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Full Length Article

Skin pattern structure and function of juvenile ages of *Chameleo chameleon*

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ABSTRACT

Little is known about the skin structure of juvenile chameleon especially its sensory function of their integumentary structure. Fifteen juvenile *Chameleo chameleon* are collected from Abu Rawash, Northern area of Giza, Egypt during Summer of 2015. It is belong to the order Squamata, family, Chamaeleonidae. Three ages are used in the present study and categorized according to the morphological criteria of head, abdomen and limb lengths. Dorsal abdominal surfaces are covered with abdominal scales of varying sizes either conical or elliptical-structures, regularly arranged in rows and imbricated with each other. Each scale possessed one cylindrical lenticular epidermal sense organ containing heavy sensillia. Histologically, the scales are characterized by wider conical surfaces and intermingled with another one by hinge region. The epidermal layer of outer scale surface is composed of five-layered stratified squamous epithelium including the stratum germinativum, intermediate zone of stratum spinosum and granulosum, α-keratin layer, β-keratin layer and outer superficial Oberhaütchen. Melanosomes are abundant in the intermediate zone as well as in the peripheral dermal layer underneath stratum germinativum layer. The melanosomes possessed long cellular processes with their content of melanin granules underneath the epidermis. The dermis is composed of upper collagenous and inner compact layer. Semithin sections revealed the presence of fibroblast cells, collagenous fibrils, nerve axons, melanosomes and mast cells in the connective tissue core. Increased immunoreaction of cytokeratin is observed in the epidermal layers of G3; meanwhile, an increased proliferation of epidermal and dermal cells was detected in G1. Transmission electron microscopy exhibited striking formation of dermal sense organs containing neuronal cells of both oligodendrocytes and Schwann cells with myelinated and unmyelinated nerve axons ensheathed externally by thin collagenous fibers. Finally, the author concluded that the juvenile chameleon skin is keratinized with obvious external and internal sensation and abundant mast cells within dermis giving characteristic immunity. The melanosomes are dispersed within epidermis and dermis allowing the animal to maintain its color alterations according to the surrounding environment.

1. Introduction

Squamata possessed varying skin structures. As a result of living in desert, the hardness of reptilian integument keeps the animal internal organs from damage and dehydration [1].

The scaly covering of epidermis represents the most adaptive function for water retention that maintain flourishing and accommodation of reptilian for a terrestrial life [2]. Characteristic variations of scale shape, size, and their overlapping are represented in reptilian species [3].

The epidermis mainly composed of five major layers, Oberhaütchen, β-keratin layer, α-keratin layer, intermediate zone and stratum germinativum [4–7]. The stratum germinativum or basale is made up of single layer of highly proliferative cuboidal cells and the source of keratinocytes progenitors [4]. After proliferation of the neo-keratinocytes, it migrates and differentiates along the way to the outer Oberhaütchen. The intermediate zone contains keratinocytes at different developmental stages where lipids and proteins are packaged in granular structures. Later, the lipid envelops are created by releasing the lipid content to form the corneocytes of the superficial layer [8]. The α-layer is soft and elastic and has specific connections to the preceding scales and the β-layer, which is relatively hard, forms the surface of the scales [4].

The dermis is mainly composed of connective tissue and collagen fibers with many important structures including chromatophores, lymphatic vessels, nerves and blood vessels [4].

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The skin of reptilian species varied in their distribution and densities of epidermal melanocytes and dermal melanophores, lipophores, and iridophores [7,9,10]. In Chameleons and some anoline lizards, the chromatophores represent the main components of a dermal chromatophoric unit in combination with melanophores [11].

Integumentary sense organs represent the mechanosensory receptors in reptilian species such as *Amphibolurus barbatus* [12] and *Iguanian lizards* [13]. These sense organs serve as mechano and thermoreceptors and possibly sensitivity to humidity [12,13]. It is also served as complex touch corpuscles on the skin surface of the head of snakes [14,15]. Small tactile mechanosensory epidermal sense organs are also reported in 13 fully aquatic and two semi-aquatic species of elapids [16].

The present study aimed to illustrate the integumentary neuroepithelial interaction of juvenile chameleon and its capacity of proliferation through light, immuno and ultrastructural studies.

2. Materials & methods

Fifteen juvenile individuals of *Chameleo chameleon*, Linnaeus (1758) (Class: Reptilia; Order, Squamata; Suborder Sauria; Family, Chamaeleonida; Genus, Chamaeleo; Species, *Chameleo chameleon*) were collected from Abou–Rawash desert, Giza Governorate, Egypt during summer 2015. The specimens were categorized into three stages according to the variations of total body length, head length, tail length, body width and limbs length as represented in Table 1 and Fig. 1.

2.1. Histological investigation

2.1.1. Hematoxylin eosin staining

Dorsal skin of trunk region of different developing stages is fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending percentages of ethyl alcohol, cleared in xylene and mounted in molten paraplast at 58–62 °C. Five μm sections were cut, stained with hematoxylin and eosin and investigated under a bright field Olympus light microscope.

2.1.2. Immunohistochemical staining of CK and PCNA

Immunostaining was performed on formalin-fixed and paraffin-embedded tissues. Hydrated tissue sections were firstly incubated in 2% hydrogen peroxide endogenous peroxidase activity for 5 min to block peroxidases. The slides were then incubated overnight at 4 °C in a humidified chamber with the primary antibodies of cytokeratin (Cat. sc-25280, 1:50, mouse, Santa Cruz) and PCNA (Cat. sc-56, 1:400, mouse, Santa Cruz). After rinsing with a phosphate-buffered solution, the specimens were incubated in biotinylated secondary antibody for 50 min at room temperature followed by treatment with Avidin–Biotin–horseradish peroxidase and staining with 0.04% 3,3′-diamino-benzidine tetrahydrochloride and Hematoxylin. Negative control was carried out by using the primary antibody.

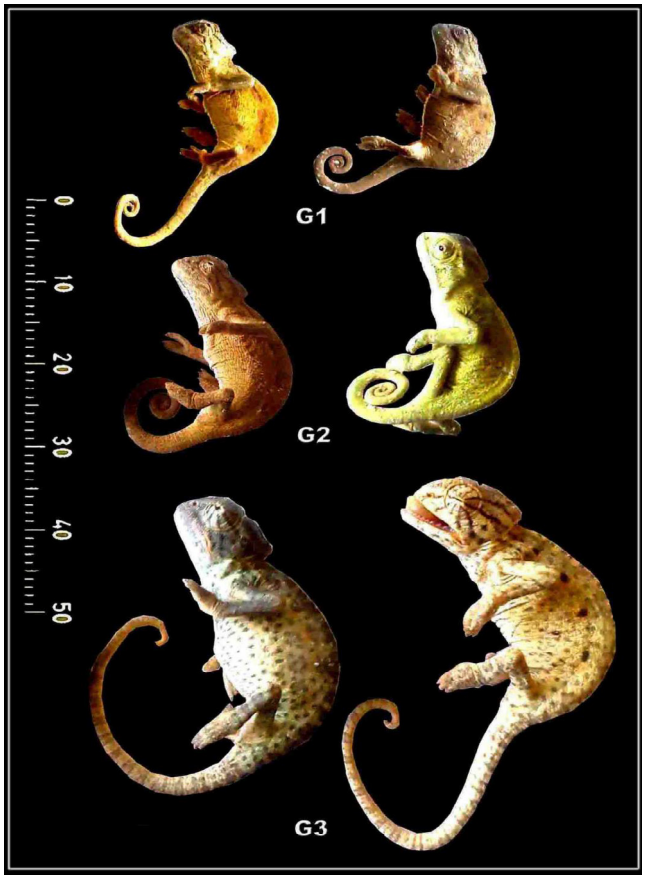

Fig. 1. Lateral view photomacrographs of different stages of developing *Chameleo chameleon.*

2.2. Scanning electron microscope

Skin samples were fixed in 2.5% glutaraldehyde in cacodylate buffer. This was followed by dehydration in ascending percentages of ethyl alcohol and critically point drying, and coating with gold in platinum-palladium ion-sputtering and investigating under Jeol scanning electron microscope, JSM-5400LV.

2.3. Transmission electron microscope

Fresh samples were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4, post-fixed in 1% osmium tetroxide, dehydrated in ascending percentages in ethyl alcohol, and cleared in propylene oxide and embedded in araldite resin and allow for complete polymerisation at 60 °C. Semithin sections (1 μm) were cut at Ultracut Reichert-Jung ultramicrotome with the aid of glass knives, stained with toluidine blue and examined under light microscope. For electron microscopy ultrathin sections were carried out, stained with uranyl acetate and lead citrate and examined with a Joel CX 100

Table 1
Morphometric analysis of different developing stages of *Chameleo chameleon* in (*mm*).

	TBL	HL		SVL	TL	BW	FLL	HLL
		Antero-posterior	Dorso-ventral					
G1	10.33 ± 0.65	2.72 ± 0.20	1.87 ± 0.16	6.08 ± 0.57	4.90 ± 0.21	2.69 ± 0.10	2.78 ± 0.33	3.37 ± 0.32
G2	13.69 ± 0.78	3.14 ± 0.17	2.22 ± 0.24	7.71 ± 0.77	6.02 ± 0.46	3.12 ± 0.09	3.16 ± 0.17	3.73 ± 0.25
G3	17.56 ± 0.56	3.48 ± 0.44	2.98 ± 0.21	10.51 ± 0.65	7.96 ± 0.54	3.52 ± 0.22	4.17 ± 0.29	4.95 ± 0.17

Each result represents the mean ± SE (n = 5). Abbreviations; BW, Body width (in the middle of trunk); FLL, For limb length; HL, Head length (from tip of snout to the corner of the neck); HLL, Hind limb length; SVL, Snout vent length; TBL, Total body length; TL, Tail length.