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A Morphological and Histological Study of the Skin in the *Bufo viridis* Frog in Iraq

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Abstract

This research was carried out to identify the layers of the skin of *Bufo viridis*. Ten adult frogs were used in the study, which was conducted in the histology and embryology laboratory at the College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad. The skin was dissected and fixed in 10% formalin, then dehydrated, cleared, infiltrated, and embedded. The sections were stained with hematoxylin and eosin. The histological structure of the skin revealed that it is composed of two layers. The epidermis layer is composed of stratified squamous epithelial tissue and consists of three strata: the Corneum, Spinosum, and Germinativum. There are no papillae in the epidermis. The dermis layer is composed of loose connective tissue with blood vessels and consists of two layers: the spongy and compact layers. The spongy tissue consists of alveolar glands, both mucous and granular. The dorsal skin and the dermis consisted of pigment cells. The skin tissue consists of a two-layered structure: the epidermis, composed of stratified squamous epithelium, and the dermis, composed of loose connective tissue. Pigment cells are present in both layers, suggesting that they serve a role as coloration or UV radiation protection.

Keywords: Frog, Skin, Amphibia.

دراسة مورفولوجية ونسيجية للجلد في ضفدع البوفو فيريديس في العراق

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الخلاصة

أُجري هذا البحث لتحديد طبقات جلد الضفدع (*Bufo viridis*). استُخدمت عشرة ضفادع بالغة في الدراسة التي أُجريت في مختبر علم الأنسجة والأجنة في كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد. شُرِّح الجلد وثُبّت في 10% فورمالين، ثم جُفِّف، رُوق، ورُشِّح، وغُمر. صُبغت المقاطع بالهيماتوكسيلين والإيوسين. أظهر التركيب النسيجي للجلد أنه يتكون من طبقتين. تتكون طبقة البشرة من نسيج طلائي حرشفي مطبق وتتكون من ثلاث طبقات: المتقرنة، والشوكية، والمولدة. لا توجد حليمات في البشرة. تتكون طبقة الأدمة من نسيج ضام مفكك مع أوعية دموية وتتكون من طبقتين: الطبقة الإسفنجية والمصمتة. يتكون النسيج الإسفنجي من الغدد السنخية، المخاطية والحبيبية. تحتوي الادمة في الجهة الظهرية للجلد على خلايا صبغية.

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يتكون نسيج الجلد من طبقتين: البشرة، المكونة من ظهارة حرشفية مطبقة، والأدمة، المكونة من نسيج ضام مفكك. توجد الخلايا الصبغية في كلتا الطبقتين، مما يشير إلى أنها تؤدي دورًا في التلوين أو الحماية من الأشعة فوق البنفسجية.

1. Introduction

The number of discovered amphibians is 8000 species, and the proportion of anura, such as frogs and toads, is 88% of them, where 150 species are discovered every year. Amphibians adapt to live in both aquatic and terrestrial environments [1-3]. The class of amphibians includes three orders: Urodela (Salamender), Anura (Frogs and toads), and Gymnophiona. The current study was conducted on genus *Bufo*, sub-class Lissamphibia, superorder Salientia, order Anura, family Bufonidae. This genus has a worldwide distribution; there are 13 species in North America, 81 in Central and South America, 3 in Europe, 77 in Asia, and 64 in Africa [4]. The skin in vertebrates covers the body and protects the underlying structures [5]. It works to remove the pathogens, as it works to repel microorganisms and parasites, as well as retain the fluids and gases of the body [6]. It is considered a sensory organ [7]. The skin is made of several layers. The epidermis is the outermost coat, and it comes from the ectoderm that forms during gastrulation. Below the epidermis is the dermis, which comes from the somites near the neural tube, while the glands in the dermis are derived from the ectoderm [8].

2. Materials and Methods

Ten (5 male and 5 female) live *Bufo viridis* were brought to the laboratory. Animals were anesthetized with ether, and the skin was removed. For a light microscope study, the pieces of skin were immersed in 10% formalin to be fixed. Then the skin was washed with tap water and dehydrated in ascending solutions of ethyl alcohol, and cleared in xylene. Then the specimens were infiltrated and embedded in paraffin wax [9]. Thin sections (5μm) were cut with a rotary microtome and stained with hematoxylin and eosin. These sections were examined and photographed using a light microscope with a camera.

This study received approval from the Scientific Ethics Committee of the University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham). The Ethical approval number is: EC-80 on 28 \4\ 2025.

3. Results and Discussion

The skin of a frog is composed of two layers. The epidermis consists of stratified squamous epithelial tissue and consists of three layers: an outer layer (stratum corneum), central layers (stratum spinosum), and a basal layer (stratum germinativum) (Figure 1). The outer layer, stratum corneum, consists of a single layer of flat cells (Figure 2). The stratum spinosum lies between the stratum corneum and stratum germinativum, and consists of several layers of distinct polygonal cells containing a central nucleus, which is surrounded by 4-8 layers of cells (Figure 1). The stratum germinativum is located above the dermis and consists of a single layer of columnar cells (Figure 1).

The cells of stratum germinativum are based on the basement membrane, which is the boundary between the epidermis and the dermis, and the epidermis does not contain papillae (Figure 1). The dermis consists of connective tissue, and it does not extend into the epidermal layers. On the dorsal skin, in the dermis, are pigment cells. The dermis consists of two layers. The superior (spongious) dermis consists of loose connective tissue containing collagenous fibers and fibroblasts. The inferior (compact) dermis, composed of dense connective tissue, contains a bundled sequence of collagenous fibers, smooth muscles, blood vessels, and nerve fibers (Figures 3 and 4). In the spongious dermis layer, there are glands that are of different sizes, and are sac-shaped; all of the glands are alveolar glands. There are two types of glands:

Mucous and granular (parotid) glands. The glands consist of a group of secretory cells, and there is a short duct connected to the secretory protein, which passes through the epidermis (Figures 3 and 4). The granular (parotid) glands are large in size and found in large numbers on the dorsal side of the skin compared to the ventral side, while the mucous glands are small in size and found in large numbers on the dorsal side of the skin (Figures 3 and 4). The dermis consists of blood capillaries and nerve fibers.

The skin in frogs has the ability to heal deep wounds quickly [10]. The skin of the reptile was very thick and dry. The skin in humans does not serve as a respiratory organ [11]. The skin of a frog consists of two layers, the epidermis and the dermis, and the epidermis layer is thin and corresponds to what was indicated by Haslam *et al.,* [12]. The surface layers of the skin, the stratum corneum, consist of flat cells and contain keratin, a protein substance formed by epidermal cells. This layer appears for the first time in amphibians and is well-formed in organisms that live on land, and is considered an adaptation for living on land, as it works to protect the body and maintain moisture. These layers in birds form a strong barrier that prevents water evaporation and loss from the body. This layer is thicker in reptiles and mammals [13, 14]. The epidermis in the skin of birds and mammals contains papilla that helps grow feathers and hair [11]. The mucus-secreting epithelial cells of the epidermis play an important role in defense against pathogens and are the rejuvenation of the mucosal cells lining the lungs and intestines in mammals [15, 16]. Egambaram *et al.,* [17] and Souak *et al*., [18] have reported that the skin contains lipids that have properties against microorganisms and ultraviolet rays. The epidermal cells of tadpoles (25-30) mm are ciliated, and the cells lose their cilia in the Metamorphosis stage, and only the cells around the eyes and nose remain ciliated [19, 20]. Birds produce their feathers, which support them, and the skin does not contain sweat and sebaceous glands, except for the uropygal gland near the tail [21]. The skin of fish is distinguished by the presence of bony scales embedded in the epidermis and dermis [22]. The spongy dermis on the dorsal side of the skin contains many granular glands compared to the ventral side of the skin, and this is also what they referred to in a rock frog [23]. Granular glands in the skin of amphibians secrete serous fluid; they secrete antimicrobial peptides and toxic alkaloids, which contain a bioactive molecule as antimicrobial peptides (AMPs)[24-26]. Shang *et al.,* [27] and Sai Kumar [28] indicated that the glands play a role in physiological activities, which include: respiration, ion regulation, water transport, and immune defense. The skin of reptiles does not contain glands [11], while the skin of fish is distinguished by the presence of goblet cells full of mucus [29]. Pigment cells are distributed differently in the skin of larvae, tadpoles, and adult frogs [30]. The skin in several colored birds is due to the carotenoid pigments that are concentrated in the skin or feathers, which the birds obtain from their food [31].

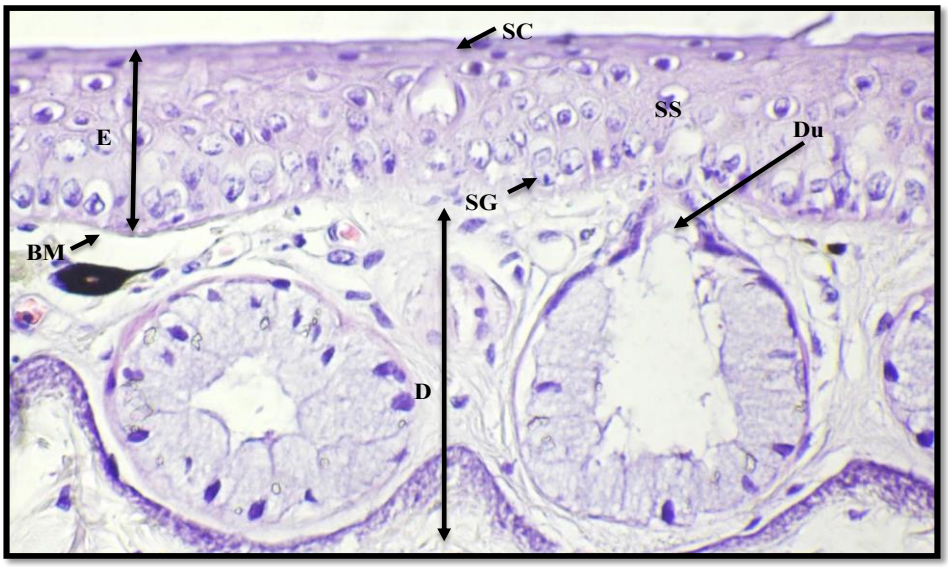

Figure 1: Longitudinal section through the frog skin showing the layers of: (E) epidermis, (BM) basement membrane, (D) dermis, (SC) Stratum corneum, (SS) Stratum spinosum, (SG) Stratum germinativum, (Du) duct, (H&E, 40x).

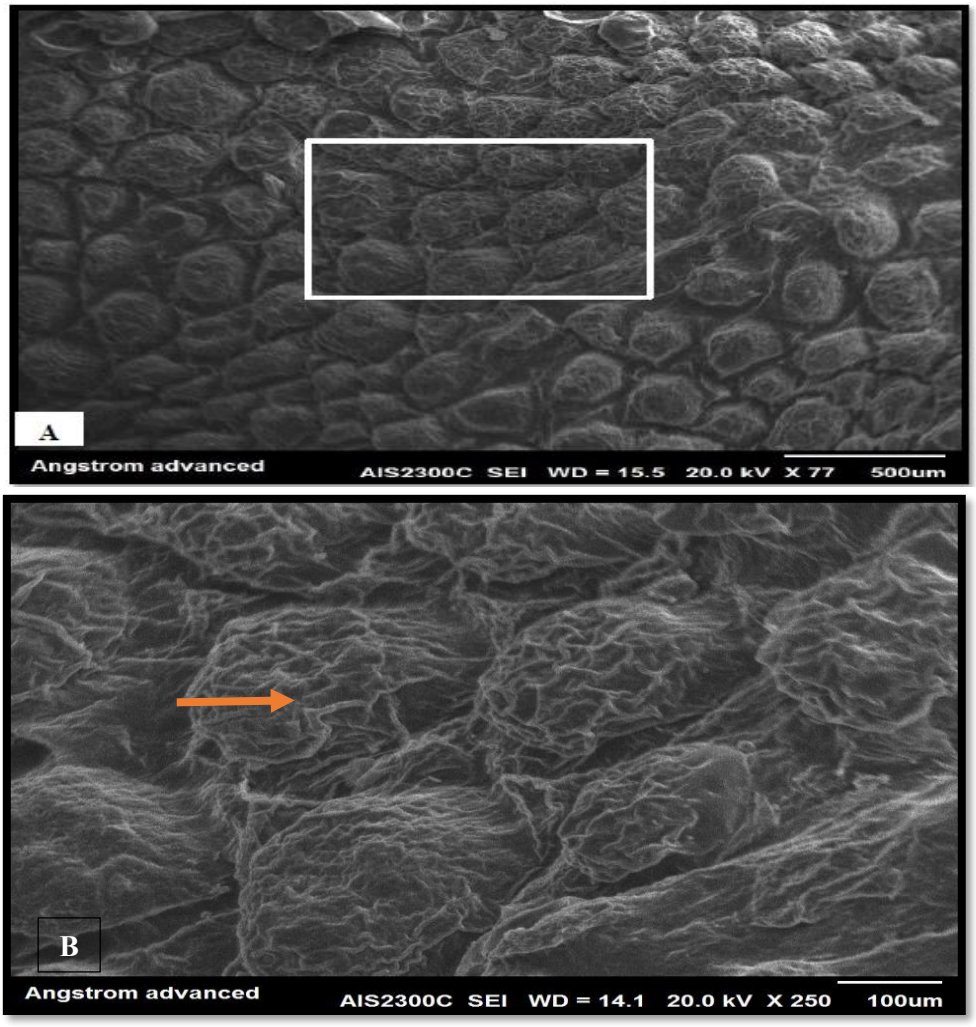

Figure 2: Scanning electron micrograph of a part of skin in the *Bufo viridis* showing that the superficial layer of skin consists of irregular-shaped epithelial cells (orange arrow), A (77x), B (250x).

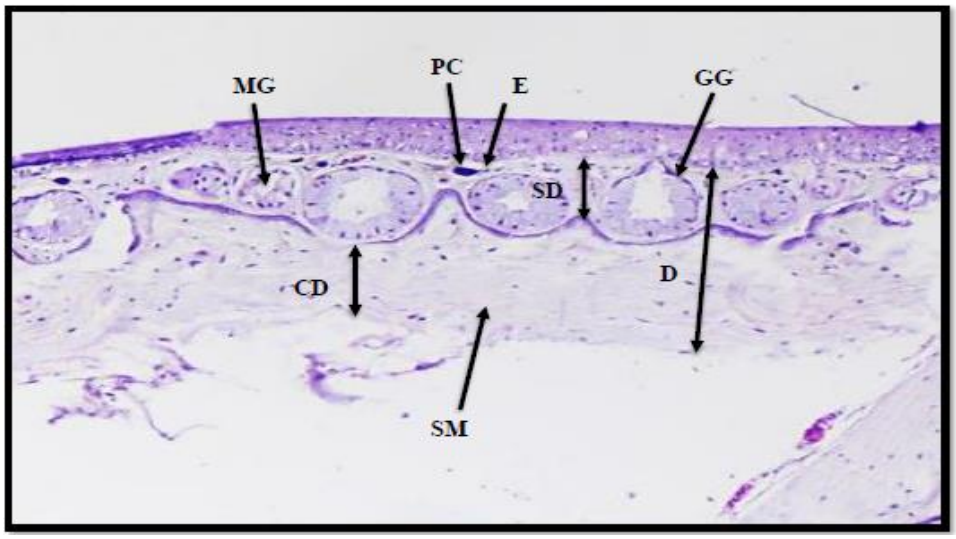

Figure 3: Longitudinal section through the frog dorsal skin side showing distribution of glands: (E) epidermis, (D) dermis, (MG) Mucous gland, (GG) granular gland, (SD) spongious dermis, (CD) compact dermis, (SM) Smooth muscles, Pigment cell (PC) (H&E, 10x).

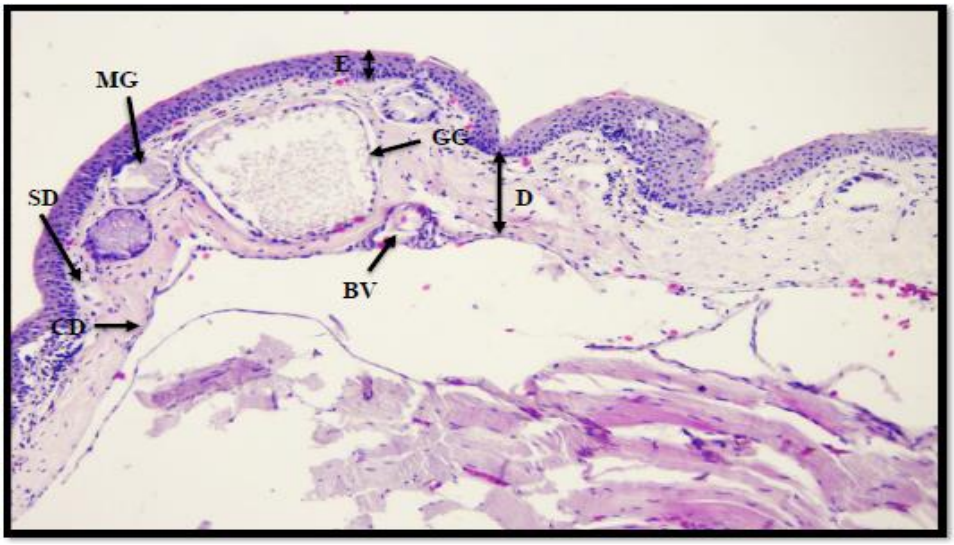

Figure 4: Cross section through the frog ventral skin side showing the frog ventral skin side, and the distribution of glands: (E) epidermis, (D) dermis, (MG) Mucous gland, (GG) granular gland, (SD) spongious dermis, (CD) compact dermis, (BV) Blood vessel. (H&E, 10x).

4. Conclusion

The skin tissue examined exhibits a typical two-layered structure, consisting of epidermis and dermis. The epidermis is composed of stratified squamous epithelium with three distinct strata: stratum corneum, stratum spinosum, and stratum germinativum. Notably, no dermal papillae were observed, which may indicate a smooth epidermal-dermal interface. In contrast, the dermis is comprised of loose connective tissue and divided into two layers: a spongy layer, which contains alveolar glands, mucous glands, granular glands, and a compact layer, which likely provides structural support. Pigment cells were present in the dorsal skin and dermis, suggesting a role in coloration or protection against UV radiation.

Conflict of interest

The authors declare that they have no conflicts of interest.

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