethanol (50%, 70%, 90%, 95% and 100%), for 15minutes each. The tissue blocks were then cleared in two changes of propylene oxide for 30 minutes each. Clearing was followed by infiltration with epoxy- resin mixture (Epon-812, MNA, DDSA and DMP-30) in three steps: Firstly, as two parts of the propylene oxide

to one part of epoxy-resin mixture for 30 minutes. secondly as one part of propylene oxide to 2 parts epoxy-resin mixture for 30 minutes. Finally, infiltrated in pure epoxy resin mixture without accelerator (DMP-30) overnight. The infiltrated tissue blocks, were then embedded using freshly prepared resin mixture with accelerator (DMP-30) in plastic capsules and finally polymerized in the oven at 60^0C for 48 hours. The resulting tissue blocks were sectioned using glass knives mounted on a Reichert ® ultra-microtome. 1µm thick, sections (semi-thin section) were cut using glass knives, floated on moistened clean glass slides and fixed by heat on a hot plate at 70^0C for 15min. The fixed sections were then stained with 3% toluidine blue in 1% borax, rinsed using distilled water and examined using leica® DM 500 light microscope. Semi-thin sections served the general purpose of screening the tissue to locate the required area for electron microscopy. Ultra-thin sections (70-100nm thick), were then cut from selected area of interest using Reichert ® ultra-microtome. The cut sections were picked with copper grids, stained with uranyl acetate, rinsed in distilled water and then counterstained with 0.5% lead acetate. The stained sections were finally rinsed with distilled water, air dried and examined and photographed using Philips ® CM 12 transmission electron microscope.

2.7 Statistical analysis

The mean and standard deviations (SD) of morphometric parameters of skin (total skin thickness, epidermal thickness, stratum corneum thickness and epidermal peg lengths) and scores of semi- quantative parameters of wound healing were first expressed from the raw data. Then, the averages and standard deviations (SD) of morphometric parameters of skin and scores of semi-quantative parameters of wound healing were used to plot bar graphs. Variations in morphometric parameters and scores of semi quantative parameters of wound

healing, between *Tachyoryctes ibeanus* and *Heterocephalus glaber* were analyzed using the Student's *t*-test. In all cases, statistical significance was set at $p < 0.05$

CHAPTER THREE

3.0 RESULTS

3.1 Comparative skin morphology of *Tachyoryctes ibeanus* and *Heterocephalus glaber*

3.1.1. General observation of the skin

Tachyoryctes ibeanus has a thick pelage, made up of soft and easily bendable hairs. In contrast, *Heterocephalus glaber* possesses few hairs and the skin is greatly folded. Both animals exhibited loose skin that does not adhere to the body compared to other members of the order Rodentia.

3.1.2. The skin structure at different topographical areas.

3.1.2.1 Nasal Skin

Tachyoryctes ibeanus has a broad rhinarium (glabrous skin of the nose) covering the lateral rim of the nostril and internasal region (Fig. 8A). The rhinarium connects with the hairy part of the nasal skin dorsally and the upper lip ventrally (Fig. 8A). The *Heterocephalus glaber* has oval shaped rhinarium and contains the nares set close together just above the base of upper incisors (Fig. 8B). The skin dorsal to the nose contains the nasal vibrissal field (Fig. 8B, D and F). The rhinarial skin surfaces of both species are very smooth without a typical dermatoglyphic pattern and are covered by keratinized stratified squamous epidermis with epidermal pegs that interdigitate with dermal papillae at epidermal-dermal junction (Fig. 9A-B). The hairy area of the nasal skin of *Tachyorcytes ibeanus* connecting with the rhinarium had a thinner epidermis than the rhinarial skin and the interdigitations of the epidermal pegs and dermal papillae were not present (Fig. 10A). A thick epidermis bearing the epidermal pegs and dermal papillae extends more rostro-caudal in the rest of the skin over the nose in *Heterocephalus glaber* (Fig. 10B).

The dermis of the nasal skin in both animal species is composed of dense irregular connective tissue and traversed by striated skeletal muscles fibres (Fig. 9A-B, 10A-B). The

dermis of hairy nasal skin in *Tachyoryctes ibeanus* contained hair follicles, located superficially in the dermis (Fig. 10A).

The dermis of both animal species contained a superficial nerve plexus situated closer to the basal layer of epidermis, a deep dermal plexus and arteries situated deeper in the skin (Fig. 10A-B, 11A- B). The nerve fibres of the deeper plexus in *Tachyoryctes ibeanus* occurred in bundles sharing a common Schwann cells sheath (Fig. 11A). A bundle contained both myelinated and non-myelinated nerve fibres (Fig. 11A). Nerve bundles in the deeper plexus of *Heterocephalus glaber* only contained non-myeleinated axons (Fig. 11B). In cross section, some of the axons were not completely surrounded by single Schwann cell lamellae. A group of non-myelinated nerve axonal terminals in association with their Schwann cells were observed in the superficial dermal plexus of *Tachyoryctes ibeanus* (Fig. 12A). Superficial nerve plexus in the dermis of *Heterocephalus glaber* composed of the basal lamina of Schwann cells at the axonal terminal juxtaposed to the basal membrane of the stratum basale and longitudinal nerve fibres running parallel to epidermis were encountered (Fig. 12B and 14B). Axon terminals and their Schwann cells were also observed in the dermal papillae of animal species (Fig. 13A and 13B). Pacinian corpuscles near the bases of epidermal pegs were observed in *Tachyoryctes ibeanus* but no lamellated corpuscles were encountered in the rhinarium of *Heterocephalus glaber* (Fig. 14A).

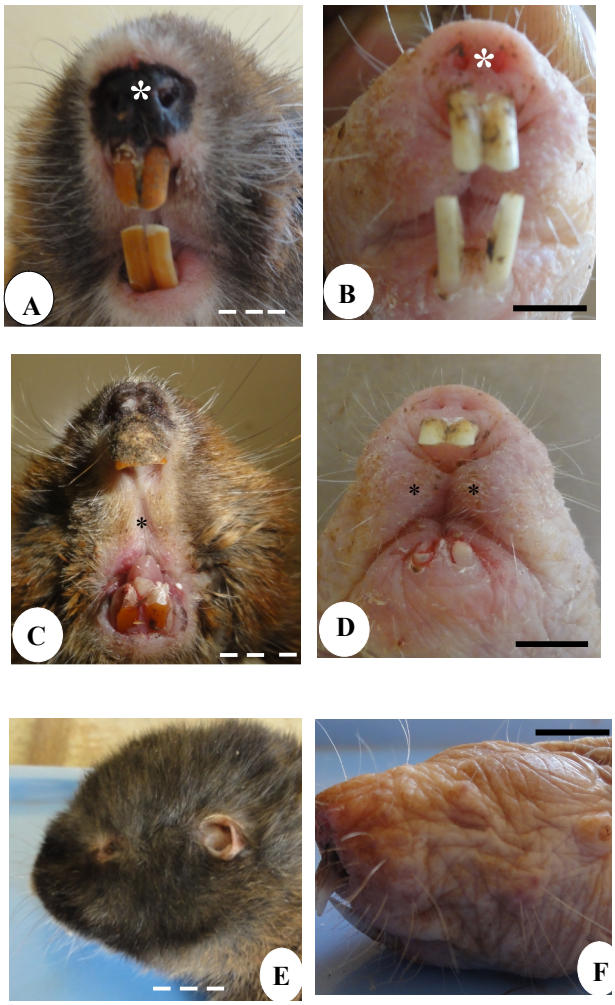


Fig. 8. Head macrographs showing the frontal, oral and lateral views of *Tachyoryctes ibeanus* (A, C and E), *Heterocephalus glaber* (B, D and F) respectively. The head of *Tachyoryctes ibeanus* and *Heterocephalus glaber* have distinctively different morphologies. The broad rhinarium (white asterisk) of *Tachyoryctes ibeanus* forms the lateral rim of the nostrils and internasal region (A), compared to *Heterocephalus glaber* (B) where the rhinarium (White asterisk) contains nares, close together and connects with the upper incisors. T he labial lobes are fused in the midline (Black asterisk) behind the incisors (C) compared to the lip folds (Black asterisk) that seal the oral cavity and are separated by a midline cleft in *Heterocephalus glaber*. Vibrissal hairs in (E) and (F). Scale bars = 1cm.

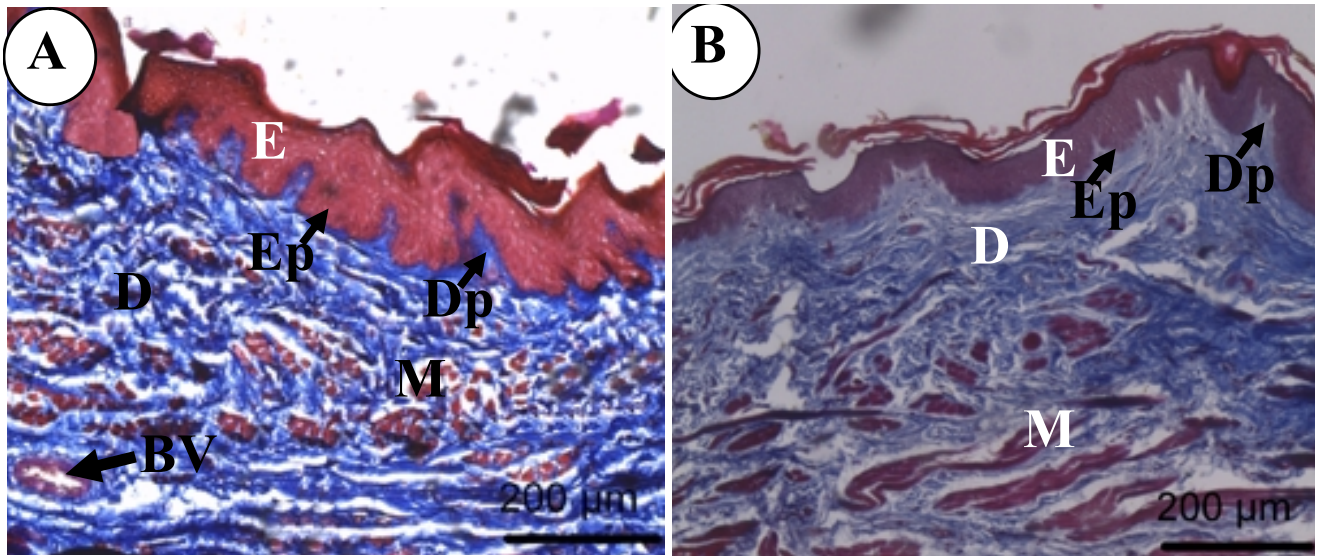


Fig. 9. Light micrographs showing the rhinarium of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). The skin layers consist of the thickened epidermis (E) and dense irregular connective tissue dermis (D) that contains skeletal muscle fibers (M). Both animal species have epidermal pegs (Ep) interdigitating with dermal papillae (Dp).The interdigitations have produced a folded epidermal surface. BV- blood vessels. Masson trichrome, Bars = 200µm

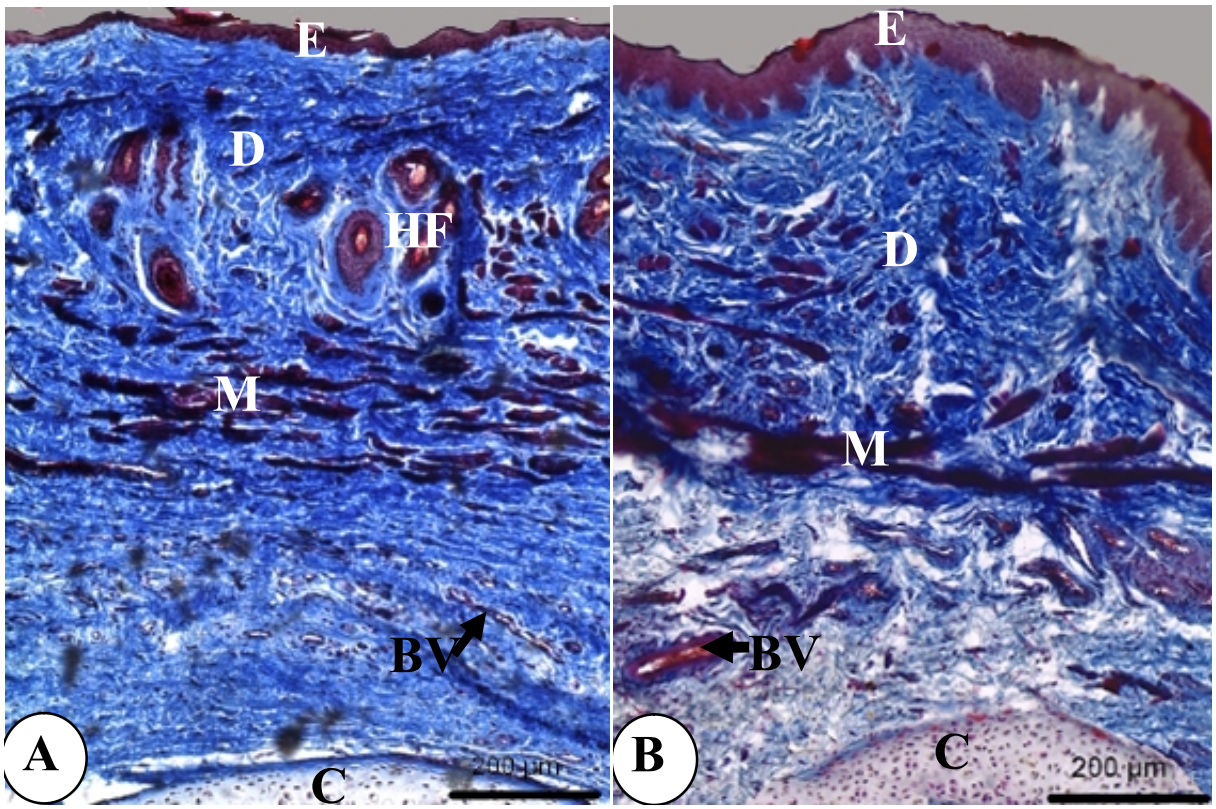


Fig. 10. Light micrographs showing the structure of nasal skin in *Tachyoryctes ibeanus* (hairy nasal skin) (A) a n d *Heterocephalus glaber* (B). The nasal skin caudal-rostral to the rhinarium of *Tachyoryctes ibeanus* and *Heterocephalus glaber* exhibits differences. Both animal species show similarities in the presence of a superficial epidermis (E), a middle dense connective tissue dermis (D) traversed by striated skeletal muscle fibres (M) and deeper in the dermis is the nasal cartilage (C). The basal dermis next to cartilage in both animals also contains blood vessels (BV). *Heterocephalus glaber* have a thicker epidermis than *Tachyoryctes ibeanus* and an epidermal- dermal junction bearing epidermal pegs and dermal papillae interdigitations which are lacking in *Tachyoryctes ibeanus*. In *Tachyoryctes ibeanus,* the upper layer of the dermis next to epidermis contains hair follicles (HF) which are lacking in *Heterocephalus glaber.* Masson trichrome, Bars = 200µm

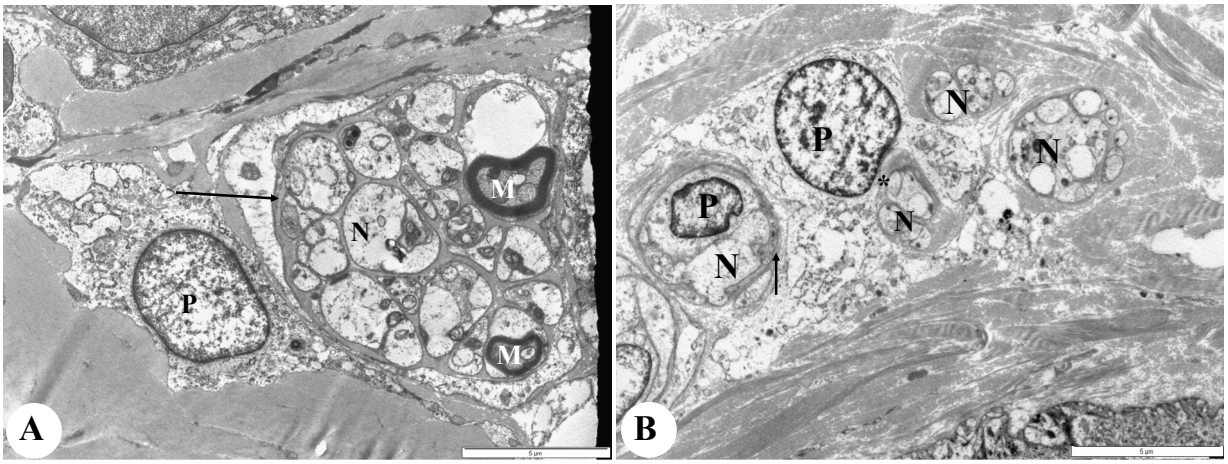


Fig. 11. Electron micrographs showing the rhinarial dermis of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Axons are organized differently in deep dermal nerve plexus in the rhinarial skin of *Tachyoryctes ibeanus* and *Heterocephalus glaber.* *Tachyoryctes ibeanus* have nerve bundles ensheathed (arrow) by Schwann cell(P) containing both myelinated (M) and non-myelinated axons (N) compared to the nerve bundles (N) ensheathed (arrow) by Schwann cells (P) containing non-myelinated axons in *Heterocephalus glaber.* Bar = 5µm

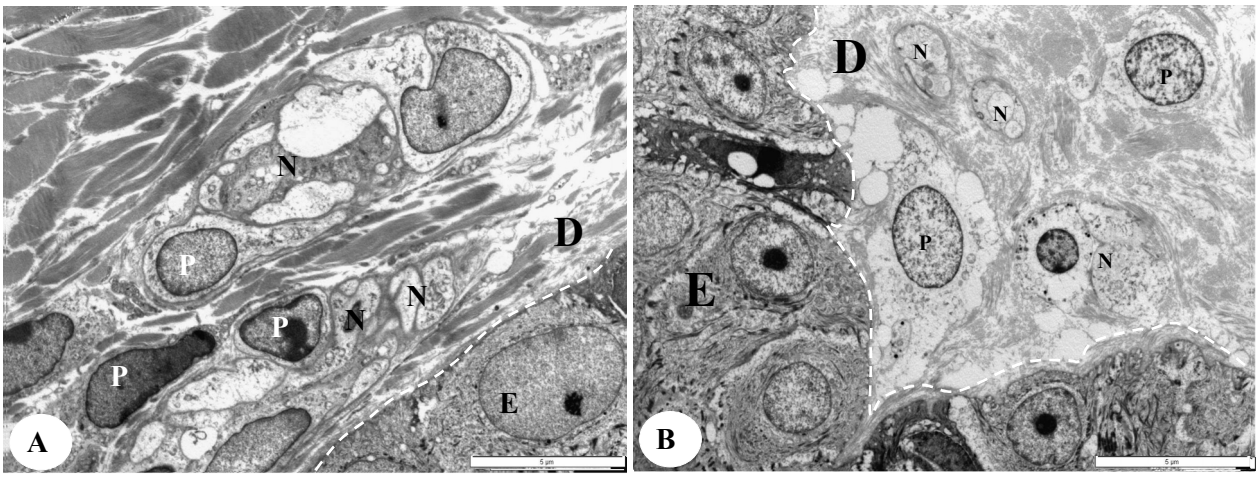


Fig. 12. Electron micrographs showing epidermis (E) and dermis (D) in the rhinarial skin in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Axons of Superficial dermal nerve plexus in the rhinarial skin in *Tachyoryctes ibeanus* and *Heterocephalus glaber* are organized differently. In *Tachyoryctes ibeanus* non-myelinated axons (N) and associated Schwann cells (P) were observed. In *Heterocephalus glaber*, Schwann cells adjacent to stratum basale and associated axon (N) was observed. Dotted lines separates epidermis (E) and dermis (D). Bar = 5µm

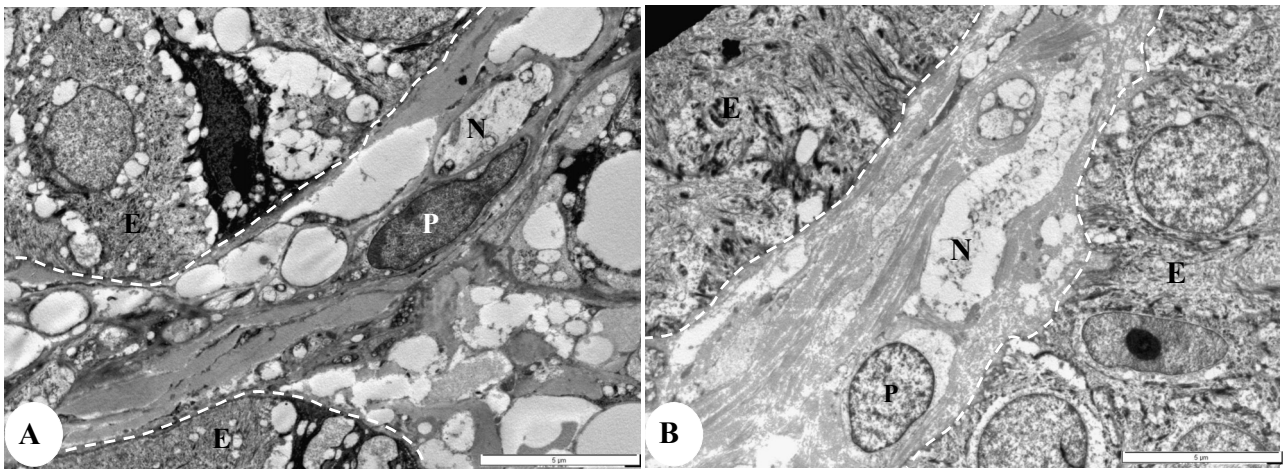


Fig. 13. Electron micrographs showing dermal papillae of the rhinarial skin in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Axonal terminals extends into the dermal papillae in both *Tachyoryctes ibeanus* and *Heterocephalus glaber*. Axonal terminals (N) and Schwann cell (P) within the dermal papillae demarcated from epidermis (E) by dotted lines. Bar = 5µm

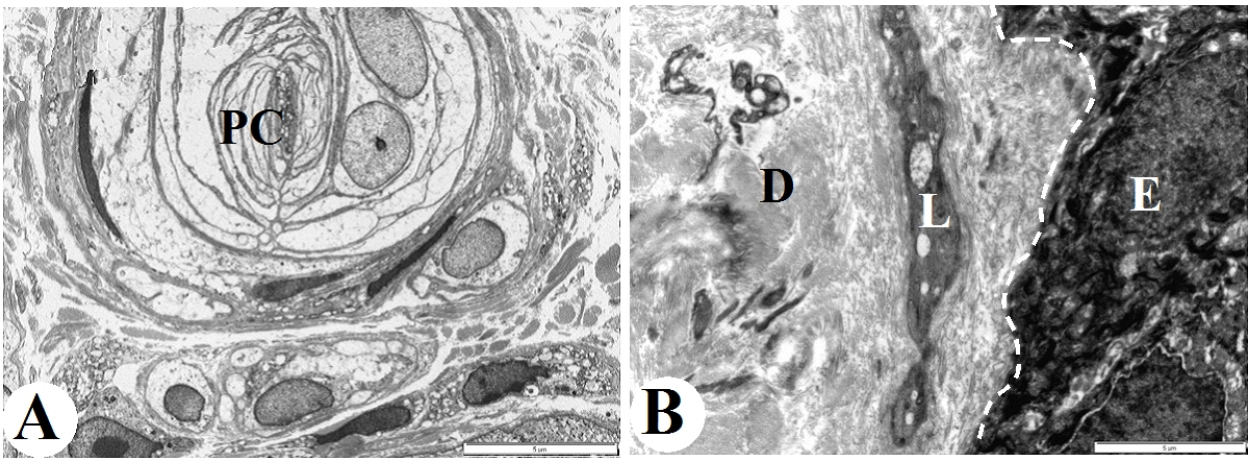


Fig. 14. Electron micrographs of the rhinarial skin in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). *Tachyoryctes ibeanus* have Pacinian corpuscles in rhinarial upper dermis but no lamellated corpuscles were encountered in this region of *Heterocephalus glaber.* Pacinian corpuscle (PC) were located near the bases of epidermal pegs and a longitudinal nerve (L) parallel to epidermis (E) in the upper dermis (D) of *Heterocephalus glaber* Bar = 5µm

3.1.2.2 Skin covering the oral cavity (labial lobes)

In *Tachyoryctes ibeanus,* the labial lobes are drawn into the space between the incisors and molars and they fuse along the midline, uniquely covering the oral cavity (Fig. 8C). The rima oris is a small hole, between the fused labial lobes and the lower incisors. The incisors lie permanently outside and in front of the oral cavity. The epidermal-dermal junctions of the labial lobes is regular with no dermal papillae and epidermal pegs interdigitations (Fig. 15A). In the superficial dermis of *Tachyoryctes ibeanus* small globular sebaceous glands that were directly connected to the epidermal layer by their ducts were identified (Fig. 15A). Small bundles of striated muscles fibres were located at the base of the secretory unit and along the ducts of sebaceous glands.

The *Heterocephalus glaber* upper lips fold behind the upper incisors and a medial cleft divides the upper lips in this species into two fairly independent antimeres (Fig. 8D). The epidermal-dermal junctions of these labial folds are regular with no dermal papillae and epidermal pegs interdigitations and had large multilobular sebaceous glands (Fig. 15B). Also at the base of these glands was a thick layer of skeletal muscle layer. Small bundles of skeletal muscle fibers traverse the connective tissue dermis between the glands.

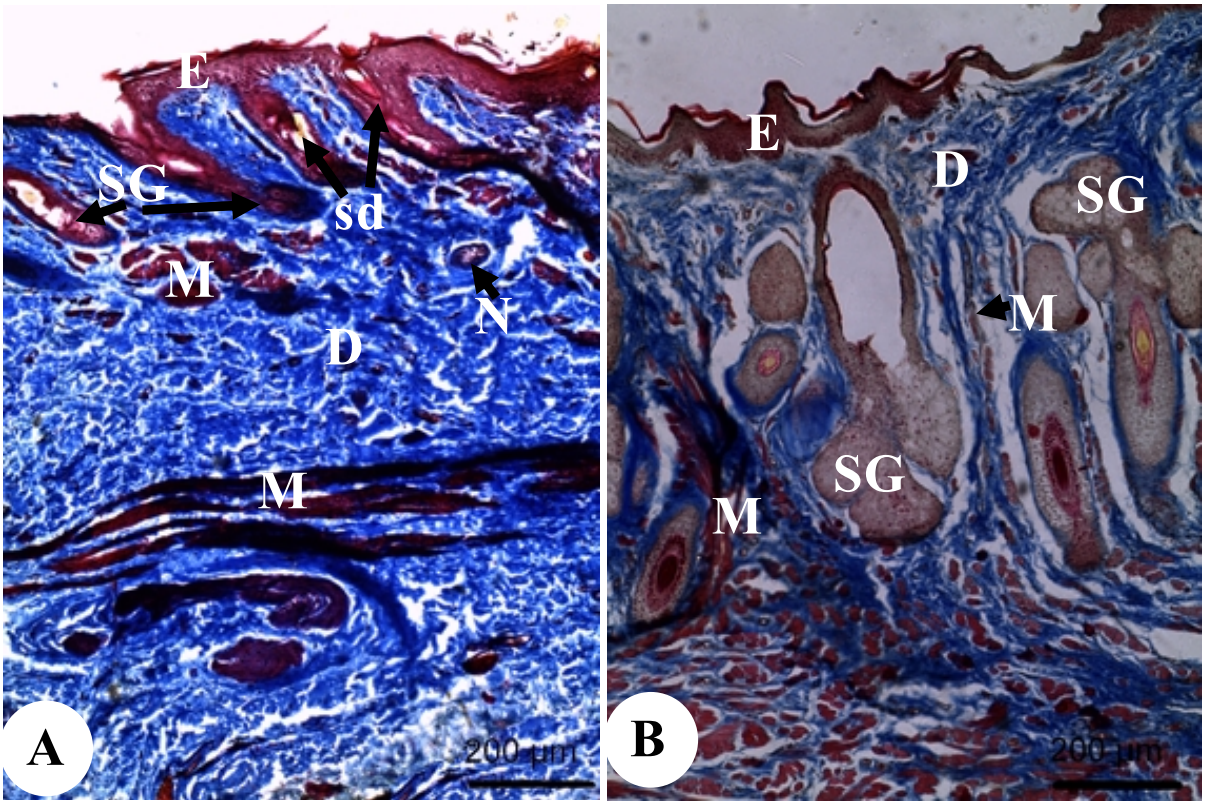


Fig. 15. Light micrographs showing the skin of structure of labial lobes in *Tachyoryctes ibeanus* (A), and *Heterocephalus glaber* (B). The size and branching of sebaceous glands in the labial lobes sealing off the oral cavity of *Tachyoryctes ibeanus* and *Heterocephalus glaber* are different. *Tachyoryctes ibeanus* have simple, small, globular sebaceous glands (SG) with their ducts (sd) opening directly to the surface in comparison to the large mutilobular sebaceous glands (SG) in the *Heterocephalus glaber*. Skeletal muscle fibers (M) are associated with sebaceous glands and others traversing dense connective tissue dermis (D) in *Tachyoryctes ibeanus*, but the skeletal muscle fibers are more wide spread in *Heterocephalus glaber*. The epidermal-dermal junction between epidermis (E) and dermis (D) lacked interdigitations in both species. Masson trichrome, Bars = 200µm

3.1.2.3 Vibrissal hairs

The vibrissae in *Tachyoryctes ibeanus and Heterocephalus glaber* are slightly longer than the rest of hairs found on the face. The vibrissal hairs in both animals are located in the nasal, buccal and mandibular region (Fig. 8A-F). The vibrissae, in both species microscopically reveal a typical hair follicle-sinus complex enclosed by dense connective tissue capsule (Fig. 16A-B). However in *Tachyoryctes ibeanus*, a cross section through the sinus hair follicle opening reveals individual hair follicles forming a ring between the thick connective tissue sheath (Fig. 17A-B). Hair follicles forming a ring between the thick connective tissue sheath are lacking in *Heterocephalus glaber*. In both species, the vibrissal hair follicle bearing hair shaft and associated sebaceous glands at this opening is enclosed by the rete pegs formed by adjacent epidermal layer (Fig. 17A-B). In both species, peripheral to the sinus hair follicles are skeletal muscle fibres (Fig. 16A-B, 17A-B).

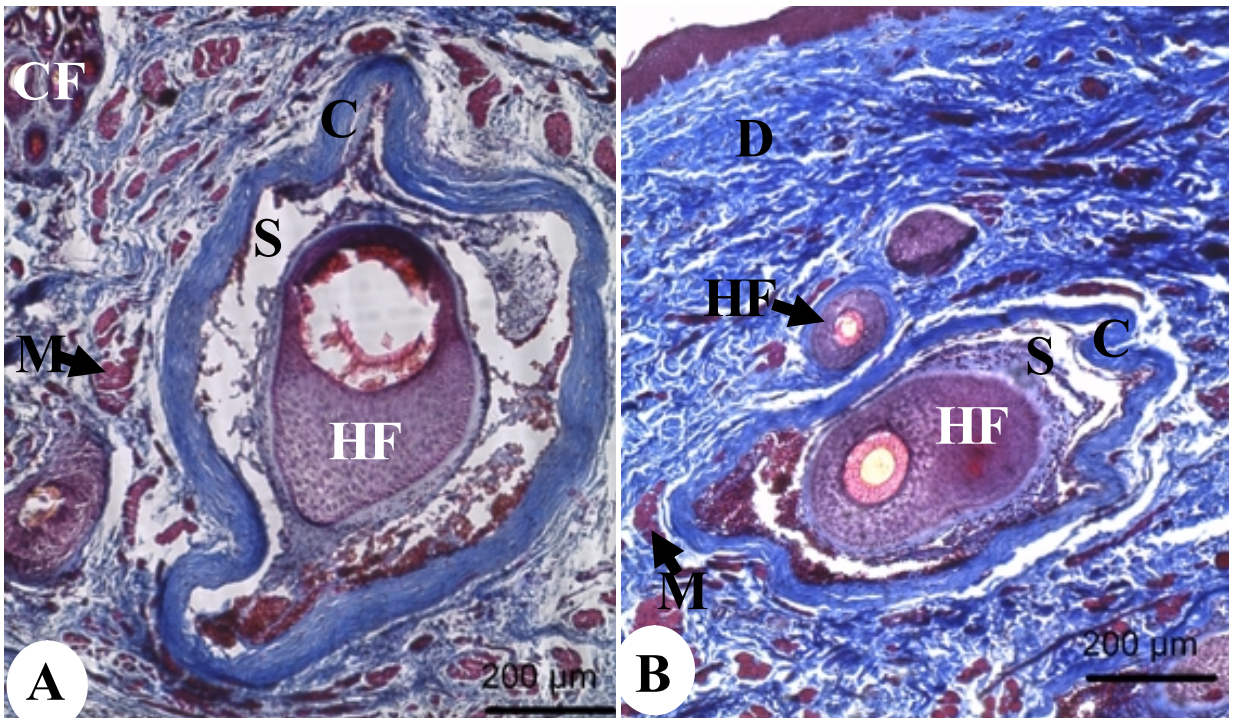


Fig. 16. Light micrographs of sinus hair follicle at the level of cavernous sinus in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Vibrassal hair follicles are structurally similar in *Tachyoryctes ibeanus* and *Heterocephalus glaber*. The sinus hair follicle is composed of connective tissue sheath (C), cavernous sinus (S) containing blood and hair follicle (HF). Peripheral to the hair follicle in the dermis is the skeletal muscle fibers (M). CF-Compound hair follicle, D- dermis. Masson trichrome, Bars = 200µm

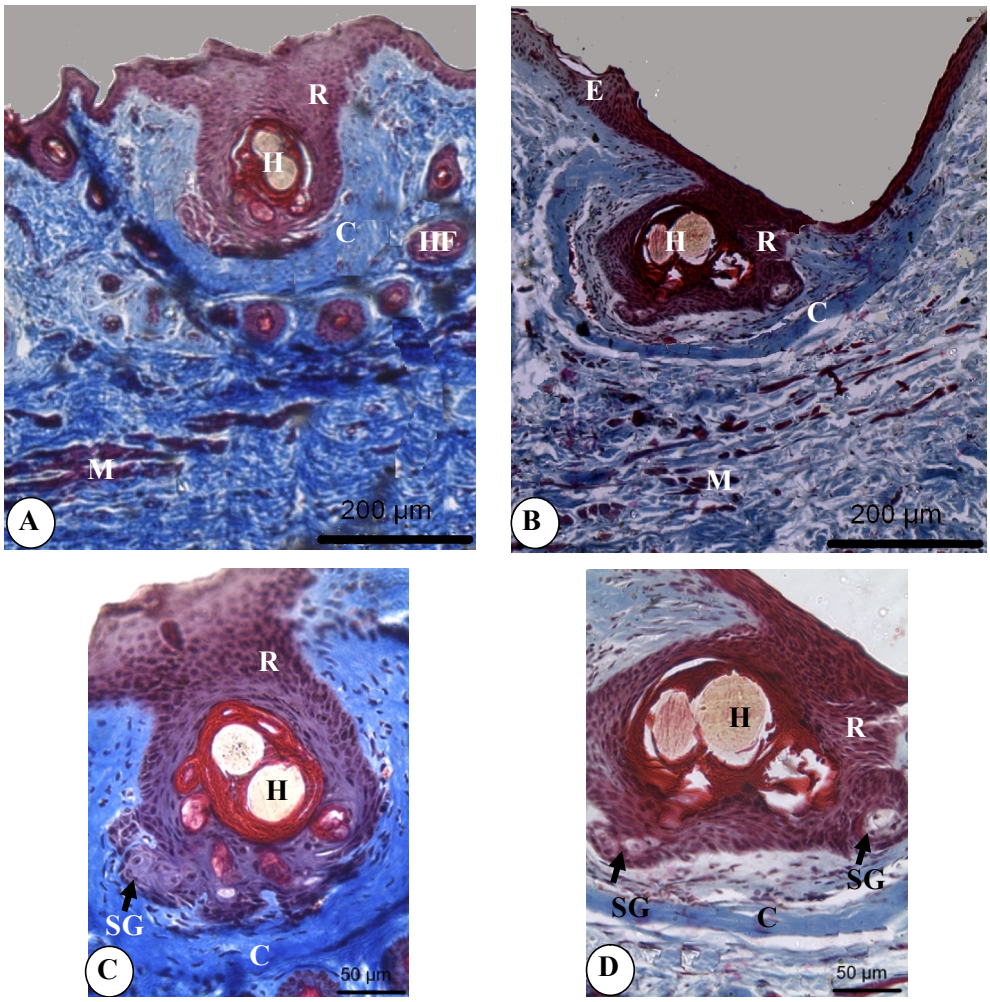


Fig. 17. Light micrographs of vibrissal hairs at the opening of vibrissal follicles in *Tachyoryctes ibeanus* (A and C) and *Heterocephalus glaber* (B and D). The vibrissal hair follicle consist of a thick connective sheath (C), rete peg (R), hair shaft (H) and associated with it is sebaceous glands (SG). Peripheral to the sinus hair follicle in *Tachyoryctes ibeanus is* a ring of hair follicles (HF) and the skeletal muscle fibers (M) while *Heterocephalus glaber* has only skeletal muscle fibers (M). C and D are insets of A and B respectively, Masson trichrome, Bars = 200µm

3.1.2.4 Skin of the cervical region

Skin of cervical region of *Tachyoryctes ibeanus* has a thick pelage like the rest of the body trunk concealing the neck while that of *Heterocephalus glaber* was highly folded (Fig. 18A-B). Both species had a smooth epidermal surface and its epidermal-dermal junction was regular, lacking epidermal and dermal papillae interdigitations (Fig. 19A-B). The dermis was densely packed with collagen, but lacked a clear demarcation into papillary and reticular layer in both species. In *Tachyoryctes ibeanus,* the dermis also contained a cluster of compound hair follicles and sebaceous glands. In the cervical region of both animal species striated skeletal muscle fibres of the panniculus carnosus as a thick and continuous layer and the hypodermis was not well expressed.

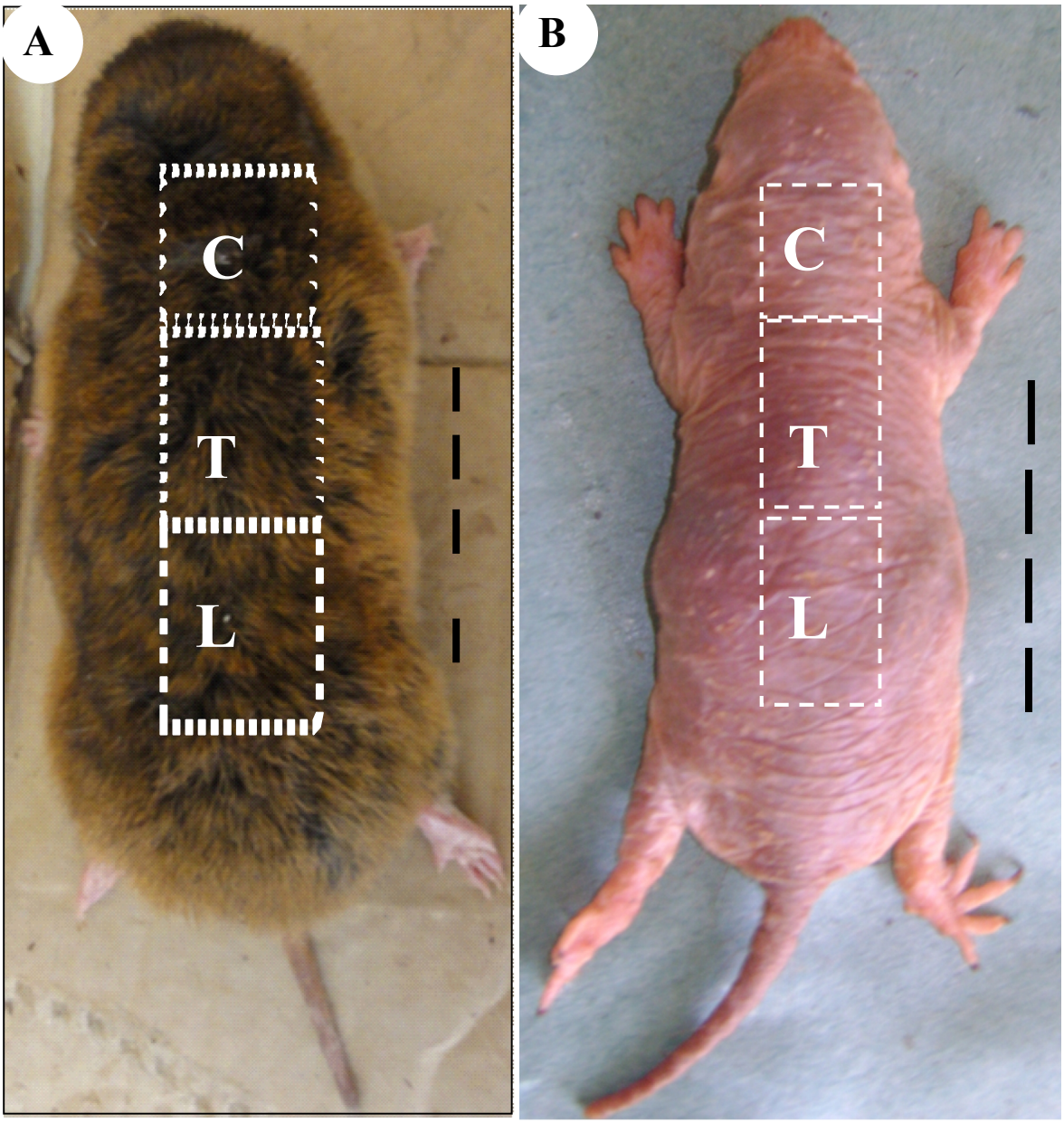


Fig. 18. Macrographs showing the dorsal view of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B) indicating cervical (C), thoracic (T) and lumbar (L) regions. The neck and dorsum of *Tachyoryctes ibeanus* and *Heterocephalus glaber* differ in terms of hair cover and folding. *Tachyoryctes ibeanus* body is covered by a thick pelage while the skin of *Heterocephalus glaber* is naked and highly folded. Bars = 10mm

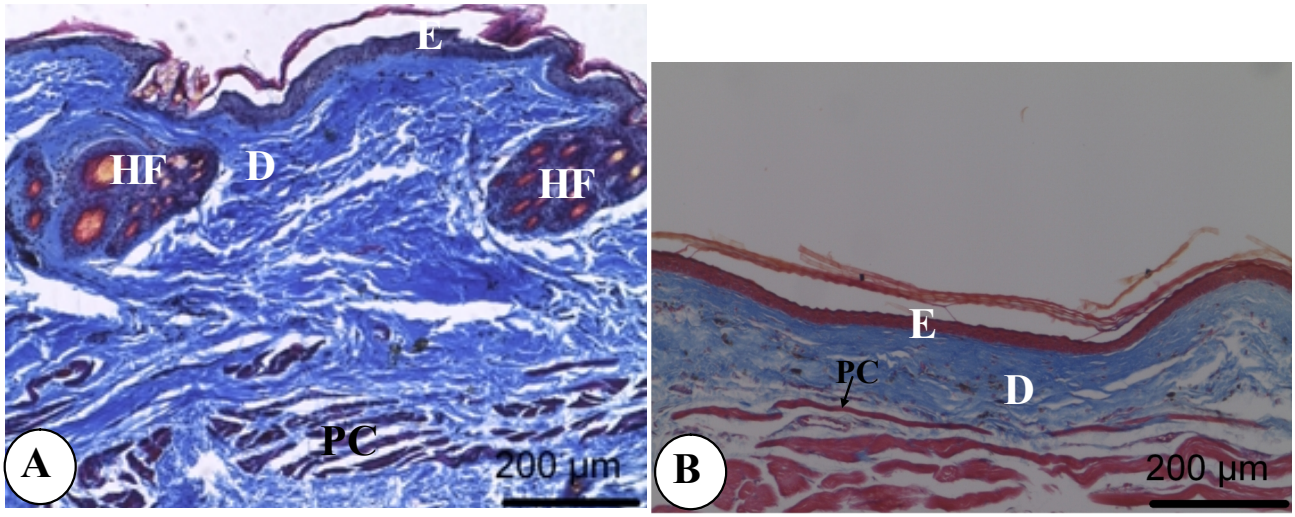


Fig. 19. Light micrographs of the skin at the cervical region in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Cervical region of *Tachyoryctes ibeanus* and *Heterocephalus glaber* have a panniculus carnosus (PC) muscle that forms a thick and continuous layer and lack a distinguishable hypodermis. The skin layers consist of epidermis (E) and dermis (D).The dermis of *Tachyoryctes ibeanus* contains clusters of compound hair follicles (HF). Masson trichrome, Bars = 200µm

3.1.2.5 Skin of dorsum

The dorsal epidermis of *Tachyoryctes ibeanus* had a stratum corneum that easily desquamates. The epidermal-dermal junction of interfollicular skin was regular without epidermal pegs or dermal papillae (Fig. 20A, C and 21A). In contrast, *Heterocephalus glaber* dorsal skin epidermis had a well-formed stratum corneum that firmly attached to the rest of the epidermis unlike that of *Tachyoryctes ibeanus* that was easily observed to desquamate (Fig. 20B, 20D and 21B). Despite *Heterocephalus glaber's* thicker epidermis, the epidermal-dermal junction was also regular without epidermal ridges or dermal papillae (Fig 21B).

Tachyoryctes ibeanus dermis is made up of loosely packed interwoven connective tissue fibres and a few cellular elements (Fig. 20A-B). Ultrastructurally the dermis exhibits large empty extracellular spaces that do not have connective fibres (Fig. 23A). The dermis does not show clear stratification into papillary layer and reticular layer. Collagen is the abundant connective tissue fibre in the dermis and substantial amount of elastic fibres separating individual hair follicles and around blood vessels (Fig. 22A). The abundant cellular elements is the spindle-shaped fibroblasts cells associated with collagen fibres (Fig. 23A). The demarcation of dermis and the hypodermis beneath occurs at point of clustering of individual hair follicles to form a compound hair follicle (Fig. 20A-B).

The *Heterocephalus glabers* dermis, in contrast to the *Tachyoryctes ibeanus's* was densely packed with collagen fibres, revealing less extracellular spaces that were not occupied by collagen fibrils ultrastructurally (Fig. 23B). Higher elastic fibres and fewer cellular elements were observed compared to *Tachyoryctes ibeanus*. The dermis did not show stratification into papillary layer and reticular layer. Blood vessels and nerves were concentrated on the basal dermis and in the adipose tissue.

Hypodermis was the thickest layer of the dorsal skin of the *Tachyoryctes ibeanus.* It was mainly composed of adipose tissue and the loosely organized connective tissue fibres. The hypodermis also contained roots of primary hair follicles (Fig. 20A, 20C and 24A). Adipose tissue of *Heterocephalus glaber* does not form a continuous layer to constitute the *panniculus adiposus* in the hypodermis (Fig. 24B).

Tachyoryctes ibeanus have obliquely set, large and deep rooted primary hair follicles (Fig. 20C and 24A). Each hair follicle is associated with a sebaceous gland. At the level of association with sebaceous gland, 9 – 18 hair follicles cluster together to form a compound hair follicle, from which their various hair shafts emerge through a single external follicular orifice (Fig. 24A). Each compound hair follicle is associated with only one arrector pilli muscle (smooth muscle) that attaches connective tissue matrix to the epidermis. Connective tissue fibers enclose a bundle of compound hair follicles and by extension, separate individual hair follicles (Fig. 25A and 26A). Elastic fibers are abundant around and within a follicular unit in the upper dermis (Fig. 22A). A thin meshwork layer of loose connective tissue and also adipose tissue of the hypodermis surrounds the basal portions of the hair follicles. Across section of the hair follicle reveals, external root sheath, internal root sheath and a hair matrix (Fig. 25C).

Heterocephalus glaber have a few tactile hair follicles that are large, deep rooted extending into the subcutaneous tissues (Fig. 24B and 25D). They are flask shaped, lack a medulla and are associated with sebaceous glands. Together with sebaceous glands, the hair follicles are enclosed by a thick connective tissue sheath. Nerve fibers ramify in this connective tissue sheath and give out longitudinal palisades of lanceolate nerve endings (Fig. 24B, 25B and 26B). Arrector pilli muscles associated with hair follicles of other mammals are lacking in *Heterocephalus glaber* . The tactile hair follicle did not show the usual layering

(internal root sheath and external root sheath not evident) in other mammals, instead they were made up of hair shaft, epithelial cells and a connective tissue sheath (Fig. 24D).

Tachyoryctes ibeanus have small globular sebaceous glands associated with each hair follicle (Fig. 25A and 26A). Sebaceous glands associated with guard hairs are much larger compared to those associated with body hairs (Fig. 26A). *Heterocephalus glaber* have relatively small sebaceous glands associated with tactile hair follicles (Fig. 24B, 25B and 26B). The secretory portion consists of a solid mass of epidermal cells enclosed by connective tissues that blends with the surrounding dermis. Both *Tachyoryctes ibeanus* and *Heterocephalus glaber* lacked sweat glands on the dorsal skin.

In *Tachyoryctes ibeanus,* a dark pigment associated with the hair shafts in the hair follicle was identifiable with Masson-Fontana reducing method for melanin (Fig. 27A). There were no pigments containing cells in either epidermis or connective dermis. *Heterocephalus glaber* had pigment cells manifesting as dark pigments in the middle layer of the dermis (Fig. 27B). Ultrastructurally these cells are spindle-shaped with long cytoplasmic processes extending between the collagen fibers (Fig. 28). The electron dense pigment containing granules are membrane bound. These pigments are distributed in the cytoplasm both around the nucleus and in the processes. In addition, the cytoplasm is drawn into thin tendril like secondary extensions which do not contain the pigment granules.

In *Tachyoryctes ibeanus,* fewer capillaries were located deep in the dermis while smaller microvessels were present superficially in the dermis (Fig. 21A). *Heterocephalus glaber* had more capillaries and larger blood vessels concentrated in the basal layer of the dermis and the hypodermis (Fig. 24B).

Tachyoryctes ibeanus have small nerves bundles comprising the dermal plexus which supply the hair follicles and run between the hair follicle group to supply the epidermis (Fig.

26A). Nerve bundles were readily identifiable in the deeper dermis of *Heterocephalus glaber* and in association with blood vessels and adipose tissue (Fig. 20D). Much larger nerves supplying the tactile hairs and piloneural complexes consisting of large and dense palisades of longitudinal lanceolate endings around the hair follicles and below the sebaceous glands were also identified (Fig. 24B, 25B and 26B).

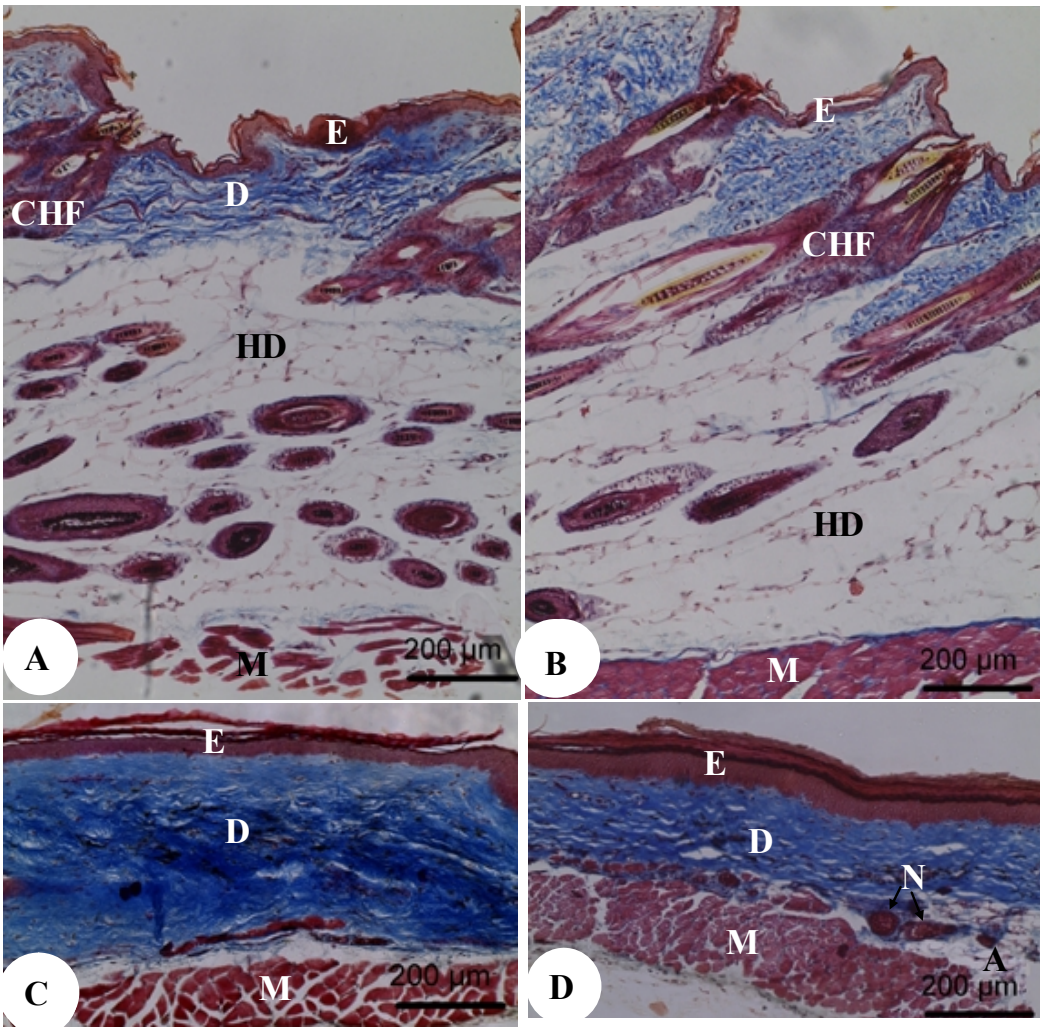


Fig. 20. Light micrographs showing the skin of thoracic and lumbal dorsum in *Tachyoryctes ibeanus* (A and B) and *Heterocephalus glaber* (C and D). The organization of dorsal skin components in *Tachyoryctes ibeanus* and *Heterocephalus glaber* is markedly different. The skin layers in *Tachyoryctes Ibeanus* constitute a thin epidermis (E), loose dermis (D) and a thick hypodermis (HD) in contrast to the thick epidermis (E), densely packed dermis (D) and adipose tissue (A) that does not form a continuous layer to constitute the hypodermis in *Heterocephalus glaber.* Compound hair follicles (CHF) run deep into hypodermis in *Tachyoryctes Ibeanus*. Nerve (N) bundles were evident in lower dermis in *Heterocephalus glaber*. Panniculus carnosus was discontinuous in both species. Masson trichrome, Bars = 200µm

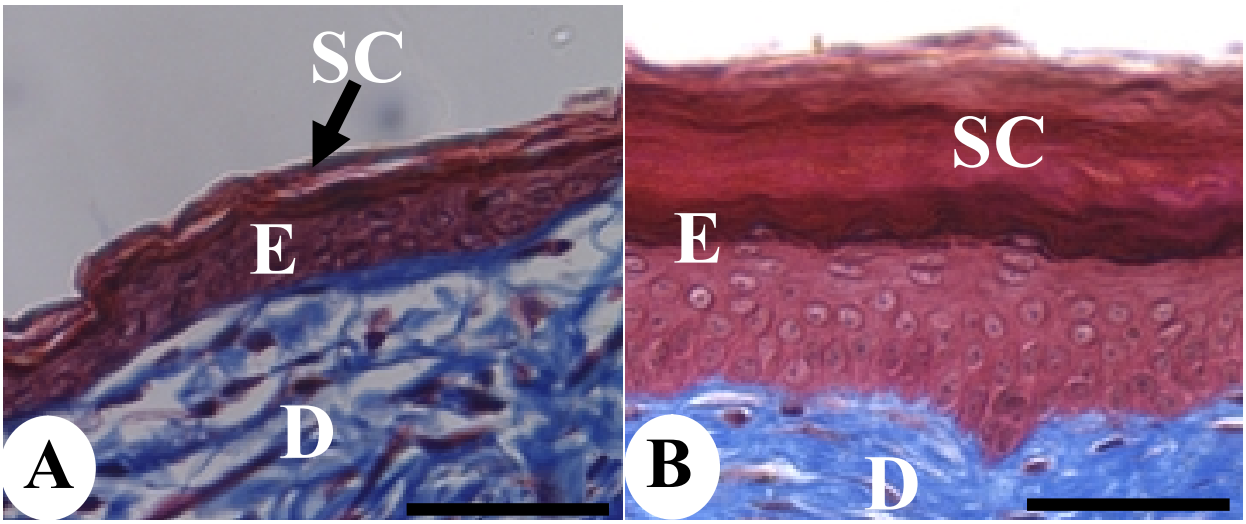


Fig. 21. Light micrographs showing the lumbal dorsum skin in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). *Heterocephalus glaber* has a thicker stratum corneum (SC) that is firmly attached to the rest of epidermis (E) compared to *Tachyorytctes ibeanus.* The dermis (D) is more densely packed in *Heterocephalus glaber* than in *Tachyorytctes ibeanus*. Masson trichrome, Bars = 50µm

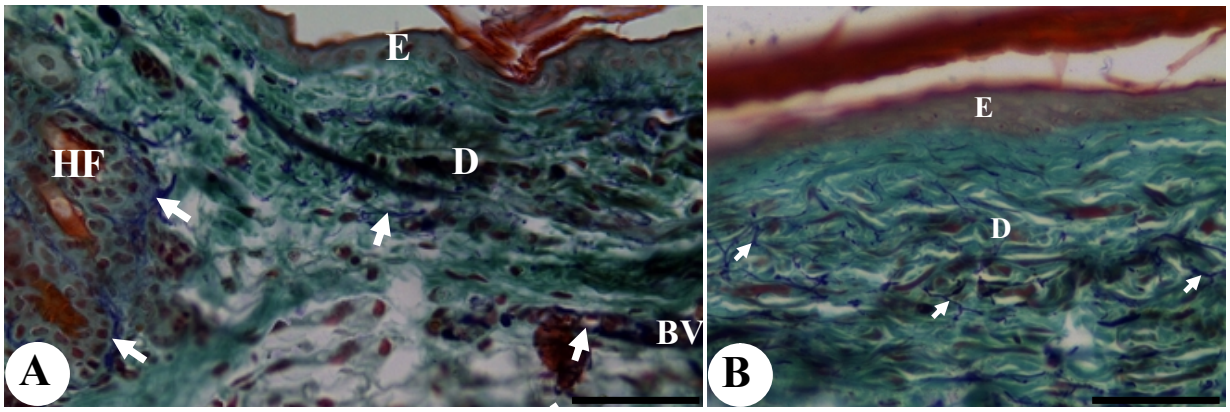


Fig. 22. Collagen and elastic fibers staining of the lumbal dorsum skin in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Collagen fibers (Green) are abundant in both animals. *Tachyoryctes ibeanus* has plentiful amount of elastic fibers (Purple - indicated by the arrow) found separating individual hair follicles and around blood vessels (BV) in comparison to the higher levels and wide spread elastic fibres in *Heterocephalus glaber*. Epidermis (E) and dermis (D). Modified Gomori Aldehyde -Fuchsin, Bars = 50µm

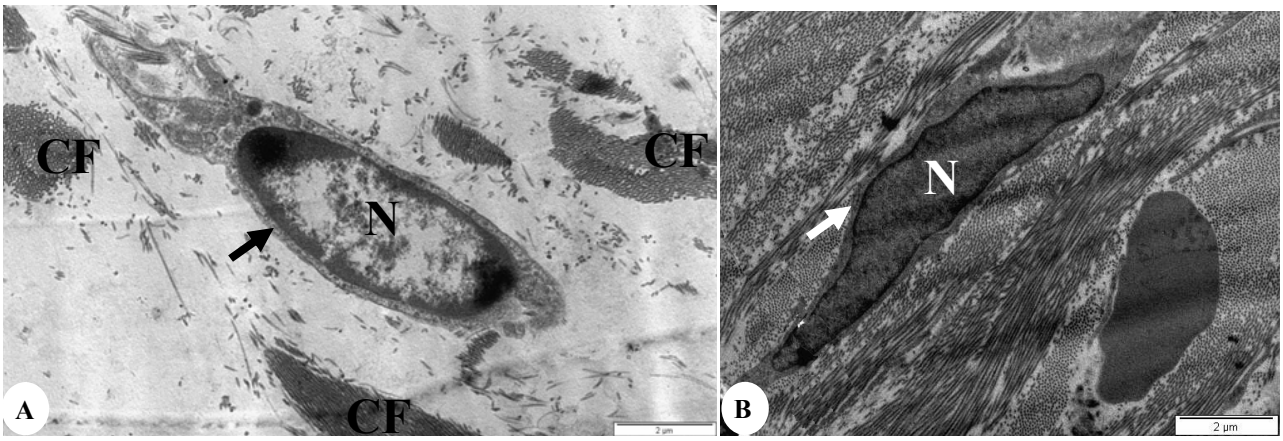


Fig. 23. **Electron micrographs of the dermis of the lumbal dorsum in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Density of extracellular fibres of dermis differs in *Tachyoryctes ibeanus* and *Heterocephalus glaber.*** There large extracellular space peripheral to the fibroblast (indicated by arrow) unoccupied by connective tissue fibres (CF) in *Tachyorytctes ibeanus* compared to the dermis of *Heterocephalus glaber* that is densely packed with collagen. N- denotes nucleus of fibroblast. Bars = 2µm

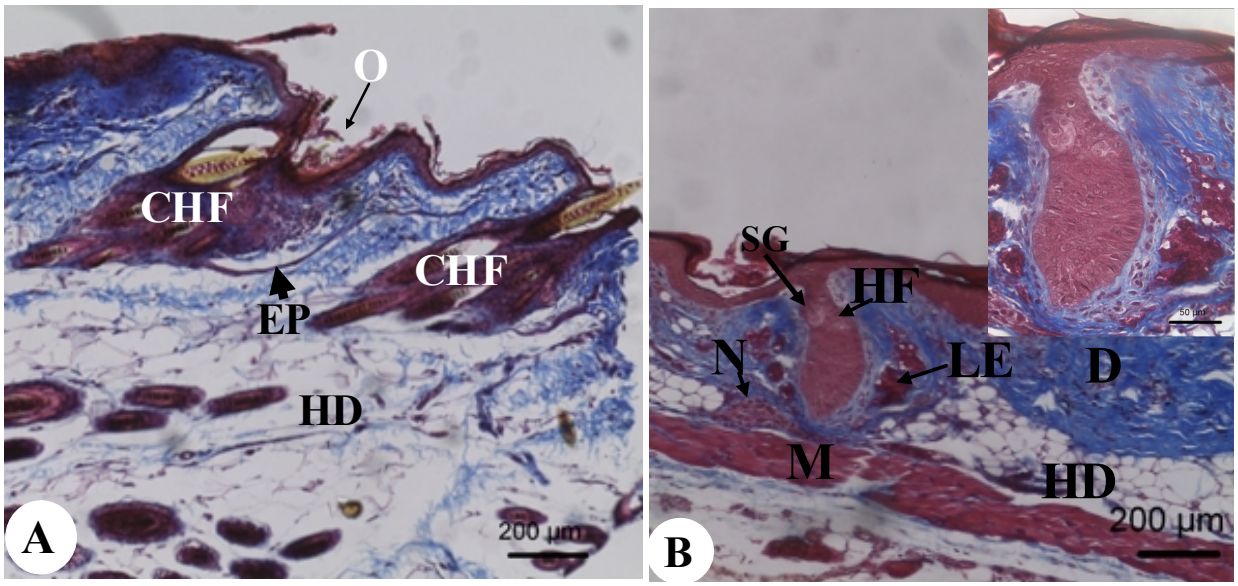


Fig. 24. Light micrographs showing the skin of the dorsum in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Hair follicles structure, number and arrangement in the dermis of *Tachyorytctes ibeanus* and *Heterocephalus glaber* is different. *Tachyoryctes ibeanus* have compound hair follicles (CHF) with a single a errector pilli muscle (EP), with the roots of their hairs extending deep in the hypodermis while their shafts emerge through a single external follicular orifice (O). *Heterocephalus glaber* in contrast has simple, flask-shaped tactile hair follicle (HF) associated with sebaceous glands (SG) extending deep in the dermis but lacking erector pili muscle. The tactile hair follicles of *Heterocephalus glaber* are supplied by a large nerve (N) that ramify in the dermis to give palisades of lanceolate nerve endings (LE) around them. Inset in **(B)** is a higher magnification of the tactile hair follicle. M-Muscle. Masson trichrome, Bars = 200µm

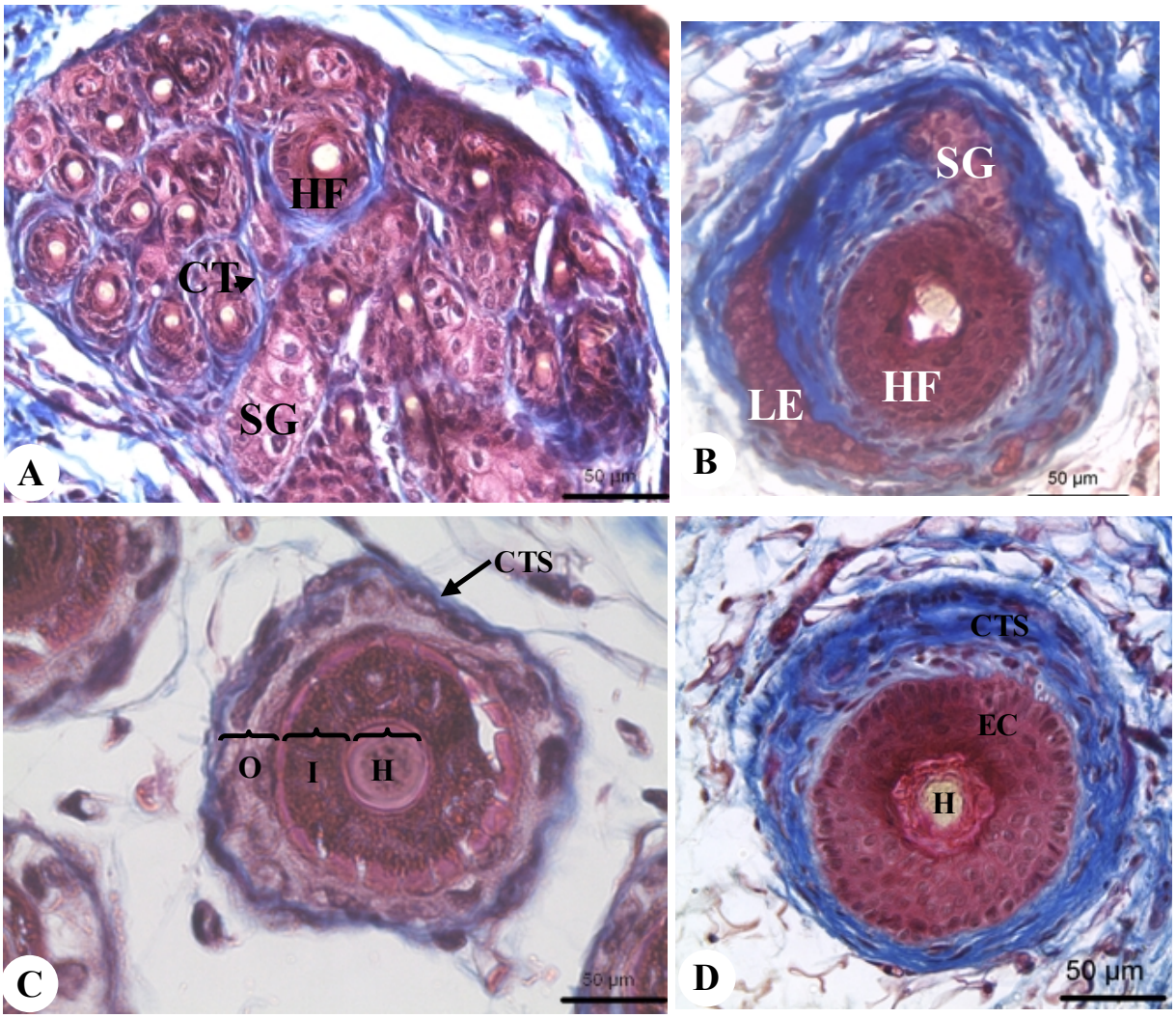


Fig 25. Light micrographs showing cross section of body hairs in *Tachyoryctes ibeanus* (A and C) and *Heterocephalus glaber* (B and D). Hair follicles in *Tachyoryctes ibeanus and Heterocephalus glaber* are associated with sebaceous glands but differ in cross section structure. Each hair follicle (HF) in a compound hair follicle of *Tachyorytctes ibeanus* **(A)** is associated with a sebaceous gland (SG). Individual hair follicles (HF) are separated from each other by connective tissue (CT) and have inner (I) and outer (O) root sheath surrounding the hair (H) **(C)**. Single tactile hair follicles **(B)** of *Heterocephalus glaber* are also associated with sebaceous gland (SG) and are supplied by Lanceolate nerve endings (LE), but lack the typical layers of a root of hair follicle (inner and outer root sheath) **(D)**. CTS- Connective tissue sheath. Masson trichrome, Bars = 50µm

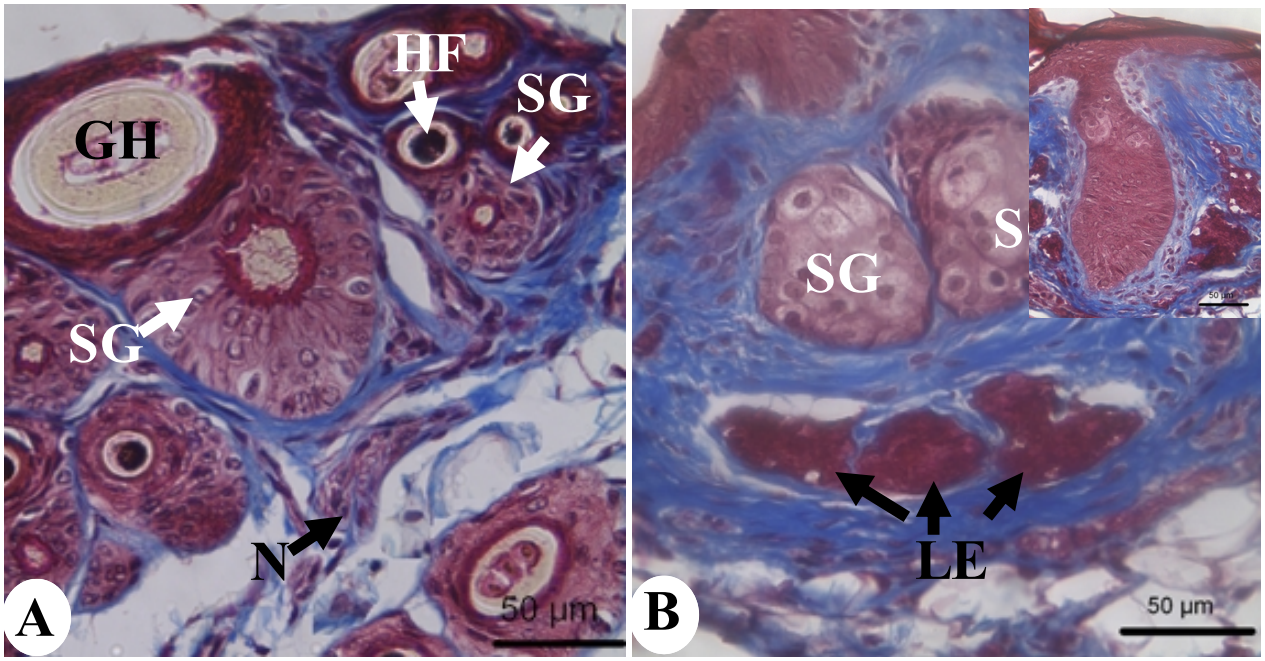


Fig. 26. Light micrographs of the skin of the dorsum showing extensive nerve supply to the hair follicles and sebaceous glands in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). In *Tachyorytctes ibeanus* nerves (N) branches are incorporated to connective tissue fibers to supply the hair follicle (HF), guard hair (GH) and sebaceous glands (SG). In *Heterocephalus glaber* palisades of lanceolate nerve endings (LE) supply tactile hair follicle (B-inset) and two sebaceous glands (SG). The sebaceous glands were revealed from tactile hair follicle (B- inset) by serial sectioning. Masson trichrome, Bars = 50µm

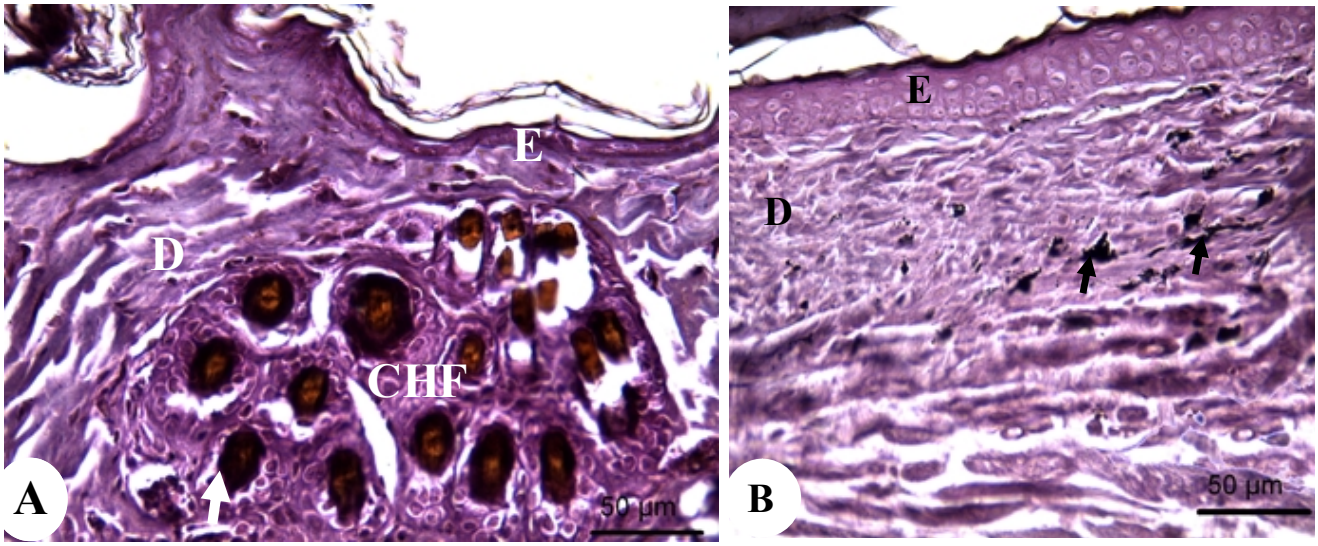


Fig. 27. Light micrographs of dorsal skin sections treated with the Masson-Fontana method for the identification of melanin in *Tachyoryctes ibeanus* (A) *Heterocephalus glaber* (B). *Tachyoryctes ibeanus* has black and dark brown pigments associated with the hair shafts of hair follicle. *Heterocephalus glaber* has dark pigments in middle layer of the dermis (D), Bars = 200µm.

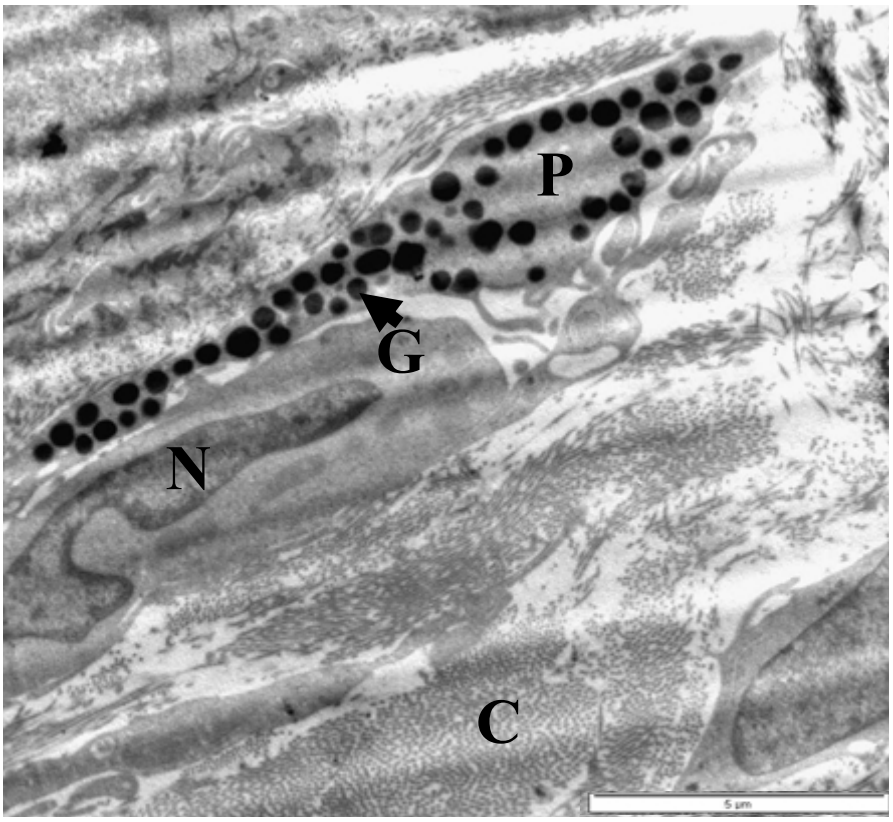


Fig. 28. Electromicrographs showing pigment cells (Melanocytes) in the dermis of *Heterocephalus glaber*. The cell (P) have long cytoplasmic extensions between the collagen fibres of the matrix that harbour numerous oval-shaped melanosomes containing pigment (G) of varying densities. Bars = 5µm

3.1.2.6 Skin of sternal region

The skin of sternal region of *Tachyoryctes ibeanus* and *Heterocephalus glaber* was organized into epidermis and dermis but lacked a distinguishable hypodermis (Fig. 30A-B). The two animal species had a smooth and regular epidermal surface without epidermal pegs and dermal papillae (Fig. 30A-B). The dermis was divided into two parts, a condensed papillary layer and a loosely organized reticular layer made of thick collagen bundles that form criss-crossing wavy pattern running parallel to epidermis (Fig. 30A-B). *Tachyoryctes ibeanus's* dermis contained a compound hair follicle comprising between 9 – 16 hairs. Individual hair follicles were associated with small globular sebaceous glands and were separated from each other by thin layers of connective tissue. Each compound hair follicle was equipped with arrector pili muscle. Neither apocrine nor eccrine sweat glands were found in the skin of the sternal region. Few blood vessels were located at the basal layer of the dermis in *Tachyoryctes ibeanus.*

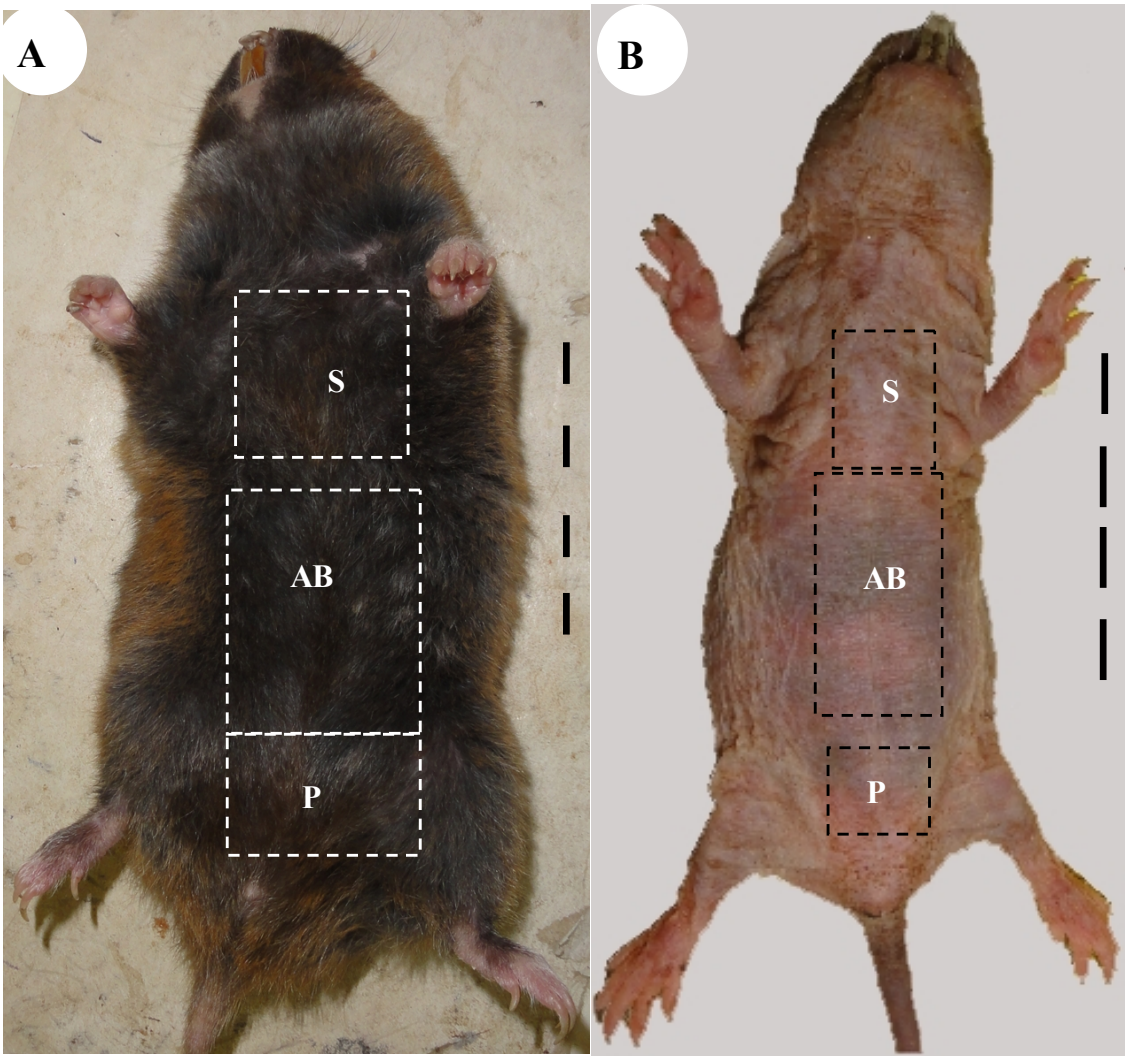


Fig. 29. Macrographs showing the ventral view of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). The skin were derived from Sternal region (S), Abdominal (AB) region and pubic region (P). Bars = 10mm

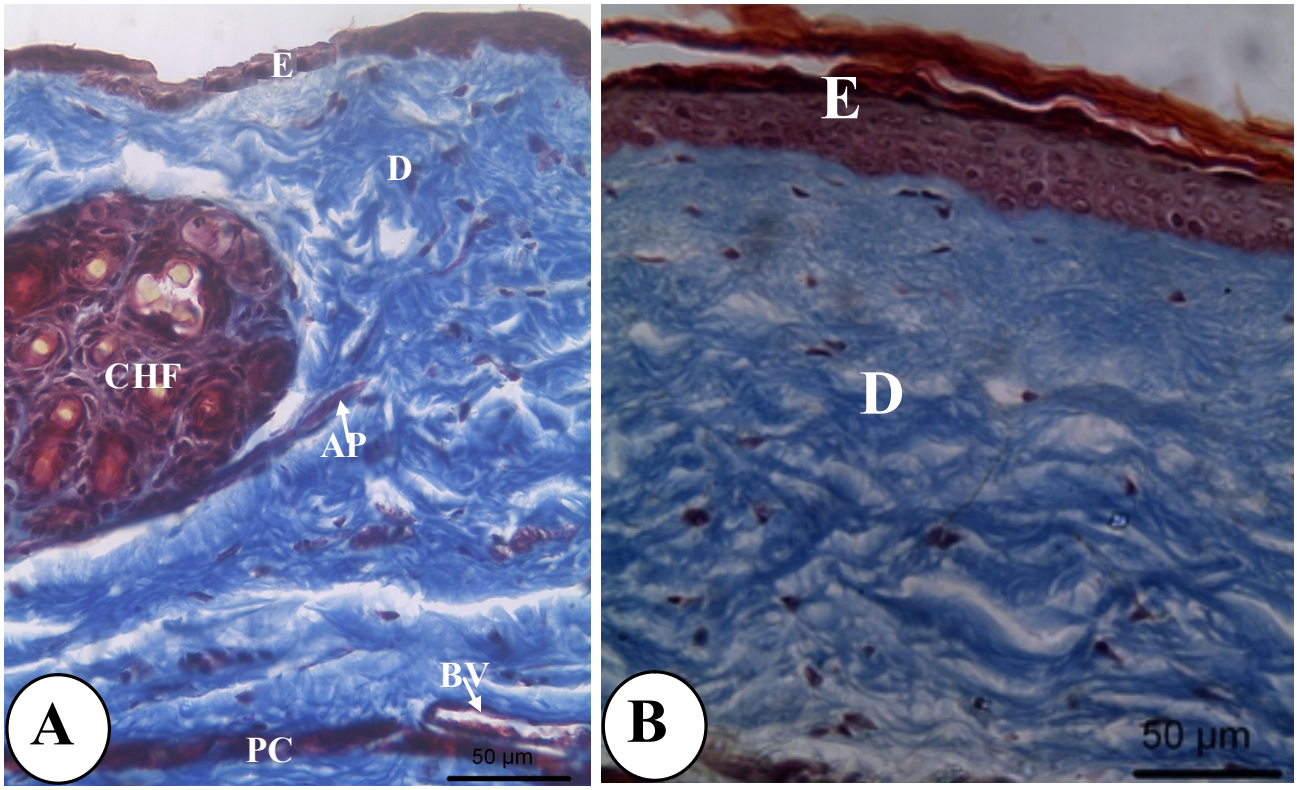


Fig. 30. Light micrographs showing the skin structure of sternal region in *Tachyoryctes ibeanus* (A) and *Heterocehalus glaber* (B). The dermis of both animal species are densely packed with collagen fibres that forms a wavy pattern. The dermis of *Tachyoryctes ibeanus* has compound hair follicle (CHF) associated with errector pili muscle (AP) in upper dermis and paniculus muscle and blood vessel (BV) in the lower dermis. Epidermis (E) and dermis (D). Masson trichrome, Bars = 50µm

3.1.2.7 Skin of Abdominal region

The skin of abdominal region of *Tachyoryctes ibeanus* constitutes a thick epidermis and dermis but lacked a distinguishable hypodermis (Fig. 31A). *Heterocephalus glaber* has a thick epidermis, dermis and a layer of adipose tissue forming the hypodermis (Fig. 31B). Stratum corneum in *Tachyoryctes ibeanus* easily desquamates (Fig. 31A). In contrast *Heterocephalus glaber* has a thick stratum corneum. The epidermal-dermal junction of *Tachyoryctes ibeanus* interfollicular skin showed a lot of undulations which contrasts the smooth border in *Heterocephalus glaber* (Fig. 31A-B). Dermis in both *Tachyoryctes ibeanus* and *Heterocephalus glaber* lacked a clear division into papillary and reticular layers. Nerves and larger blood vessels were located deeper in the dermis of *Tachyoryctes ibeanus*, while smaller blood capillaries traversed the rest of the dermis and get to epidermis. Connective tissue fibers of the basal dermis in *Tachyoryctes ibeanus* blend with striated muscles fibers of abdominal wall.

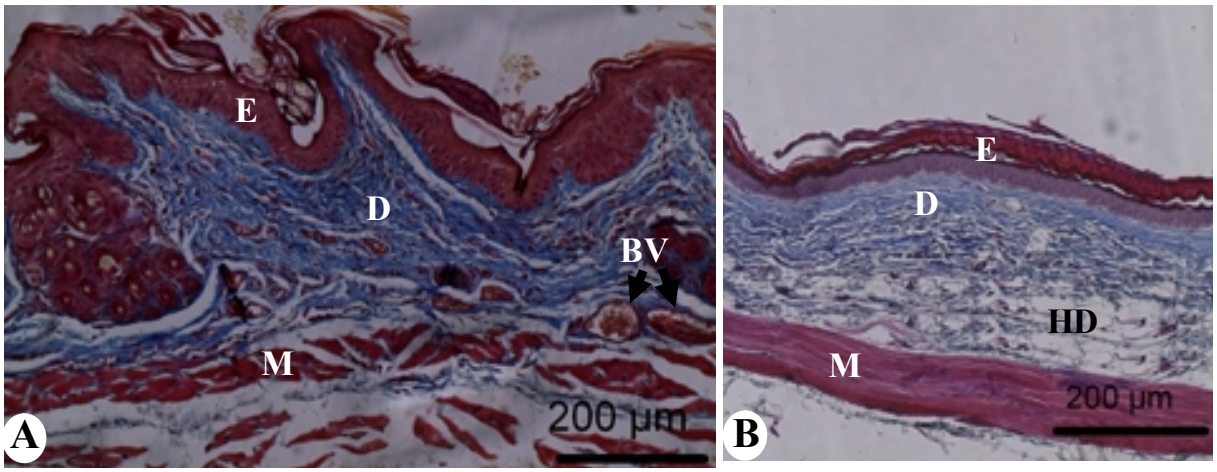


Fig. 31. Light micrographs showing the skin in the abdominal region in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). The skin of the abdominal region in *Tachyoryctes ibeanus* and *Heterocephalus glaber* differ in structure. The skin layers consists of epidermis (E) and dermis (D). *Tachyoryctes ibeanus* has a folded external epidermal surface, thick Epidermis (E) and large blood vessels (BV) and skeletal muscle at basal layer of dermis. *Heterocehalus glaber* epidermis lacks external epidermal surface folding, and have a hypodermis (HD) anchoring the skin on the skeletal muscle (M). Masson trichrome, Bars =200µm

3.1.2.8 Skin of pubic region/groin

Both *Tachyoryctes ibeanus* and *Hetercephalus glaber* had a smooth epidermal-dermal junction , lacking epidermal pegs and dermal papillae and a dermis that does not show clear division into papillary and reticular layer (Fig. 32A-B).The deeper aspect of the dermis in *Tachyoryctes ibeanus* contained striated skeletal muscle fibers and apocrine sweat glands (Fig. 32A). The deeper layer of the dermis in *Hetercephalus glaber* contained blood vessels and characteristic large multilobular sebaceous glands but sweat glands were lacking (Fig. 32B).

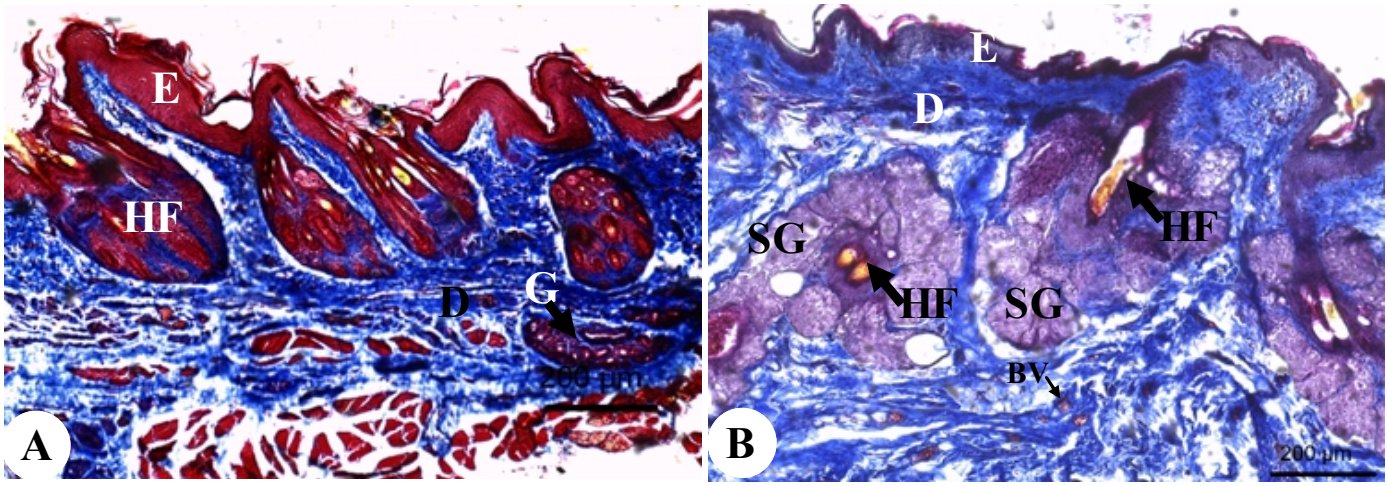


Fig. 32. Light micrographs showing the skin structure of the pubic region in *Tachyoryctes ibeanus* (A) *Heterocehalus glaber* (B). Skin of pubic region in *Tachyoryctes ibeanus* possesses apocrine sweat glands while *Heterocehalus glaber* has a large multilobular sebaceous gland. Both animals have smooth epidermal-dermal junction, lacking epidermal pegs and dermal papillae. The dermis of *Tachyoryctes ibeanus* contained the compound hair follicles (HF) and sweat glands (G). The characteristic feature in the dermis of *Heterocehalus glaber is* the large multilobular sebaceaous glands (SG). E-Epidermis, D- dermis, BV-blood vessels. Masson trichrome, Bars = 200µm

3.1.2.9 Skin of the palmar surface of feet.

The palmar surfaces of *Tachyoryctes ibeanus* and *Heterocephalus glaber* have a thick hairless epidermis consisting of several cell layers and the thickest being stratum corneum that has a smooth surface (Fig. 33A -B). The epidermal-dermal junction of the palmar surface exhibits a pattern of regular epidermal pegs, fitting between the prominent dermal papillae (Fig. 33 A-B). The dermis is composed of dense connective tissue, blood vessels and nerve fascicles traversed the dermis and extended to dermal papillae (Fig. 33A -B). In *Tachyoryctes ibeanus* the dermal papillae contained the sensory corpuscles and blood capillary loops. Sensory corpuscles were not encountered in dermal papillae of *Heterocephalus glaber*. No glands were identified in the palmar surface of the foot pad in both animals.

3.1.2.10 Skin of the Plantar surface of feet.

The plantar surfaces of *Tachyoryctes ibeanus* and *Heterocephalus glaber* had a similar structure to palmar surface with a thick epidermis, interdigitations at epidermal-dermal junction and a dense connective tissue dermis (Fig. 34A -B). Meissner's corpuscles were easily identifiable in the dermal papillae of *Heterocephalus glaber* (Fig. 34B).

3.1.2.11 Skin of the dorsum of feet

The dorsum of the forefeet and hind feet in *Tachyoryctes ibeanus* and *Heterocephalus glaber* had much reduced epidermal thickness compared with the palmar and plantar surfaces (Fig. 33C-D and 34C-D). *Tachyoryctes ibeanus* lacked epidermal pegs and dermal papillae interdigitations but their dense connective tissue dermis contained hair follicles that occurred singly or in pairs.The dorsum of forefeet in *Heterocephalus glaber* showed shallow epidermal and dermal papillae interdigitations which lacked on the dorsal surface of the hind feet (Fig. 33D and 34D). The dermis of dorsum of hind feet in *Heterocephalus glaber* was more loosely packed and had a rich blood supply compared to forefeet (Fig. 33D and 34D).

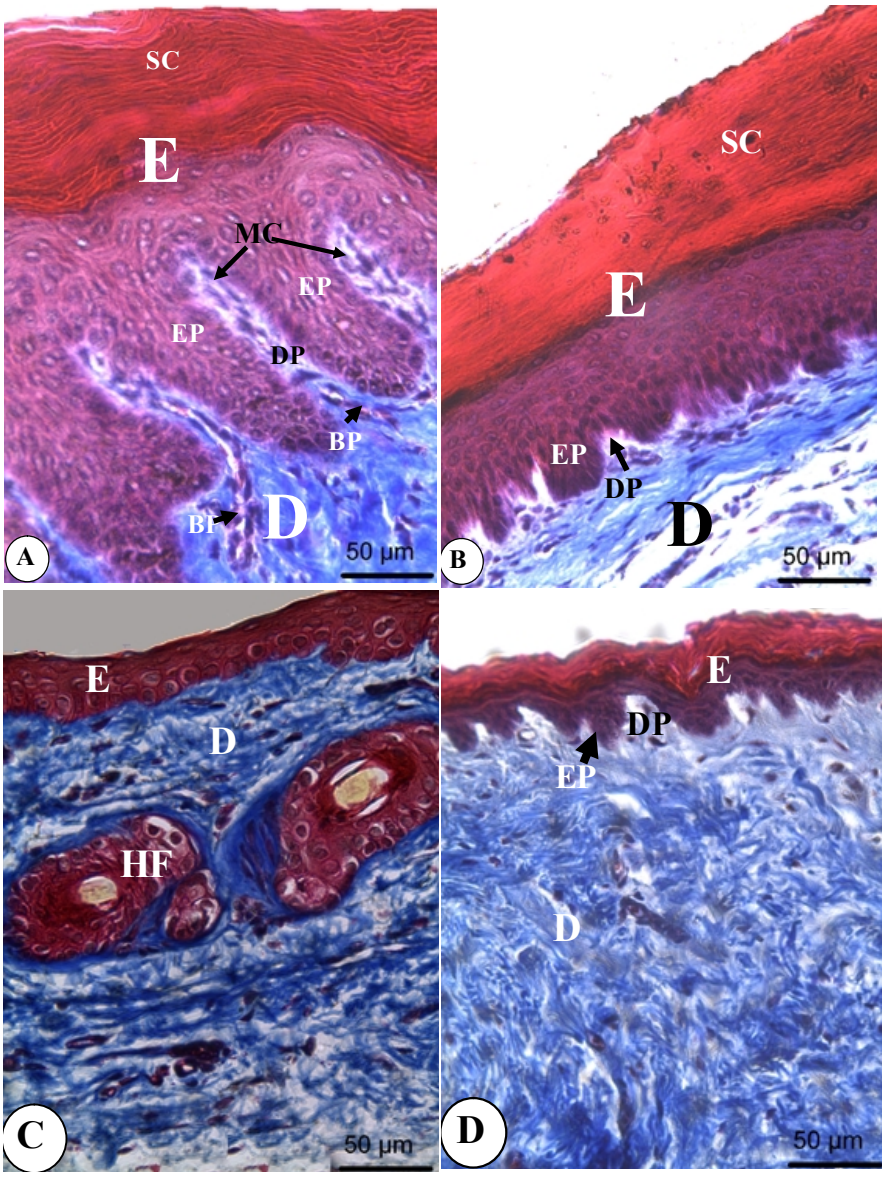


Fig. 33. Light micrographs showing the skin of palmar pads and dorsum of the fore feet in *Tachyoryctes ibeanus* and *Heterocephalus glaber.* The palmar pads have a thick keratinized epidermis and interdigitating epidermal pegs (EP) and dermal papillae (DP) in both *Tachyoryctes ibeanus* **(A)** and *Heterocephalus glaber* **(B).** The dermal papillae of *Tachyoryctes ibeanus* contained Meissners (MC) and blood capillary loop (Bp). The epidermal thickness of dorsum in both *Tachyoryctes ibeanus* **(C)** and *Heterocephalus glaber* **(D)** was thinner compared to the palmar pads, however, *Heterocephalus glaber* had shallow epidermal pegs and dermal papillae interdigitations. The dermis of dorsum of fore feet of *Tachyoryctes ibeanus* contained simple hair follicles (HF). Masson trichrome, Bars = 50µm

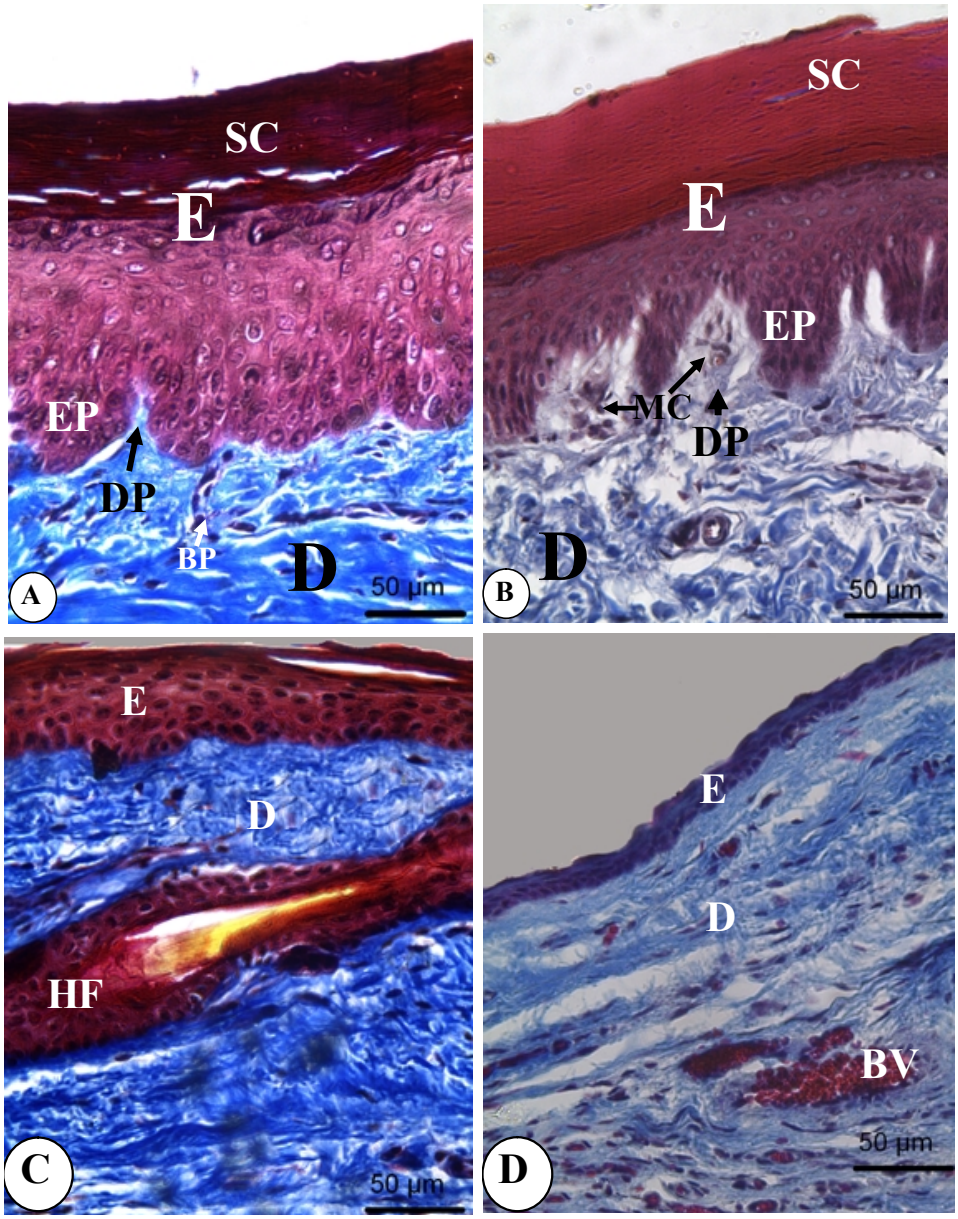


Figure 34. Light micrographs showing the skin of plantar pads and dorsum of the hind feet in *Tachyoryctes ibeanus* and *Heterocephalus glaber.* The plantar pads have a thick keratinized epidermis and interdigitating epidermal pegs (EP) and dermal papillae (DP) in both *Tachyoryctes ibeanus* **(A)** and *Heterocephalus glaber* **(B).** The dermal papillae of *Heterocephalus glaber* contained Meissners corpuscle (MC) and blood capillary loop (Bp). The epidermal thickness of dorsum of forefeet in both *Tachyoryctes ibeanus* **(C)** and *Heterocephalus glaber* **(D)** was thinner compared to the plantar pads. *Tachyoryctes ibeanus* had a dense connective dermis of dorsum of fore feet of contained simple hair follicles (HF) in contrast to the loosely packed dermis of *Heterocephalus glaber* that was in rich in blood supply. Epidermis (E), Dermis (D) and stratum corneum. Masson trichrome, Bars = 50µm

3 .2 Comparative skin morphometry of *Tachyoryctes ibeanus* and *Heterocephalus glaber*.

Figure 35 and table 1, shows the total skin thickness at various regions of the body in *Tachyoryctes ibeanus* and *Heterocephalus glaber*. Total skin thickness in *Tachyoryctes ibeanus* ranged from 962.27 ± 11.35 µm (thoracic dorsum) to 281.25 ± 69.80 µm (sternal) compared to 962.66 ± 28.36 µm (labial lobes) and 269.03 ± 5.06 µm (sternal) in *Heterocephalus glaber*. The overall skin thickness was greater in all regions in the body in *Tachyoryctes ibeanus* (Rhinarium 566.25 ± 8.21 µm, Neck 691.19 ± 9.10 µm, Thoracic 962.27 ± 11.35 µm, Lumbar 973.81 ± 80.27 µm, Sternal 281.25 ± 69.80 µm, Abdominal 405.39 ± 10.51 µm, Pubic 646.97 ± 17.69 µm, Palmar 558.14 ± 16.87 µm and Plantar 481.17 ± 14.79 µm) compared to (Rhinarium 536.88 ± 7.78 µm, Neck 303.89 ± 13.95 µm , Thoracic 367.58 ± 9.63 µm , Lumbar 333.29 ± 6.62 µm , Sternal 269.03 ± 5.06 µm , Abdominal 267.11 ± 6.49 µm , Pubic 599.93 ± 19.98 µm, Palmar 481.13 ± 14.73 µm and Plantar 433.02 ± 13.37 µm) in *Heterocephalus glaber* except at labial lobes. Significant difference was noted in the neck, thoracic, lumbar and abdominal regions. The skin of *Heterocephalus glaber* was only significantly thicker at labial lobes (962.66 ± 28.36 µm) compared to the same region (661.25 ± 21.23 µm) in *Tachyoryctes ibeanus.*

Table 1: Comparison of skin thickness at different cutaneous sites *Tachyoryctes ibeanus* and *Heterocephalus glaber*

	Rhinarium	Labial lobes	Neck	Thoracic	Lumbar	Sternal	Abdominal	Pubic	Palmar	Plantar
T. ibeanus	566.25 ± 8.21	661.25 ± 21.23	691.19 ± 9.10	962.27 ± 11.35	973.81 ± 80.27	281.25 ± 69.80	405.39 ± 10.51	646.97 ± 17.69	558.14 ± 16.87	481.17 ± 14.79
H. glaber	536.88 ± 7.78	962.66 ± 28.36	303.89 ± 13.95	367.58 ± 9.63	333.29 ± 6.62	269.03 ± 5.06	267.11 ± 6.49	599.93 ± 19.98	481.13 ± 14.73	433.02 ± 13.37

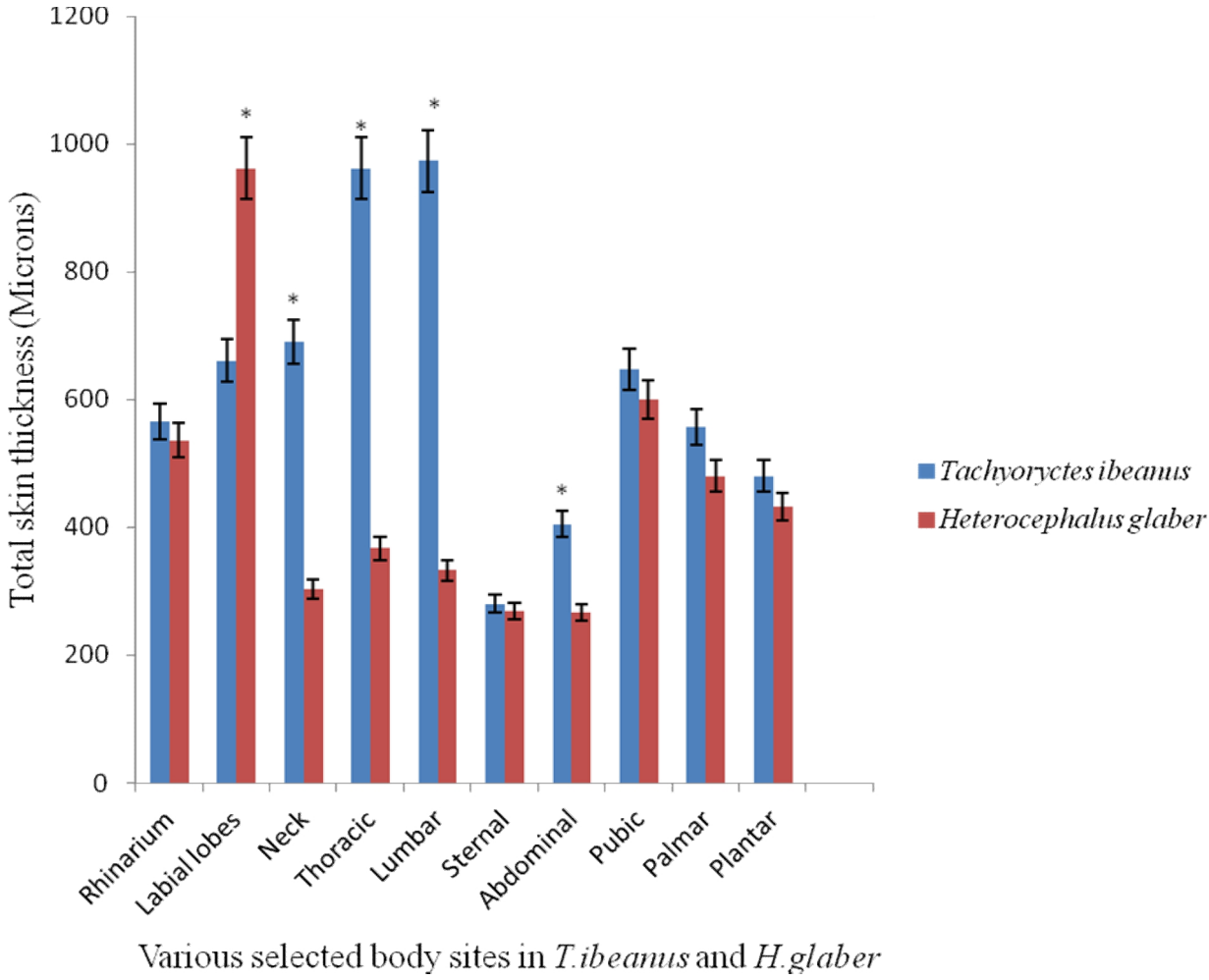


Fig. 35. Graphic representation of mean overall skin thickness in *Tachyoryctes ibeanus* and *Heterocephalus glaber* of skin samples of various regions. The skin was significantly thicker (indicated by asterisk*) at neck, thoracic, lumbar and abdominal region in *Tachyoryctes ibeanus* compared to *Heterocephalus glaber*. The skin was only significantly thicker (indicated by asterisk *) in *Heterocephalus glaber* at the labial lobes. Data represented as mean ± S.e.m at $P > 0.05$.

The average epidermal thickness varied in the various regions of the body in the two species (Fig. 36 and table 2). Epidermal thickness in *Tachyoryctes ibeanus* range from 212. 94 ± 12.27 µm (palmar pads) to 29.36 ± 1.39 µm (Sternal) compared to 220.04 ± 9. 13µm (palmar pads) and 45.25 ± 3.78 µm (Pubic) in *Heterocephalus glaber*. The epidermis was significantly thicker in Rhinarium 155.27 ± 10.21 µm, labial lobes 61.12 ± 3.33 µm, abdominal 98.59 ± 3.13 µm and pubic region 91.35 ± 5.27 µm of *Tachyoryctes ibeanus* compared to Rhinarium 122.63 ± 13.66 µm, labial lobes 45.33 ± 3.69 µm, abdominal 77.05 ± 2.08 µm and pubic region 45.25 ± 3.78 µm of *Heterocephalus glaber* .Conversely, the epidermis was significantly thicker in the thoracic dorsum 60.42 ± 3.08 µm, lumbal dorsum 72.55 ± 4.03 µm and sternal region 47.48 ± 1.71 µm of *Heterocephalus glaber* compared to thoracic dorsum 46.76 ± 2.97 µm, lumbal dorsum 46.72 ± 2.35 µm and sternal region 29.36 ± 1.39 µm of *Tachyoryctes ibeanus*.

Table 2: Comparison of epidermal thickness at different cutaneous sites in *Tachyoryctes ibeanus* and *Heterocephalus glaber*

	Rhinarium	Labial lobes	Neck	Thoracic	Lumbar	Sternal	Abdominal	Pubic	Palmar	Plantar
T. ibeanus	155.27 ± 10.21	61.12 ± 3.33	52.18 ± 2.00	46.76 ± 2.97	46.72 ± 2.35	29.36 ± 1.39	98.59 ± 3.13	91.35 ± 5.27	212. 94 ± 12.27	127.51 ± 7.28
H. glaber	122.63 ± 13.66	45.33 ± 3.69	54.89 ± 1.91	60.42 ± 3.08	72.55 ± 4.03	47.48 ± 1.71	77.05 ± 2.08	45.25 ± 3.78	220.04 ± 9. 13	125.27 ± 6.76

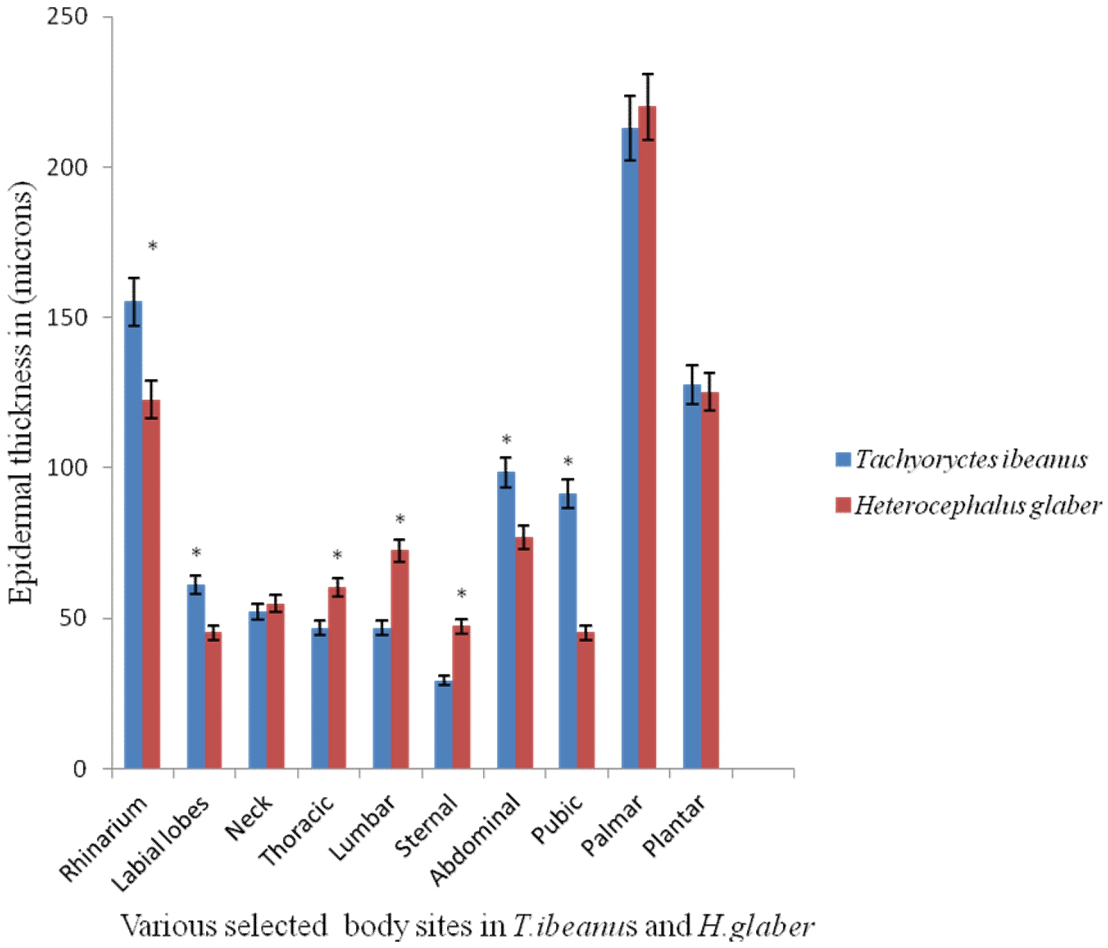


Fig. 36. Graphic representation of mean epidermal thickness in *Tachyoryctes ibeanus* and *Heterocephalus glaber* of skin samples of various regions. The epidermis was significantly thicker [indicated by asterisk (*)] at rhinarium, labial lobes, abdominal and pubic regions in *Tachyoryctes ibeanus* compared to *Heterocephalus glaber.* Significantly thicker [indicated by asterisk (*)] epidermis in *Heterocephalus glaber* was recorded at the thoracic, lumbar and sterna compared to *Tachyoryctes ibeanus.* Data represented as mean ± S.e.m at P > 0.05.

Figure 37 and table 3, shows the average stratum corneum thickness at various regions of the body in the two species. Stratum corneum thickness of *Tachyoryctes ibeanus* range from 110.03 ± 9.12 µm (Palmar pads) to 15.84 ± 1.69 µm (Sternal) compared to 118.21 ± 8.86 µm (Palmar pads) and 19.04 ± 1.77 µm (Sternal skin) of *Heterocephalus glaber*. Stratum corneum was significantly thicker in the neck 25.75 ± 1.97 µm, thoracic dorsum 29.61 ± 4.03 µm , lumbar dorsum 38.19 ±2.11 µm and abdominal regions 47.23 ± 1.80 µm of *Heterocephalus glaber* compared to neck 21.18 ± 1.55 µm, thoracic dorsum 20.75 ± 2.85 µm, lumbar dorsum 18.18 ±1.08 µm and abdominal regions 22.89 ± 2.21 µm of *Tachyoryctes ibeanus.* Stratum corneum was only significantly thicker in the rhinarium (63.59 ± 3.28 µm) of *Tachyoryctes ibeanus* compared to 40.41 ± 2.76 µm of *Heterocephalus glaber.*

Table 3: Comparison of stratum corneum thickness at different cutaneous sites in *Tachyoryctes ibeanus* and *Heterocephalus glaber*

	Rhinarium	Labial lobes	Neck	Thoracic	Lumbar	Sternal	Abdominal	Pubic	Palmar	Plantar
T. ibeanus	63.59 ± 3.28	20.12 ± 1.90	21.18 ± 1.55	20.75 ± 2.85	18.18 ±1.08	15.84 ± 1.69	22.89 ± 2.21	23.94 ± 2.30	110.03 ± 9.12	72.02 ± 8.04
H. glaber	40.41 ± 2.76	20.64 ± 2.12	25.75 ± 1.97	29.61 ± 4.03	38.19 ±2.11	19.04 ± 1.77	47.23 ± 1.80	22.54 ± 2.09	118.21 ± 8.86	74.14 ± 7.65

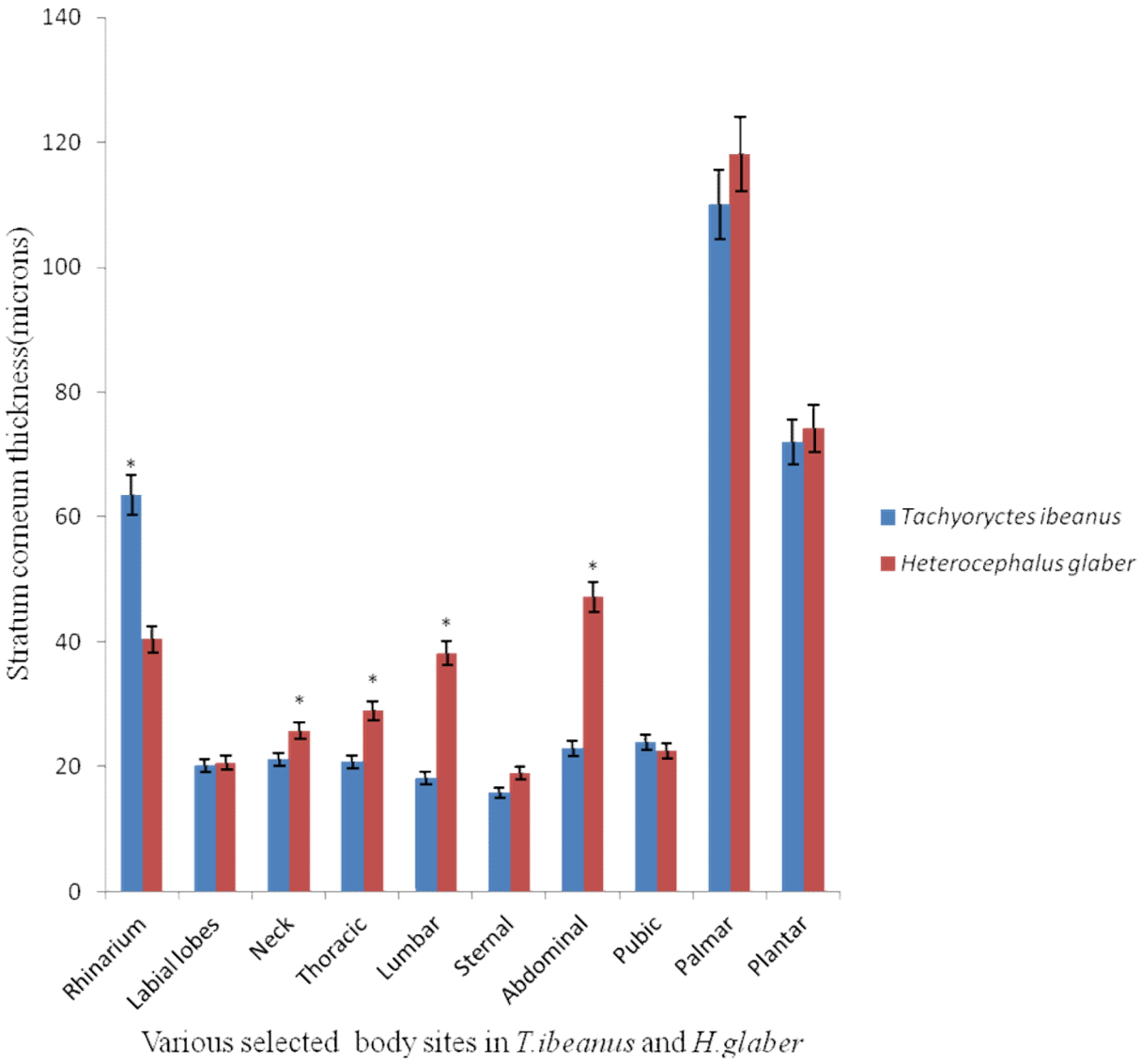


Fig. 37. Graphic representation of mean stratum corneum thickness in *Tachyoryctes ibeanus* and *Heterocephalus glaber* of skin samples of various regions. Stratum corneum was significantly thicker [indicated by asterisk (*)] at neck, thoracic, lumbar and abdominal region in *Heterocephalus glaber* compared to *Tachyoryctes ibeanus.* Stratum corneum was only significantly thicker [indicated by asterisk (*)] at the rhinarium of *Tachyoryctes ibeanus.* Data represented as mean ± S.e.m at $P > 0.05$.

The mean length of epidermal pegs in rhinarial (60.42 ± 5.11 μm), palmar (81.65 ± 5.57 μm) and plantar pads (77.33 ± 4.26 μm) were significantly greater in *Tachyoryctes ibeanus* compared to rhinarial (47.63 ± 3.37 μm), palmar (39.54 ± 3.21 μm) and plantar pads (38.95 ± 2.89 μm) of *Heterocephalus glaber* (Fig. 38 and Table 4).

Table 4: Mean length of epidermal pegs in rhinarium, palmar pads and plantar pads in *Tachyoryctes ibeanus* and *Heterocephalus glaber*

	Rhinarium	Palmar	Plantar
Tachyoryctes ibeanus	60.42 ± 5.11	81.65 ± 5.57	77.33 ± 4.26
Heterocephalus glaber	47.63 ± 3.37	39.54 ± 3.21	38.95 ± 2.89

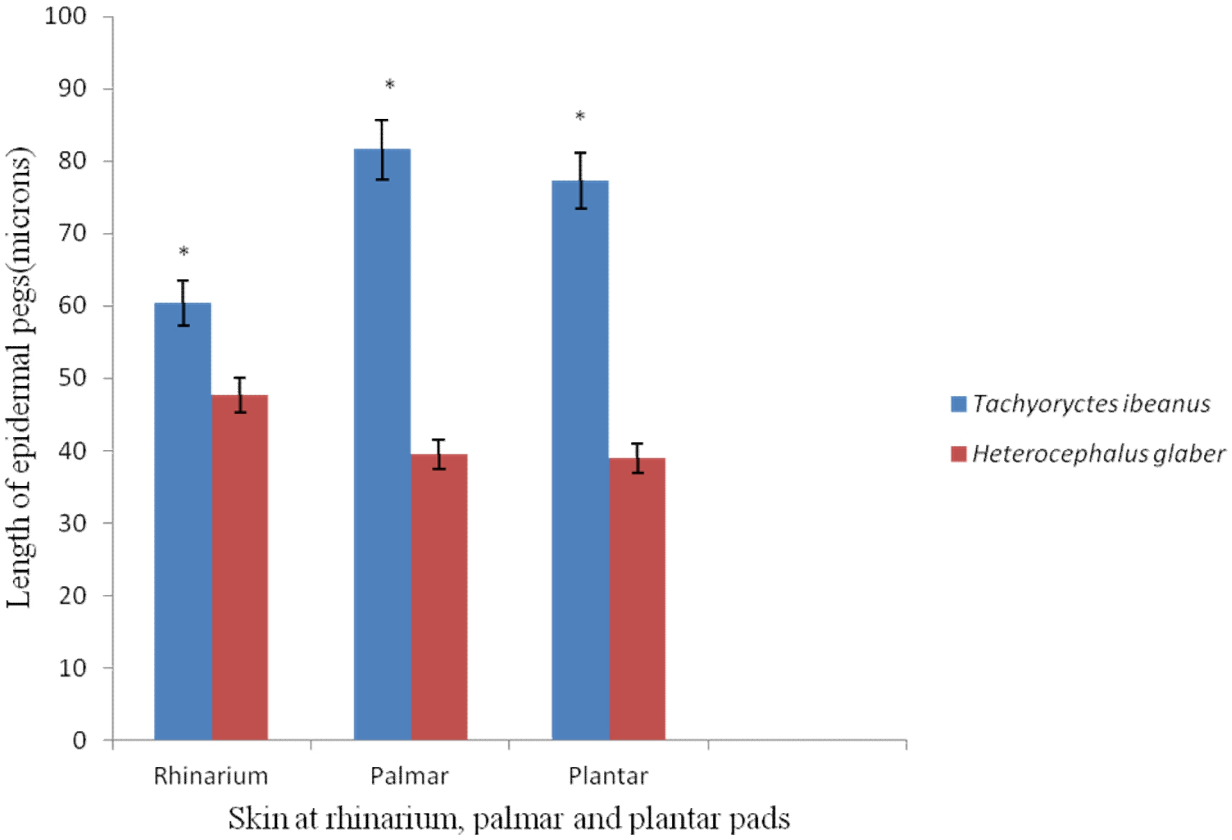


Fig. 38. Graphic representation of mean length of epidermal pegs in *Tachyoryctes ibeanus* and *Heterocephalus glaber* of skin samples of various regions. In *Tachyoryctes ibeanus*, epidermal pegs were significantly longer in rhinarium, palmar and plantar [indicated by asterisk (*)] compared to those of *Heterocephalus glaber.* Data represented as mean ± S.em at $P > 0.05$.

3.3 Comparative wound healing in *Tachyoryctes ibeanus* and *Heterocephalus glaber*

3.3.1 Macroscopic and histological evaluation of wound area

Day 0

Immediately following full thickness excisional wounding in *Tachyoryctes ibeanus*, there was no significant indication of bleeding (n=5) (Fig. 39). In contrast, *Heterocephalus glaber* showed substantial bleeding after wounding, followed by clot formation (n=5) (Fig. 39).

Day 3

Tachyoryctes ibeanus wounds on D3 were covered by pale scabs with smooth surfaces, while *Heterocephalus glaber* had a brown scab, with uneven surfaces and crusty edges (Fig. 39). At a histological level, the wound edges of *Tachyoryctes ibeanus* indicated commencement of re-epithelization under the scab as thin sheets of epidermal cells (Fig. 40A, inset). In *Heterocephalus glaber* wound edge keratinocytes had begun migrating (Fig. 40B, inset). Beneath the migrating epithelium in both species, granulation tissue contained many inflammatory cells that were embedded in the provisional wound matrix leading to a clear demarcation between the wound area and unwounded area.

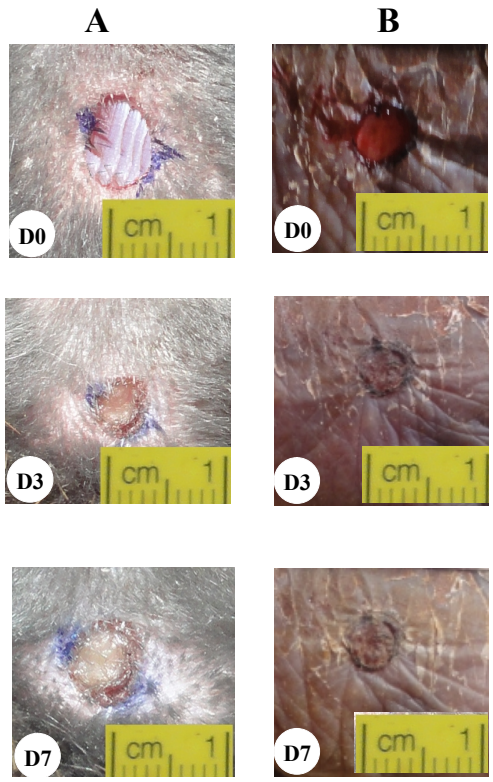


Fig. 39. Macroscopic observations of excisional wounds of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B) at days 0, 3 and 7 after wounding. Following full thickness excisional wounding (D0) with 4mm biopsy punch there was no significant indication of bleeding in *Tachyoryctes ibeanus*. In contrast *Heterocephalus glaber* there was bleeding. The excisional wounds (D3 and D7) then progressed to form pale scabs in *Tachyoryctes ibeanus* and brown scabs in *Heterocephalus glaber.*

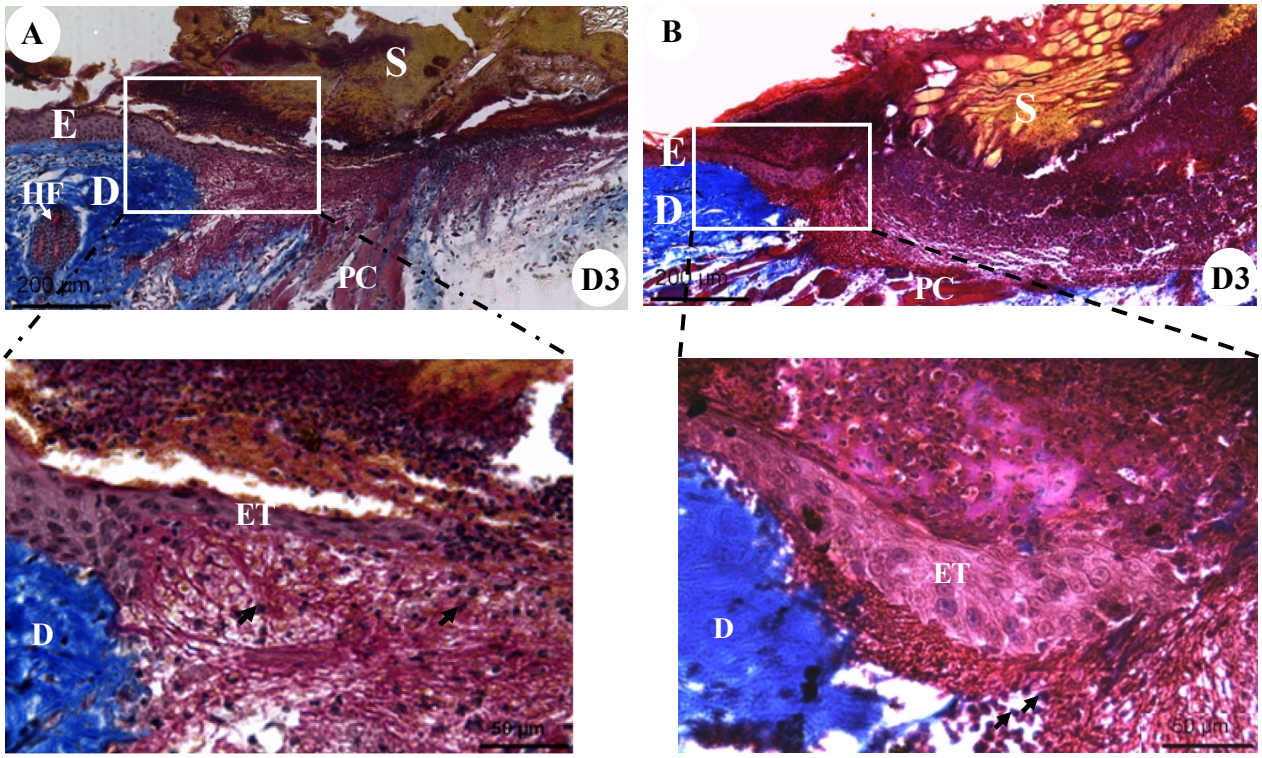


Fig. 40. Light micrographs showing D3 wounds in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). Re-epithelization begins by D3 in both *Tachyoryctes ibeanus* and *Heterocephalus glaber.* Epithelial tongues (ET) formed beneath scabs (S) in both species. There was clear demarcation between the unwounded area of epidermis (E) and dermis (D) from the wound area consisting of epithelial tongues and provisional matrices containing inflammatory cells (Arrows). Hair follicles (HF) of *Tachyoryctes ibeanus* in the unwounded area. PC- Panniculus carnosus muscle. Masson trichrome, Bars = 50µm

Day 7

Although re-epithelialization was complete by D7, scabs still covered the wound bed in both species (Fig. 39 and 41). There was a substantial reduction in leucocyte infiltration, an increase in fibroblast recruitment, new blood vessels and newly synthesized collagen in D7 wounds of *Tachyoryctes ibeanus* compared to D3 wounds (Fig. 41). Comparing D7 wounds of *Heterocephalus glaber* to *Tachyoryctes ibeanus,* I observed higher leucocyte numbers, though not as great as D3 wounds. Due to ongoing inflammation, the wounded area could easily be demarcated from the unwounded area in *Heterocephalus glaber* (Fig. 41). Below the layer of leucocytes infiltrate, some collagen and large new blood vessels were found resting on the subcutaneous layer in *Heterocephalus glaber* (Fig. 41).

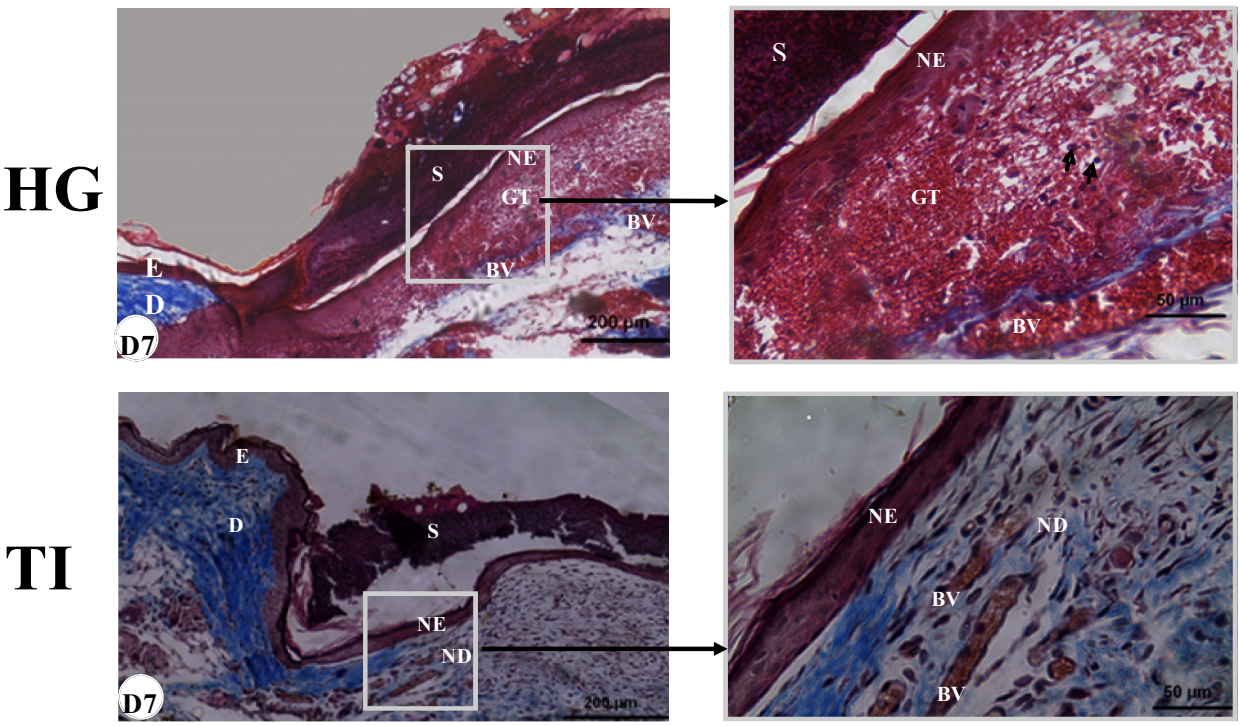


Fig. 41. Light micrographs showing day 7 wounds in *Tachyoryctes ibeanus* (TI) and *Heterocephalus glaber* (HG). The forming dermis of Day 7 wounds in *Tachyoryctes ibeanus* and *Heterocephalus glaber* had marked differences. Both wounds were covered by a scab (S) and epidermal closure was complete to constitute the new epidermis (NE). New dermis (ND) matrix remodelling had advanced more in *Tachyoryctes ibeanus* with very high fibroblast cells proliferation for laying new collagen contained in granulation tissue (GT) compared to *Heterocephalus glaber* which had higher inflammatory cells infiltrate. BV-Blood vessels, D-dermis, E-Epithelium. Masson trichrome Bar =200µm

Day 14

Scabs in *Tachyoryctes ibeanus* were shed between D12 – 14 post wounding (Fig. 42). Histologocally, a regular and thin epithelium devoid of ingrowths was evident (Fig. 43). The completion of epidermal differentiation was confirmed by formation of stratum corneum. There was more collagen deposition in the whole wound gap and the number of fibroblast and leucocyte cells were markedly reduced compared to D7 wounds (Fig. 43). Collagen fibers were horizontally organized. Very few strands of elastic fibres were evident. There was no sign of hair follicle or sebaceous gland regeneration.

By D14, *Heterocephalus glaber* still possessed a dark scab raised above the skin (Fig. 42). Detachment of the scab occurred between D21 – 24 post wounding. The epidermis was thicker in *Heterocephalus glaber* compared to *Tachyoryctes ibeanus* above the wound bed similar to unwounded skin (Fig. 43). The number of fibroblast cells and newly synthesized collagen fibers in *Heterocephalus glaber* had increased compared to D7 wounds (Fig. 43).

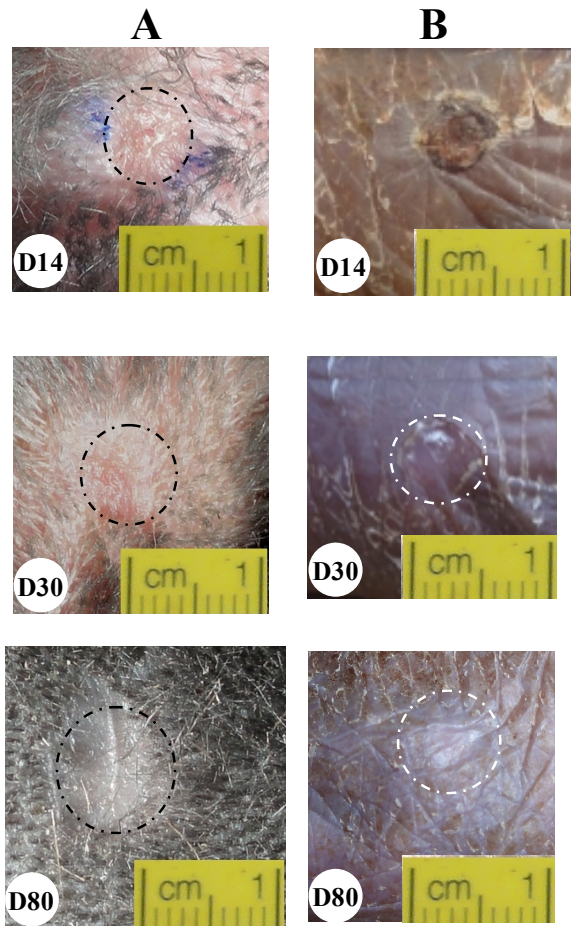


Fig. 42. Macroscopic observations of excisional wounds of *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B) at days 14, 30 and 80 after wounding. *Tachyoryctes ibeanus* shed there scabs by day 14 after wounding and they progressed to form hairless area (D30) and eventually a stable white scar without hair as observed by day 80. In contrast, *Heterocephalus glaber* scab detachment occurs between days 21-24 after wounding and progresses to form a less prominent scar (D30 and D80) when compared to the rest of the skin.

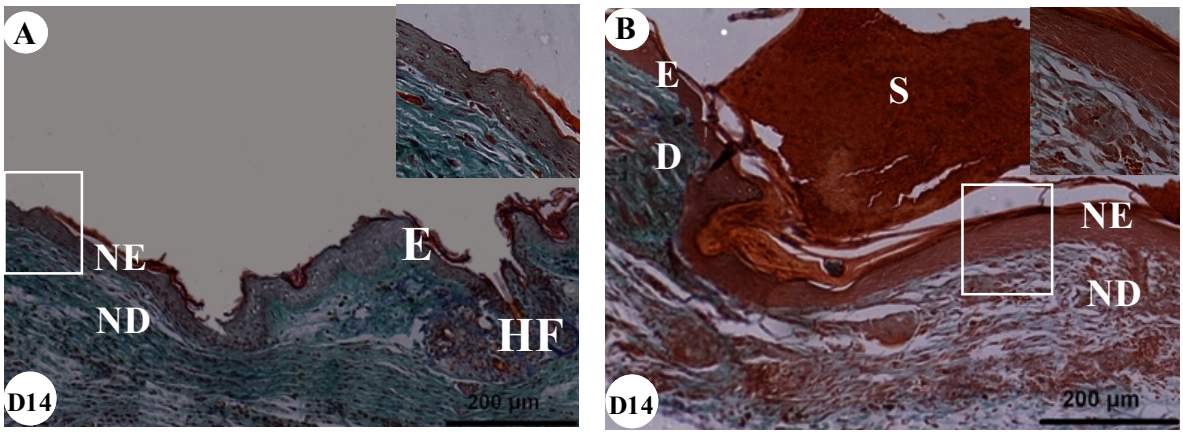


Fig. 43. Light micrographs showing day 14 wounds in *Tachyoryctes ibeanus* (A) and *Heterocephalus glaber* (B). By day 14 healing had progressed more in *Tachyoryctes ibeanus* compared to *Heterocephalus glaber*. At day 14 post wounding, *Tachyoryctes ibeanus* scab had detached on the wound, epidermis (NE) had completely regenerated and more collagen deposited in the new forming dermis (ND). In *Heterocephalus glaber* by day 14 scab (S) had not detached, complete re-epithelization (NE) had occured but the forming dermis (ND) had higher number of fibroblast cells and less collagen deposited compared to *Tachyoryctes ibeanus*. HF- hair follicle, D-dermis. Insets are high magnification Bar = 50µm. Modified Gomori alydehyde, Bar = 200µm

Day 30

At day 30, wounds of *Tachyoryctes ibeanus* macroscopically showed a hairless area (Fig. 42). The newly formed epidermis was fully differentiated by presence of stratum corneum (Fig. 44). The epidermal-dermal border was even without showing any in-growths. The dermis was densely packed with collagen organized in bundles laid parallel to epidermis and elastic fibers content had increased compared to day 14.

At day 30, wounds of *Heterocephalus glaber* were paler compared to the rest of the skin (Fig. 42). The newly formed epidermis had developed stratum corneum and the keratinocytes were flattened (Fig. 44). The epidermal-dermal border was smooth, lacking any ingrowths. The amount of collagen deposited had increased compared to day 14, but not horizontally oriented like in *Tachyoryctes ibeanus*. Blood vessels were numerous in *Heterocephalus glaber* compared to *Tachyoryctes ibeanus.*

Day 80

At day 80, wounds of *Tachyoryctes ibeanus* had a white scar that lacked hairs (Fig. 42). On histological evaluations of the scar tissue, the epidermal-dermal border was smooth, the deposition of collagen and elastic tissue in the dermis had increased but lacked hair follicles and the adipose tissue constituting the hypodermis was also lacking (Fig. 44). Blood vessels and cellular elements were scanty in the scar tissue dermis.

In *Heterocephalus glaber,* the scar was less prominent and only showing some slight difference from the rest of uninjured skin (Fig. 42). The scar tissue had smooth epidermal-dermal border, lacking down-growths (Fig. 44). The dermis had collagen as the abundant connective tissue fibers with substantial increment of the elastic tissue compared to the day 30 observations. Blood vessels and cellular elements were scanty.

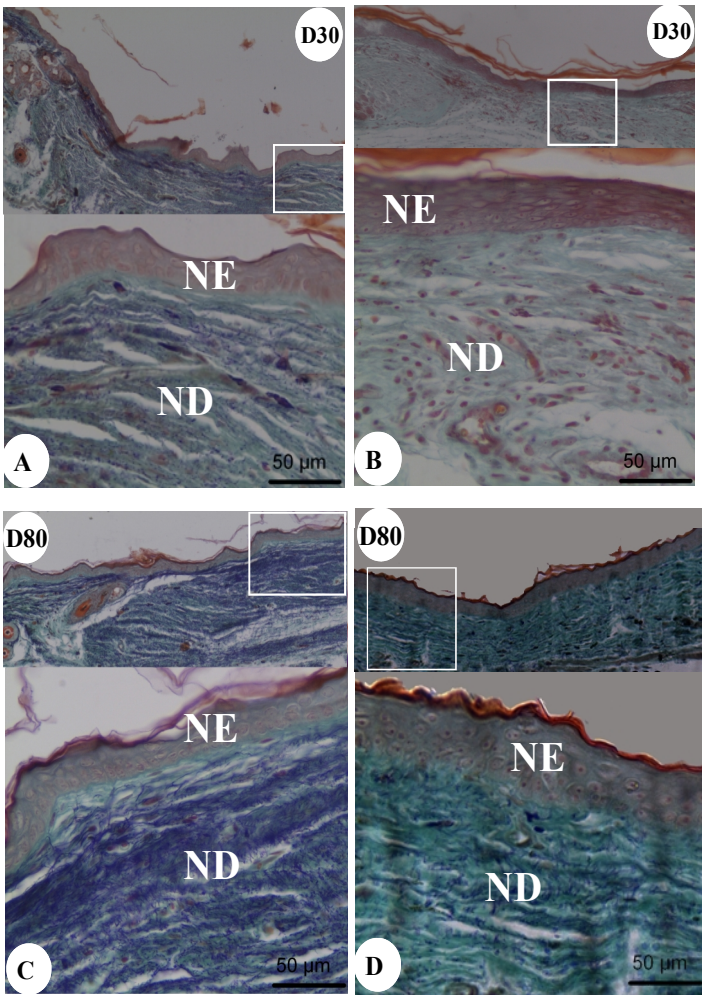


Fig. 44. Light micrographs of day 30 and 80 postwounding in *Tachyoryctes ibeanus* (A and C) and *Heterocephalus glaber* (B and D). At day 30**,** *Tachyoryctes ibeanus* wounds, the epidermis had completely differentiated with presence of stratum corneum and the dermis was densely packed with collagen (green) organized in bundles laid parallel to epidermis and with higher elastic fibers (purple). At day 30 the dermis was more porous in *Heterocephalus glaber* and blood vessels were numerous as compared to *Tachyoryctes ibeanus.* At day 80 *Tachyoryctes ibeanus* had higher content of elastic fibers than *Heterocephalus glaber.* NE-New epidermis, New dermis. Insets are high magnifications Bar= 50µm. Modified Gomori alydehyde, Bar= 200µm

3.3.2 Semi-quantitative microscopic analysis of wound repair

Day 3

At day 3 post-wounding in *Tachyoryctes ibeanus* the process of epidermal regeneration, proliferation and migration of fibroblast was significantly accelerated compared to *Heterocephalus glaber* (Fig. 45). There were no significant differences on inflammatory process and no new collagen was observed in the wound.

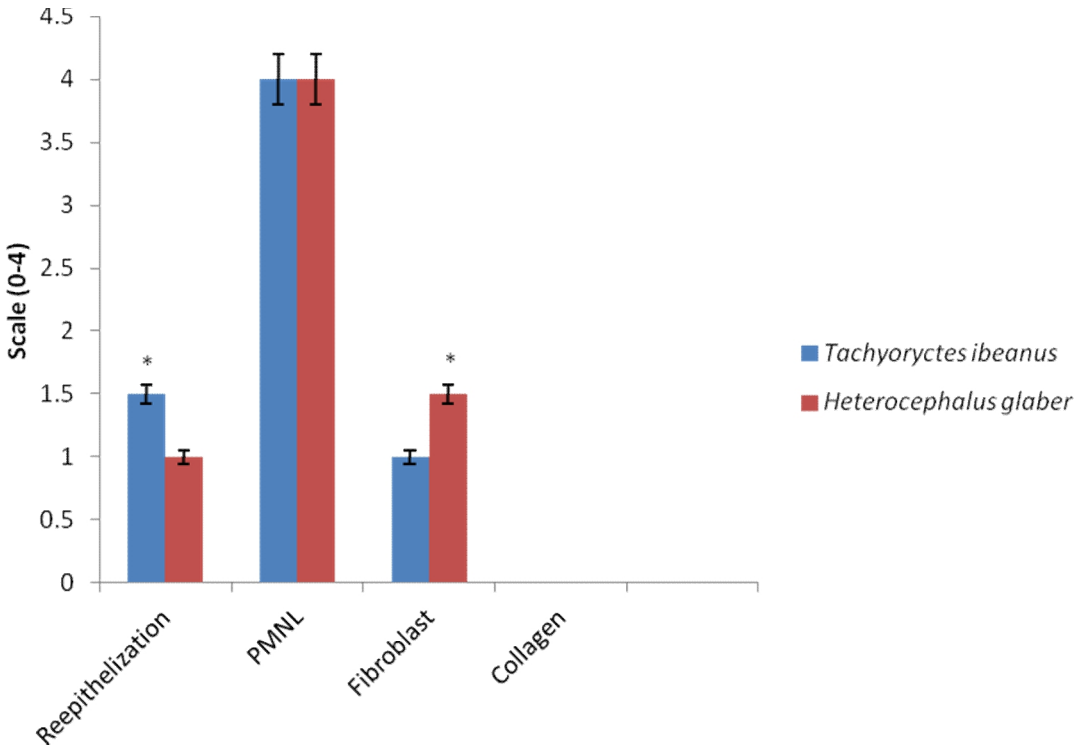
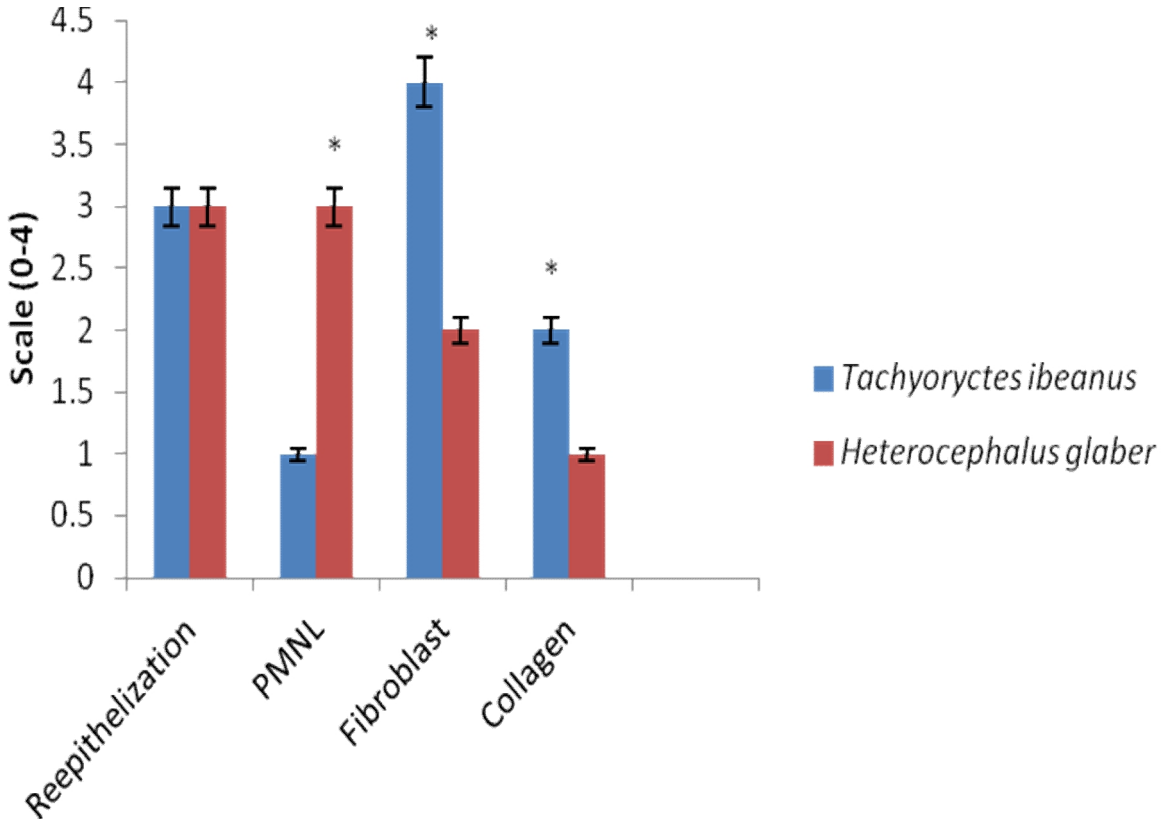


Fig. 45. Semi-quantitative analysis of histological wound healing parameters of *Tachyoryctes ibeanus* and *Heterocephalus glaber* at day 3 post-wounding. Significantly different parameters are indicated by asterisk (*). Polymorphonuclear cells(PMNL). Data represented as mean ± S.e.m at $P > 0.05$.

Day 7

At day 7 post wounding, epidermal closure was observed in both animals. However, in the *Tachyoryctes ibeanus* the proliferation and migration of fibroblast and collagen deposition in the wound was manifested earlier than in to *Heterocephalus glaber* (Fig. 46). There was still significant amount of polymorphonuclear cells (PMNL) in *Heterocephalus glaber* (Fig. 46).



Fig, 46. Semi-quantitative analysis of histological wound healing parameters of *Tachyoryctes ibeanus* and *Heterocephalus glaber* at day 7 post-wounding. Significantly different parameters are indicated by asterisk (*). Polymorphonuclear cells (PMNL). Data represented as mean± s.e.m at $P > 0.05$.

Day 14

At day 14 postwounding the regenerated epidermis had undergone differentiation in both animals (Fig. 47). Polymorphonuclear cells infiltration and proliferation of fibroblast was significantly higher in *Heterocephalus glaber* than *Tachyoryctes ibeanus*. Collagen deposition was abundant in *Tachyoryctes ibeanus* and scanty in *Heterocephalus glaber*.

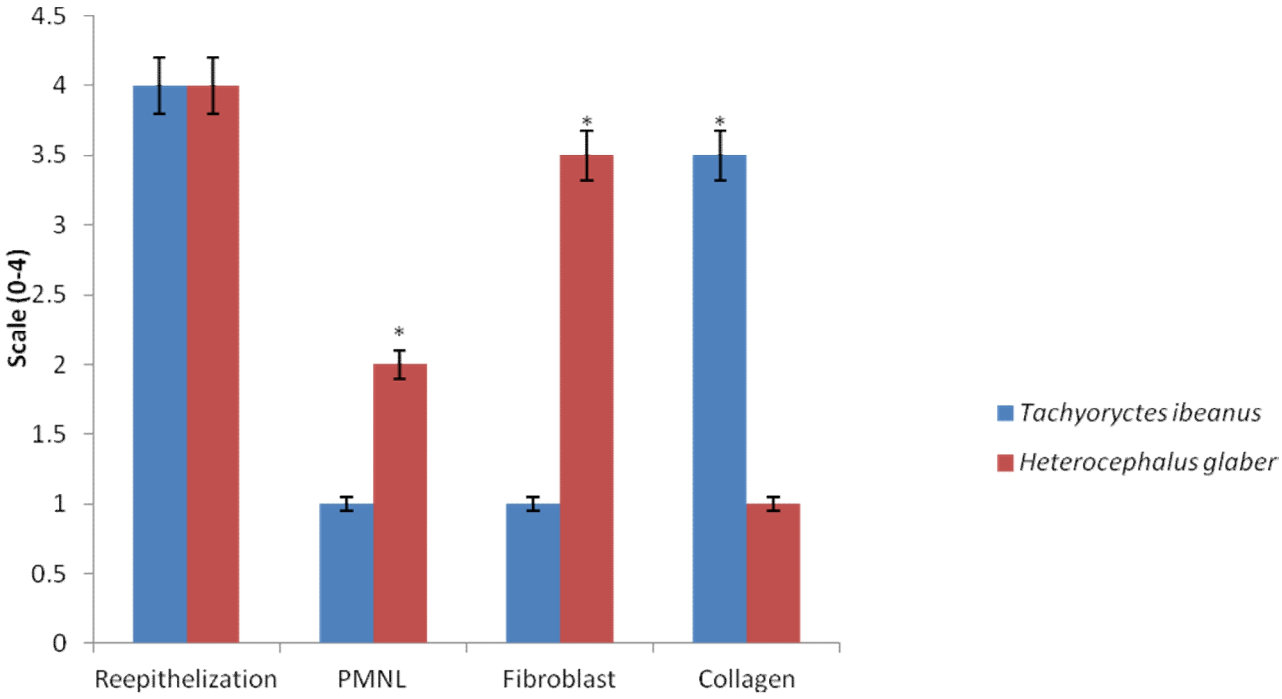


Fig. 47. Semi-quantitative analysis of histological wound healing parameters of *Tachyoryctes ibeanus* and *Heterocephalus glaber* at day 14 post-wounding. Significantly different parameters are indicated by asterisk (*) Data represented as mean at $P > 0.05$.

CHAPTER FOUR

4.0 Discussion

4.1 Skin morphology

Mammals shows tremendous diversity in their skin morphology which enables them to adapt successfully to a wide variety of environmental demands. Different skin adaptations of mammals reflect diverse habitats and modes of life such as terrestrial, arboreal, subterranean and aquatic habitats. Moreover, the skin as an organ has anatomically different areas that differ in organization of structural and cellular constituents. These areas have to fulfil different biological functions which are in part reflected by specific macromolecular organization of constituent molecules. In the present study, we compared the skin topography and microanatomy of two fossorial rodents, *Tachyoryctes ibeanus* and *Heterocephalus glaber,* that inhabit unique subterranean environments. Our results showed notable morphological similarities and differences on the skin of these two animal species.

The integument of *Tachyoryctes ibeanus* is covered by a thick soft pelage and is loosely attached to the underlying body musculature except in the rhinarium, labial lobes, palmar and plantar pads. The skin of the *Heterocephalus glaber* also has a loose attachment to the underlying musculature but additionally has large integumetal folds.The loose skin has been reported in other subterranean mammals including Geomyidae (Pocket gopher) **,** Bathyergidae mole rats and Ctenomyidae **(**Tuco-tuco) (Hildebrand, 1974; Tucker, 1981; Lacey *et al.*, 2000). The loose skin is believed to be an adaptation to reduce friction when subterranean rodents come into contact with the walls of the tunnels **(**Sokolov, 1982; Lacey *et al.,* 2000). Otherwise were the skin to be tightly fixed to the body, friction caused by squeezing through the narrow tunnels would lead to lacerations and abrasions. In *Heterocephalus glaber,* the impact of surface friction is further reduced by large integumental folds that deflect in response to a suddenly applied force. Fluffy and often upright fur is a

feature that has been noted in some of the subterranean rodents, such as bamboo rats (*Cannomys* and *Rhizomys*) and pocket gophers (lacey *et al.,* 2000). The soft fur bend easily in both directions, and it may represent an adaptation for locomotion in burrows. From these observations in the hairy species, it is likely that the integumentary folding in *Heterocephalus glaber* substitutes for the protective function of the pelage.

Another notable surface feature on the skin of the two animal species is the way the labia have been modified to close the mouth. In *Tachyoryctes ibeanus* the lips constitute a skin fusing between the upper and lower incisors leaving a small rima oris whereas in *Heterocephalus glaber,* buccal folds from the left and right side approximate to seal off the oral cavity. The labial lobes drawn into the space between the incisors and molars, where they meet and join in *Tachyoryctes ibeanus,* has also been reported in elliobus (Ognev, 1950) while where the lips fold behind the precumbent incisors as in *Heterocephalus glaber* has been observed in other chisel diggers such as *Geomyids* and *Ctenomys* (MacDonald, 1984; Lacey *et al.,* 2000). It has also been observed that in P*rometheomys* (Long-clawed Mole Vole) and *Nannospalax* (blind mole rats) the labial lobes are drawn into the space between the incisors and molars, where they meet but they do not join (Lacey *et al.,* 2000). The fact that the labial lobes are drawn into the space between the incisors and molars, whether fused or unfused has a common role of closing the mouth. This therefore suggests that, while digging when the incisors approximate, the labial lobes cover the mouth and seal off the oral cavity effectively to exclude soil from getting to mouth. Entry of particulate matter in the respiratory tract and alimentary canal may cause irritation and impede digestion .

The overall skin thickness and the thickness of specific skin layers in different parts of the body showed a wide variation in both *Tachyoryctes ibeanus* and *Heterocephalus glaber*. The skin was thicker on the dorsum (thoracic and lumbar) in both *Tachyoryctes ibeanus* and *Heterocephalus glaber* compared to the ventrum (sternal and abdominal). *Spalax*

microphthalmus(Greater mole rat) and zokors(Eospalax and Myospalax) just like the two mole rats examined have thicker skin on the back compared to the breast (Sokolov, 1982). However, other members of *Talpidae* family and gophers have a thicker skin on the chest area (Sokolov, 1982). The difference in skin thickness is related to the mode of digging the animal species employs, which could lead to some area of the skin being more prone to wear as a result of rubbing on the walls compared to others. The *Tachyoryctes ibeanus*, *Heterocephalus glaber*, *Spalax microphthalmus* and zokors employ the head and procumbent incissors to break up the soil and the claws of their limbs to kick the soil below the belly and out of the tunnel (Lacey *et al.,* 2000; Stein 2000). This mode of digging exposes the back to a lot of mechanical stress. *Geomyidae* and *Talpidae* break and loosen the soil by using the fore claws, after which it is pushed posterioly with the pads of forefeet thus exposing the skin at the chest region to a lot of mechanical stress.

The overall skin thickness was significantly higher in all regions of the body in *Tachyoryctes ibeanus* than in *Heterocephalus glaber*, except at the labial regions. Despite the greater overall skin thickness in all body regions in *Tachyoryctes ibeanus,* the skin of *Heterocephalus glaber* had significantly thicker epidermis and a well expressed stratum corneum on the dorsum but lacked epidermal pegs and dermal papillae interdigitations. The thick epidermis of the dorsum of *Heterocephalus glaber* compensates for the lack of a thick hair coat that confers mechanical protection enjoyed by *Tachyoryctes ibeanus*. This argument is reinforced further by the fact that the areas with shorter fur in *Tachyoryctes ibeanus* such as labial lobe, abdominal and pubic regions have thicker epidermis compared to the dorsum. The inverse relationship between the thickness of the pelage and epidermis was well illustrated by Sokolov (1982), who showed that sparsely furred wild boar, bore thicker epidermis compared with furred members of order artiodactyla. Pinkus, (1963), also observed that human epidermis is relatively thicker compared to that of other mammals because of lack of

pelage and suggested that epidermal thicknening is a compensation of loss of regenerative functions of the hair follicles.

The greatest epidermal and stratum corneum thickness was noted in the rhinarium, palmar and plantar pads in both *Tachyoryctes ibeanus* and *Heterocephalus glaber*. The integument surfaces at these regions were smooth without a typical dermatoglyphic pattern and the dermal-epidermal junction exhibited well developed interdigitations. The dermatoglyphic patterns have been documented in primates palmar and plantar skin, the rat snout pad and muzzle of different breeds of cows (Macintosh, 1975; Kimura *et al*., 2004). The smooth surface, thicknened epidermis and interdigitated epidermal-dermal border in the two species of animal examined may be an adaptation to match the high shearing forces resulting from bulldozing, compacting tunnels and kicking of the soil.

The dermis of both animal species were similar in that they lacked distinct papillary and reticular zones throughout the integument. Differences were however noted, on the content and architectural arrangements of the dermis. One of the variant component of the dermis was the distribution of the extracellular matrix. For instance, the dermis of the dorsal skin in *Tachyoryctes ibeanus* notably consisted of more loosely arranged collagen fibers, organized in a thin feltworks compared to any other region in the body. The ground substance appeared as large empty spaces between collagen bundles. On the contrary, *Heterocephalus glaber* had a dense and massive three dimensional meshwork of collagen fibres and fibre bundles dominating the bulk of its dermis, leaving little space for ground substance.The dermis of the footpads and rhinarium showed similarity in bearing most connective fiber content and much less ground substance than any other region of the body. The variation in arrangement and proportion of collagen fibres as the most abundant structural constituent of the dermis from different body regions was similar to that of other mammals (Kumar *et al*., 2012). The fact that the dorsum of *Tachyoryctes ibeanus* had a loosely organized dermis with

less collagen fiber content compared to *Heterocephalus glaber* may indicate that their dermis respond differently to mechanical stress due to compressive forces. Compressive forces due to subterranean environment on the dorsum of *Tachyoryctes ibeanus* would result in much reduction of thickness of the dermis, since collagen fibers will be closely packed together and the viscous ground substance will be displaced to adjacent region without pressure, as observed by Tregear (1969). On the contrary there will be less reduction in the thickness of the dorsal dermis of the *Heterocephalus glaber* if deforming compressive forces were to be applied since there is less ground sunstance to be displaced and the shock absorbing capacity is enhanced by three dimension network of collagen fibres. In compression, rhinocerous skin has a very high impact resistance ensured by its dermis containing densely and highly ordered three dimensional array of straight and highly crosslinked collagen fibers (Shadwick *et al.,* 1992). The loosely organized dermal architecture as noted in *Tachyoryctes ibeanus* has also been noted in the hairy skin of laboratory animals such as mice, rats, guinea pigs, rabbits, dogs and non-human primates (Winkelmann, 1961; Spearman, 1973). On the contrary, reticular dermis of hairless micropig, Chinese bama miniature pig, domestic pig, hairless guinea pig, hairless mice, and human integuments are densely packed with thick collagen bundles (Montagna *et al.,* 1952,1954; Lavker *et al*., 1991; Sueki *et al*., 2000; Sullivan *et al*., 2001; Liu *et al*., 2010) as in *Heterocephalus glaber*.

Elastic fibres in the dermis of *Tachyoryctes ibeanus* had abundant connective tissue matrix binding together a group of primary hair follicles and associated sebaceous glands. A single erector pili muscle embedded in the connective tissue matrix extended from just under the basement of epidermis to attach one hair follicle in a group. On the other hand elastic fibres were more widespread in distribution in *Heterocephalus glaber*. The sparsely distributed tactile hairs were associated with sebaceous glands but lacked erector pili muscle. *Tachyoryctes ibeanus* just like terrestrial wool bearing and densely haired animals such as

rabbit, sheep, mouse, rat, fox and raccoon, the elastic fibres in the dermis are predominantly allied with hair group and associated single arrector pili muscle, despite the difference in size and arrangements in individual hair follicles (Sokolov 1982; Meyer *et al.* 2000; Starcher *et al*. 2005; Oznurlu *et al.* 2009). This in contrast to the sparsely-haired mammals such as humans, opossum, pigs, nonhuman primates and micropig among others which have variant elastic fibres distribution in the dermis and each hair follicle is associated with erector muscle (Hirose and Kligman, 1988; Lavker *et al*., 1991; Meyer *et al.* 2000; Starcher *et al*. 2005). The advantage of such a specific elastic interweaving of hair follicles in the dermis as found in terrestric densely-haired mammals is that individual hair follicles of a group can be moved together and simultaneously along the entire body, so that a better and rapid insulation by the hair coat is achieved considering that a follicular unit share a single erector pili that erects them during very low temperatures. Thus, the hair follicles to which erector pilli muscle is not attached could easily follow this movement. When the erector pili muscles relax, all hair follicles are brought back to their former position by the contraction abilities proper of the elastic fibre system. This functional aspect of normal skin biology seems important, in particular, for small mammals, because they have a comparatively larger body surface area than medium-sized and large mammals for thermoregulation. The skin of unrelated genera of subterranean rodents, *Crytomys, Geomys* and *Spalax* had compound hair follicles but they differed from *Tachyoryctes ibeanus* in the sizes of individual hair follicles and their attachments to skeletal muscles (Klauer *et al*., 1997; Daly and Buffenstein 1998). The thermoregulatory function of arrangement of hairs into groups in subtarranean rodents may be difficult to deduce despite having hair groups like other terrestrial mammals since they live in warm, temperature buffered burrows. The well innervated and widely spaced hair follicles of *Heterocephalus glaber*, may function to increase tactile sensitivity and not as erectile organs since they lack erector pili muscle.

The structure of vibrissae in both animals species shared the characteristics of other subterranean rodents of genera *Spalax* and *Crytomys* in being short and presence of skeletal muscles fibres attaching to the vibrissal capsule (Klauer *et al*., 1997). The short vibrissae in both *Tachyoryctes ibeanus* and *Heterocephalus glaber* may indicate vulnerability to friction during burrowing and only may be used for passive monitoring of the tunnel walls. The striated muscle fibers attaching at the vibrissal capsule may be involved in erecting the vibrissal hair follicle. Small globular sebaceous glands were associated with ordinary hairs and vibrissal hairs in both species of mole rats. Additionally, *Heterocephalus glaber* had large multilobular sebaceous gland located at the labia and pubic region skin. The skin of small mammals of the orders insectivora, chiroptera and rodentia have been found to be rich in sebaceous glands in the head region (Haffner, 1998, 2000). They are those associated with the hair follicles and are used to lubricate the hair and therefore prevent it from mechanical damage, and those that open directly to the skin surface. Enlarged sebaceous glands around the mouth area like those of *Heterocephalus glaber* have been found in some families of order rodentia such as Soricidae, Talpidae, Rhinolophidae, Vespertilionidae, Muridae and Arvicolida ensures more sebum production and enhanced protection of the skin (Haffner, 1998; Sokolov 1982).

A notable feature of dermis of *Heterocephalus glaber* is the presences of melanocytes located midway in the dermis and not associated with cells of epidermis. *Tachyoryctes ibeanus* had dark pigments only associated with hair shafts in their hair follicle and lacked pigment containing cells in the dermis. The melanophores in most mammals provide pigmentation in the epidermis by sending out long, slender processes which make contact with the keratinocytes and so allow for the transfer of pigment melanin that protects animals from the sun's rays (Romer and Parsons, 1977). Poikilothermic and homeothermic vertebrates have different molecular mechanisms that are involved in pigment cells morphogenesis and

migration (Kelsh *et al.,* 2009). Specific molecular cues determine where pigment cells stop their migration. Mice, rats, some prosimians and birds pigments have been noted in hair follicles and feather germs helps the animals blend into the background (Montagna, 1967). Pigment granules are translocated within chromatophores of poikilothermic vertebrates and crustaceans in response to photic, thermal and/or neurohormonal stimuli, allowing the animal to rapidly change color for thermoregulation, adaptation to light and background, and social behavior display (Deoliveira *et al*., 1996). The functional significance of presence of melanocytes in the dermis of naked mole rat is unknown and therefore fundamental molecular mechanisms that lead to pigment cells morphogenesis and migration in this species need to be established.

Apocrine sweat glands were only noted in the pubic regions of *Tachyoryctes ibeanus* and were completely lacking in *Heterocephalus glaber*. Both animal species lacked eccrine glands throughout their integument. Depending on distribution of apocrine sweat glands, they may be involved in temperature regulation, lubrication and moistening the pelage, creation of a specific odour or in excretion (Sokolov, 1982). For instance sweat glands in domestic sheep are involved in lubricating the pelage and are not essential in temperature regulation since they are few, and panting is the principal method of evaporative cooling (Montagna, 1967). Eccrine sweat glands are distributed all over the primates body and are involved in thermoregulation while apocrine in the axillar and anogenital region are regarded as scent glands (Shelley, 1951). Apocrine glands have been located in the opening of the urogenital organs in rodent, (Hyman, 1973) whereas eccrine sweat glands are in the underside of the paw (Kennedy *et al*., 1984). Both eccrine and apocrine sweat glands in rodents lack thermoregulatory function (Sperman 1973). The eccrine sweat glands on the footpads have been intimated to enhance tactile sensitivity (Wechsler and Fisher 1968). Sweat glands in glaborous skin of footpads and snout in the dog and cats increase friction and enhance grip

and rely on panting for thermoregulation (Bell and Montagna, 1972).The apocrine sweat glands localized at the anogenital area of *Tachyoryctes ibeanus* could serve to lubricate and moisten the pelage between the hind legs as friction may result due rubbing as the animal kicks the soil.

4.2 Wound healing.

Given the structural differences reported in the dorsal skin of *Tachyoryctes ibeanus* and *Heterocephalus glaber* in terms of the thickness of the various layers(epidermis, dermis and hypodermis) and the content of the dermis, the ability of the two species to heal skin wound was assessed. On evaluation of macroscopic and histological parameters of wound healing between the *Heterocephalus glaber* and *Tachyoryctes ibeanus* showed a significant difference on the time course and quality of healing.

On wounding with 4mm biopsy punches, there was no significant indication of bleeding in *Tachyoryctes ibeanus,* where as in *Heterocephalus glaber* there was substantial amount of bleeding. This difference could be attributed to the rich blood supply encountered in the dermis of *Heterocephalus glaber* compared to *Tachyoryctes ibeanus* which may serve a thermoregulatory role. In both animals a scab had formed over the wound by day 3 postwounding. Scab detachment was delayed in *Heterocephalus glaber,* occurring between days 21-24 postwounding, compared to days 12-14 in the *Tachyoryctes ibeanus*. Detachment of the scab in both animals grossly revealed a well healed wound. The duration of scab detachment in the *Tachyoryctes ibeanus* was consistent with that of mice (Braiman-Wiksman *et al*., 2007) where detachment was reported to occur between days 8 -12 postwounding. Scabs on wounds have been found to be essential, in that if removed before their time to detach, the scabs come out with new regenerated tissue underneath, therefore interfering with the healing process (Galko and Krasnow, 2004). Scabs also serve as a sink for inflammatory

cells and bioactive inflammatory mediators (Goren *et al*., 2003). There is evidence that in wounds treated with bacterial biofilm, the scab lasted upto 6 weeks before detaching, indicating prolonged inflammation (Zhao *et al.,* 2011). Since dermal closure occurred quite slowly in *Heterocephalus glaber* as indicated by the histological section at day 7 and 14, delayed scab detachment provided the wound with stability by ensuring the tissue integrity was restored.

On examination of reepithelization in both animals epidermis had completely bridged the gap by day 7 in majority of the wounds. Since the two animal had different dermal closure times, our studies showed that keratinocytes migration is independent of granulation tissue formation just as has been reported in mice (Braiman - Wiskman *et al*., 2007).

Slower scab detachment, minimal wound contraction, prolonged inflammation, slower collagen deposition and slower deposition of elastic fibers are indicative of much slower wound healing processes as noted in *Heterocephalus glaber* compared to *Tachyorystes ibeanus* and other rodents earlier studied (Brainsman-waksman *et al*., 2007; Richardson, 2004). The cause of the slower wound healing process is not clear at the moment, perhaps molecular studies may reveal the contributing factors. However earlier studies in the *Heterocephalus glaber* in relation to pain have shown that this species lacks neuropeptide substance P and calcitonin gene-related peptide in their cutaneous sensory fibers (Park *et al*., 2003, 2008). Substance P has been shown to play major roles in wound repair. Substance P has been reported to amplify the inflammatory response by inducing inflammatory mediators such as cytokines, oxygen radicals and histamines that potentiates tissue injury and stimulate leucocytes recruitment (Holzer and Holzer-Petsche, 1997). Some studies have detailed the proliferative effect of substance P on a variety of cells, where it acts as mitogen for smooth muscle cells, fibroblasts and endothelial cells (Ziche *et al.,* 1990; Rameshwar *et al.,* 1997). Other researchers, reported that substance P enhances wound healing through a complex

network of mediators and also by inducing mobilization of a stromal-like cell at site of tissue injury (Delgado *et al.,* 2005, Hong *et al.,* 2009). Collectively, these studies suggest that substance P has a primary role in tissue repair but not as a sole factor. Systematic studies are necessary to determine the full repertoire of factors that participates in wound healing in absence of substance P in *Heterocephalus glaber*.

On examining the wound at day 30 postwounding in *Tachyoryctes ibeanus* its bed had collagen deposited parallel to the epidermis and a significant deposition of elastic fibers was noted. In contrast, the new dermis of *Heterocephalus glaber* was less packed with collagen and there was no deposition of elastic fibers. These differences in deposition of extracellular matrix suggests remodeling was faster in *Tachyoryctes ibeanus* compared to *Heterocephalus glaber*. The findings, that wound extracellular matrix is deposited slowly and its porous configuration noted in *Heterocephalus glaber* was also reported in adult Acomys and axotols (Seifert *et al,* 2012). Seifert and colleagues indicated the deposition of extracellular matrix in this manner favours regeneration over fibrosis during skin repair in both Acomys and axotols.

At day 80 postwounding, the wound in *Tachyoryctes ibeanus* had healed completely but by scar formation as grossly indicated by lack of hair and histologically as a gap in the hair follicle distribution. In contrast, the wound in *Heterocephalus glaber* had grossly and histologically healed with less scarring. Scarring has been thought of as a late event in wound healing, predorminantly involving extracellular matrix remodeling (Ferguson and Leigh 1998; Cherry *et al.,*2001). Rodents have been reported to heal by forming a scars which mature at about 80 days postwounding (Ferguson and O'Kane, 2004). Embryonic scar-free healing compared to healing with scar formation in adults is attributed to differences in the type and amount of growth factors present during the repair process (O'Kane and Ferguson, 1997, Shah *et al.,* 2000, Cowin *et al.,* 2001). The factors that contribute to less scarring in the

wounds of *Heterocephalus glaber* could not be deduced at the level of light microscopy we carried out our study. Perhaps the healing factors that are present in mammalian embryos are also present in the *Heterocephalus glaber*. Whether or not the lack of substance P influences the quality of healing in the naked mole rat is a question that requires further investigations.

5. 0 CONCLUSIONS AND RECOMMENDATIONS

Tachyoryctes ibeanus and *Heterocephalus glaber* showed topographical and histological differences on their skin morphology both structurally and in terms of thickness. These observations may be a reflection of the selective pressures exerted by mechanical, temperature, and sensory aspects of their unique subterranean environments.

Wound repair process was slower but resulted in much better quality healing in *Heterocephalus glaber* than in *Tachyoryctes ibeanus*, where the repair process was faster but resulted in a stable and mature scar. In this study it is speculated that the differences in healing between the two species could be attributed to their markedly different skin structure. Further experiments could be carried out to establish the molecular mechanisms that may be involved in the healing process of *Heterocephalus glaber* and *Tachyoryctes ibeanus*.

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