**Alexandria Journal of Veterinary Sciences**
www.alexjvs.com

**AJVS. Vol. 53: 1-10. April 2017**
*DOI: 10.5455/ajvs.261838*

# Micro-Morphological investigation of the Skin of the Larval and Adult Stages of the African Catfish (*Clarias gariepinus)*

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**Key words:**
Catfish, Skin, Immunohistochemistry, SEM, TEM

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## Abstract

The current study aimed to explore the skin sructure at the larval and adult stages of the African catfish. Of the previous studies on the skin morphology of fish, none has researched the detailed skin differentiation at the larval and adult stages of the African catfish. Here by light microscopic investigation, the skin of the larva after hatching consisted of two layers of simple squamous epithelium. The skin of the 5-6 day old larva consisted of more than one layer of flattened to oval cells, continuous layer of melanocytes and club cells showed up. The skin of the 15-days old larva distinguished into epidermis, dermis and hypodermis. While the skin of the adult catfish, the epidermis consisted of three layers a basal layer of cuboidal or columnar cells, middle layer of polyhedral cells and surface layer of flattened cells. Its mucous cells were oval in shape having foamy cytoplasm. Its club cells had acidophilic cytoplasm and some cells were binucleated. At the lateral line, a group of elongated neuromasts viewed between the club cells. Langerhans like cell showed a positive reaction with CD 1a. The dermis divided into stratum spongiusm and stratum compactum. At lateral line beneath the hypodermis, the neuromast canal detected. By SEM, no any evidence of taste buds pores and neuromast detected at the newly hatched larva. Different forms of neuromast appeared on the surface as small epidermal protrusions at the 6-day old larva. In addition, the neuromast at the epidermal protrusion appeared with long and short hair bundles. While, the skin of the adult catfish, the surface epithelium showed many projections, the broken epidermis showed a large club cells, melanocytes with cytoplasmic processes and pores. By TEM of the adult catfish, the surface cells had (microridges), desmosomes distinguished between the neighboring epidermal surface cells. The club cells cytoplasm had scanty organelles, two types of club cells identified; the first type with no vesicular secretion and the second type with fibrillar cytoplasm. The plasma membrane showed many invaginations. The study recorded two types of neuromast; superficial neuromast and canal neuromast set in the dermis. No neurmast detected at the 1st day larva afterthat it appeared and incresead in number and its shape changed from simillar hair cells to long and short hair bundles.

## 1. INTRODUCTION

Teleost skin in special was unique and histologically divergent (Fast et al., 2002). It was distinct from that of mammals, because it secreted mucus which winded in immune functions (Salinas et al., 2011). The skin of fish is the first line of defense against invading pathogens and the cutaneous diseases are common in fish than in vertebrates (Groff, 2001).

The skin of the catfish (*Clarias gariepinus*) consisted of three layers epidermis, dermis and hypodermis. The epidermis comprised of stratified aquamous epithelium with many malpigian, club and mucus cells and the dermis composed of stratum

adiposum or spongiosum and compactum (El Zoghby et al., 2016).
The lateral line system always contains mechanoreceptors and often electroreceptors (Webb, 1989). The superficial neuromasts contained few hair and support cell and were smaller than canal neuromasts (Northcutt and Bleckmann, 1993), while Dijkgraaf (1963) stated that the superficial neuromasts distinguished within canal neuromasts or on papillae and they appeared by projections of cupula whether they were in grooves or pits.
Many researches focused on the use of the fish skin secretions in wound healing in animals and foot ulcers in human and this point need more studies to explain the mechanism of wound healing by theise secretions (Al-Hassan et al., 1985; Al-Hassan, 1989).
The study aimed to discover the morphology of the African catfish the skin distinguishing from larval to adult stages. The study focused on the head, trunk and lateral line regions with special reference to the pattern of the sensory organs distribution and immunohistochemistry of langerhans like cells.

## 2. MATERIAL AND METHODS

### 2.1. Samples

Nine larvae of the African catfish (*Clarias gariepinus*) three after hatching, three at the 5-6-days old larvae, three at the 15-days old larvae and six of the adult collected from the local fish hatchery at the Faculty of Veterinary Medicine, Alexandria University, Egypt.

### 2.2. For light microscopy

The whole larva and small pieces from the skin of the trunk and head of the adult catfish were fixed in 10% neutral buffered formalin for 48hrs and dehydrated in ethyle alcohol ascending grades. Then were cleared in xylene and embedded in paraffin. The paraffin blocks were cut at 6 µm slices thickness and stained by harris hematoxylin and eosin stain (H&E) (Harris, 1900).

### 2.3. For scanning electron microscopy

The whole larva and small pieces from different parts of the skin of the trunk and head of the adult catfish were immediately immersed in a fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) at 4°C. Once fixed, the samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, and finally dehydrated in increasing concentrations of ethanol (15 min each in 50, 70, 80, 90, 95 and 100% ethanol). The samples were critical point dried in carbon dioxide, attached to stubs with colloidal carbon and coated with gold palladium in a sputtering device. Specimens were examined and photographed with a JEOL scanning electron microscope operating at 15 Kv.

### 2.4. For transmission electron microscopy

Small cubes (1 $mm^3$) of the skin of the trunk of the adult catfish were immediately fixed in 6% solution of phosphate-buffered glutaraldehyde, pH 7.4, at 4°C for 6 hrs (McDowell and Trump, 1976). After initial fixation, tissues were washed in several changes of cold (4°C) 0.1 M phosphate buffer every 15 min for 2 hrs. Samples were then rapidly dehydrated through increasing concentrations of ethanol, transferred to propylene oxide and placed over-night in a 1:1 mixture of propylene oxide and epoxy araldite. Semi-thin sections (1 mm) were first cut and stained with toluidine blue and viewed with light microscopy to specify areas suitable for transmission electron microscopy. Ultrathin sections (60–100 nm) were then cut by a glass knife with an L.K.B. micro-tome and stained with uranyl acetate followed by lead citrate (Hayat, 1986). The ultrathin sections were examined with a JEOL transmission electron microscope operating at 100 Kv.

### 2.5. Immunohistochemistry

Paraffin sections of 5 micrometre were prepared on positive charged microscope slides. Sections were deparaffinised in xylene, rehydrated in descending grades of ethanol then distilled water and rinsed in phosphate buffered saline. Antigen retrieval was done by heating the tissue section in 10mM citrate buffer, PH 6.0 for 10 minutes followed by cooling at room temperature for 20 minutes. Monoclonal Mouse Anti-Human CD1a (Dako Agilent Technologies, (Dako, 2012) is used for identification of langerhans' cells. The reaction located in the membrane and cytoplasm.
This study followed the guidelines for the care and use of animals and it was approved by the animal welfare and Ethics Committee of the Faculty of Veterinary Medicine, Alexandria University according to the Egyptian´s laws.

## 3. RESULTS

### 3.1. Histolog and Immunohistochemistry

After hatching, the larval skin consisted of one layers of simple squamous epithelium covering the entire larvae, their nuclei varied in shape from

flattened to oval (Figs.1-2). At the 5-6-day old larvae, the skin consisted of more than one layer of flattened to oval cells, continuous layer of melanocytes appeared under the basal lamina of epithelium. The club cells showed up with acidophilic cytoplasm (Figs. 3-4). At the 15-day old larvae, the skin distinguished into epidermis, dermis and hypodermis. The epidermis composed of stratified squamous epithelium (Fig. 5). In the adult catfish, the skin comprised of epidermis, dermis and hypodermis (Fig. 6). The epidermis contained three layers: a basal layer of cuboidal or columnar cells, middle layer of polyhedral cells and surface layer of flattened cells. The melanocytes distinguished between the middle polyhedral cells (Figs. 6-7). The basal cells distinguished cuboidal or columnar and had spherical basophilic nucleus. The surface cells were oval to flatten in shape with oval darkly stained basophilic nuclei, the surface cells arranged in 3-4 layers (Fig. 8). The mucous cells viewed among the surface cells oval in shape and had foamy cytoplasm. The club cells (alarm cells) found between the intermediate cells, they were oval, rounded to elongated in shape with acidophilic cytoplasm and some cells were binucleated (Figs. 8-9). Langerhans like cells appeared among the surface cells (Fig. 10) and showed positive reaction with CD 1a (Fig. 11A). At the lateral line, a group of elongated neuromast cells appeared between the club cells (Fig. 11b). The dermis comprised of stratum spongiusm and stratum compactum. The stratum spongiosum composed of loosely arranged collagen fibers, many melanocytes and blood vessels, while the stratum compactium comprised of densely packed collagen fibers. The hypodermis consisted of white adipose tissue (Fig. 12A). At the lateral line beneath the hypodermis neuromast canal distinguished (Fig. 12B).

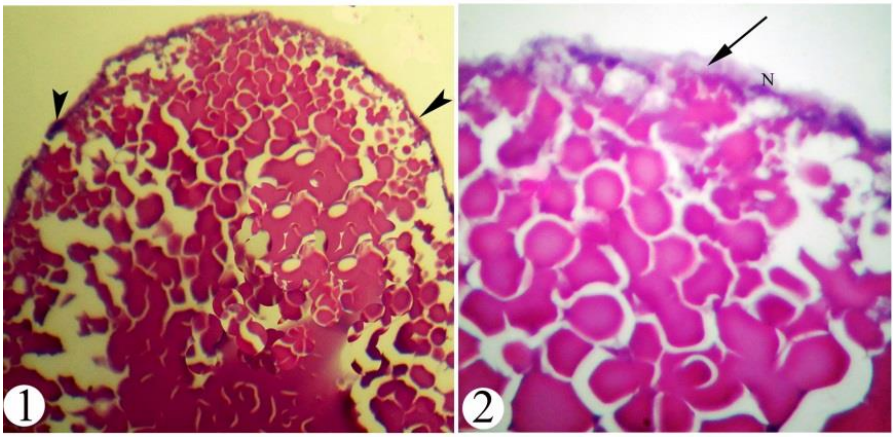


Fig.1. Light photomicrograph of the larvae of the catfish after hatching showing the skin consists of one layers of simple squamous epithelium (arrowhead). Micro.Mag.x100. Stain H&E.
Fig. 2. Higher magnification of the fig.1 showing the simple squamous epithelium (arrow) and nucleus (N). Micro.Mag.x400. Stain H&E.

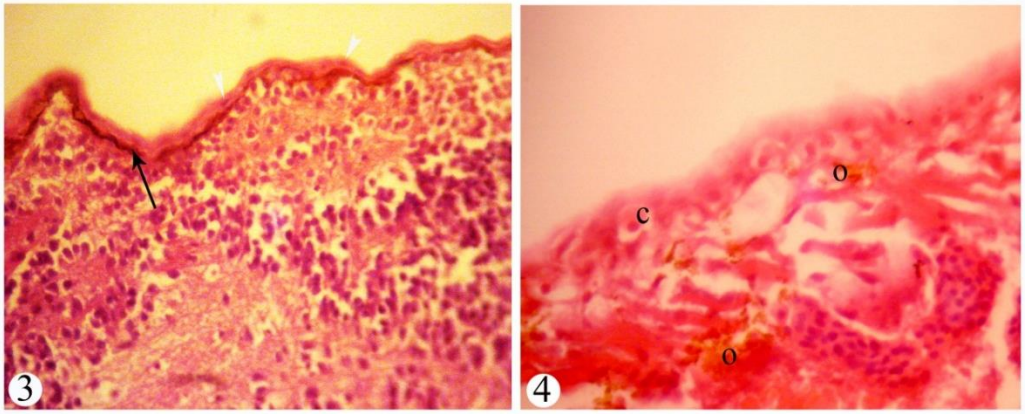


Fig. 3. Light photomicrograph of the 5-6 day old larvae of catfish showing the skin consists of more than one layer of flattened to oval cells (arrowhead) and melanocytes (arrow). Micro.Mag.x100. Stain H&E.
Fig, 4. Another light photomicrograph of the 5-6 day old of the larvae of catfish depicting club cell (C) melanocytes (O). Micro.Mag.x400. Stain H&E.

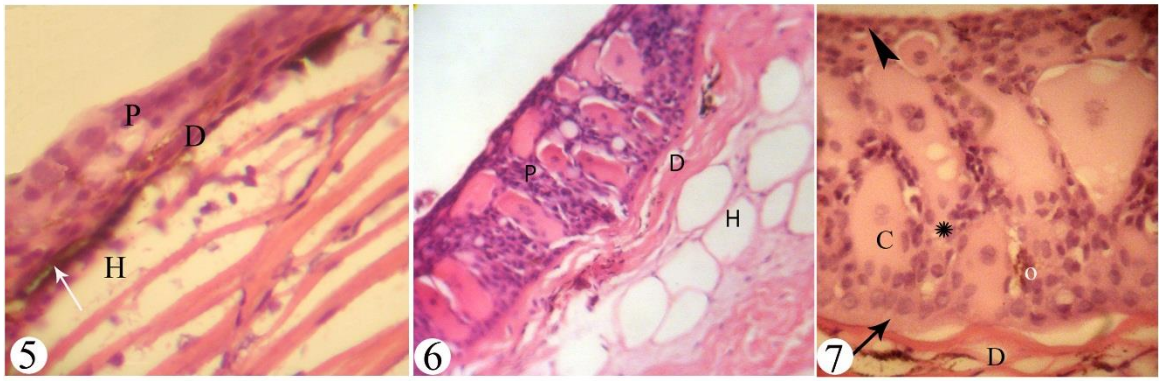


Fig. 5. Light photomicrograph of the 15 day old of the larvae of catfish showing the epidermis (P), dermis (D), hypodermis (H) and melanocytes (arrow). Micro.Mag.x1000. Stain H&E.

Fig. 6. Light photomicrograph of the skin of the adult catfish showing epidermis (P), dermis (D) and hypodermis (H).Micro.Mag.x100.Stain.H&E.

Fig. 7. Another photomicrograph of the skin of the adult catfish depicting basal cell of epidermis (arrow), middle polyhedral cell (asterisk), flattened surface cell (arrowhead), dermis (D), melanocytes (O) and club cells (C). Micro.Mag.x1000.Stain.H&E.

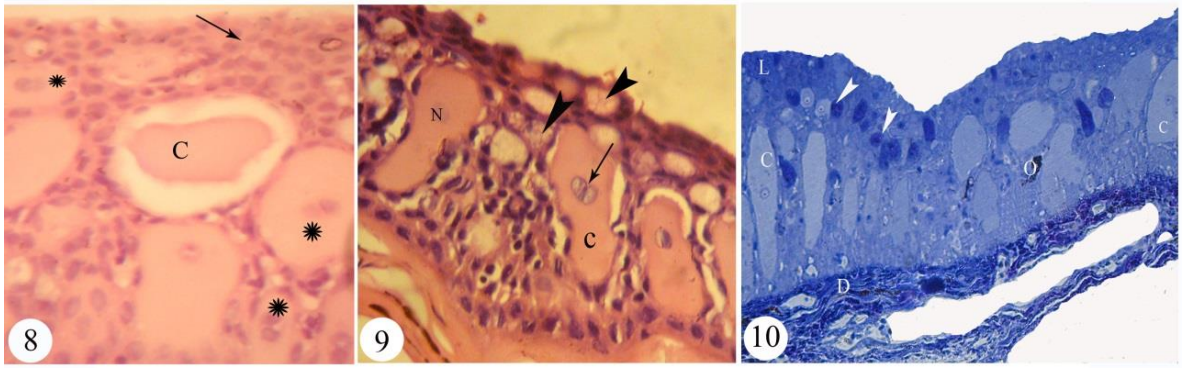


Fig. 8. Light photomicrograph of the skin of the adult catfish depicting surface cell of epidermis (arrow) and club cells (C). Note the binucleated club cell (asterisk). Micro.Mag.x1000.Stain.H&E.

Fig. 9. Light photomicrograph of the skin of the adult catfish showing mucus cell (arrowhead), nucleus (N) and club cells (C). Note the binucleated club cell (arrow). Micro.Mag.x400.Stain.H&E.

Fig. 10. Light photomicrograph of the skin of the adult catfish showing club cells (C), Langerhans like cells (L), melanocytes (O), mucus (arrowhead) and Dermis (D). Micro.Mag.x100.Stain.Toludine blue stain.

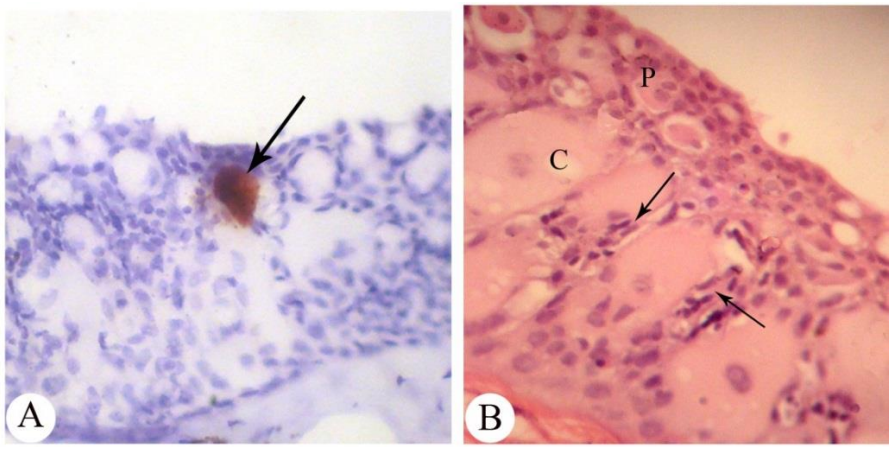


Fig. 11. Light photomicrograph of the skin of the adult catfish showing positive reaction of langerhans like cells (arrow). Micro.Mag.x400.Stain.CD 1a. (B) Light photomicrograph of the skin of the adult catfish at the lateral line depicting epidermis (P), neuromasts (arrow) and club cell (C). Micro.Mag.x1000.Stain H&E.

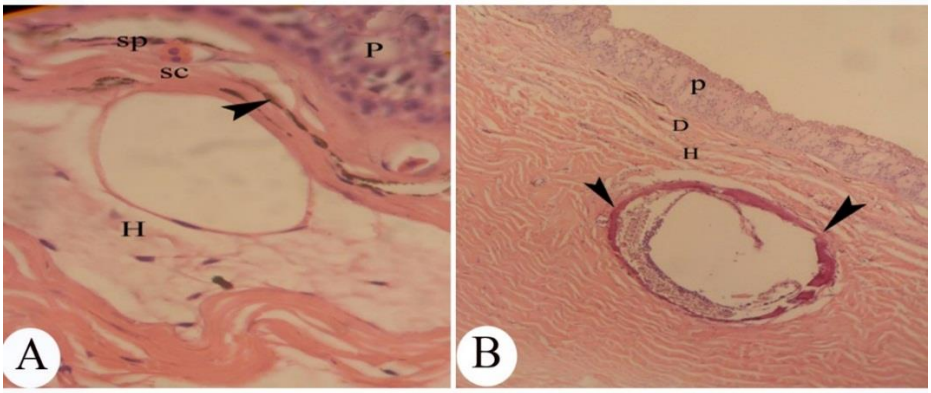


Fig. 12. Light photomicrograph of the skin of the adult catfish showing epidermis (P), stratum spongiosum (sp), stratum compactum (sc), melanocytes cells (arrowhead) and hypodermis (H). Micro.Mag.x1000.Stain H&E. B. light photomicrograph of the skin of the adult catfish lateral line showing epidermis (P), dermis (D), hypodermis (H) and neuromast canal (arrowhead). Micro.Mag.x100.Stain H&E.

### 3.2. SEM findings

SEM at the first day old larva, the lateral line viewed, polygonal shape cells appeared on the skin and there was not any evidence of taste buds pores or neuromasts at this age (Fig. 13).

SEM at the head region of the 6-day old larva showed different forms of neuromast that appeared on the surface as small epidermal protrusions. The supraorbital groove showed the neuromast in pores and on the papillae while the lateral line showed the neuromast at pits, however the neuromast at epidermal protrusion appeared with long hair bundles and short hair bundles (Fig.14A-D). The neuromast in pores or on papillae were small and round, while that on the epidermal protrusion was large and oval with demarcated hair cell bundles, many microvilli distinguished around the hair cell bundles (Fig.14/C).

SEM of the skin of the head region at the 15-day larva showed many pores containing neuromast on the lateral line (Fig.15).

SEM at the skin of the head region of the adult catfish, the surface epithelium showed many projections (Fig.16A), the broken epidermis showed a large club cells, melanocytes with cytoplasmic processes and pores (Fig.16B). The lateral line of the adult catfish had a prominent undulating surface with many projections and grooves, neuromasts and pores contained neuromast (Fig.17A). The neuromasts appeared well developed and had different sizes of hair bundles (Fig.17B).

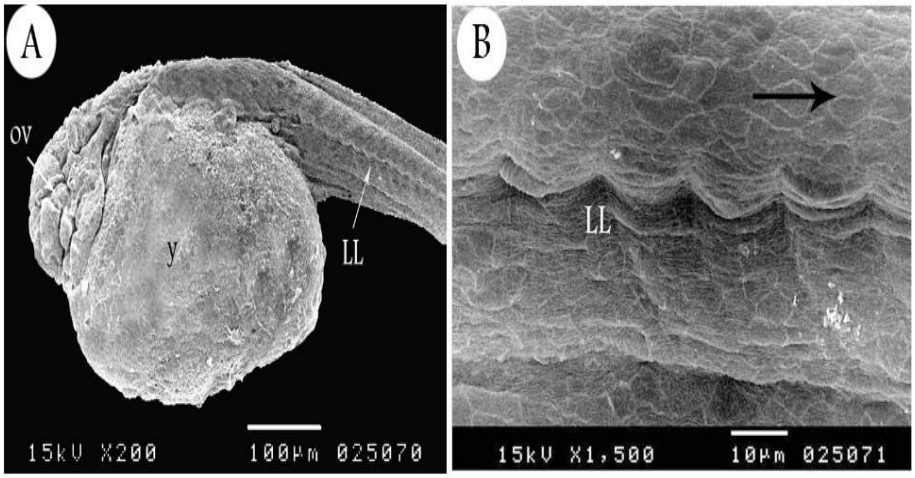


Fig.13. SEM of the one-day-old larva skin showing lateral line (LL), yolk sac (Y), eye (ov) and polygonal shape cells cover the skin (arrow).

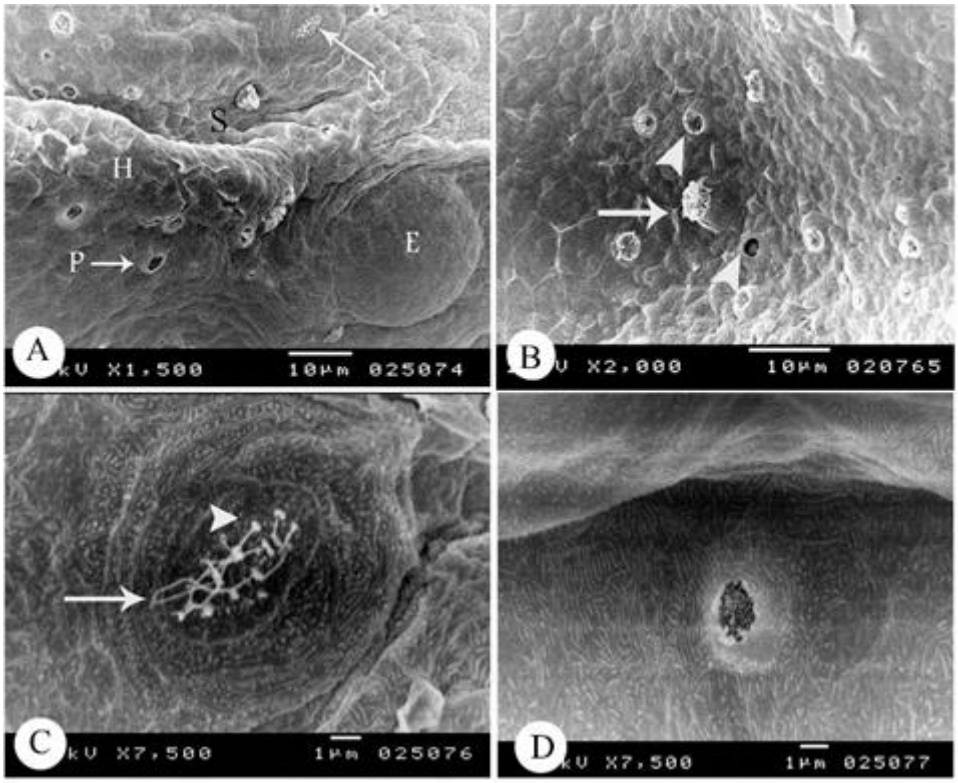


Fig.14. SEM at the skin of the 6 day old larva. (A) At the head region, eye (E), supraorbital groove (S), neuromast (N), pores contain neuromast (P), polygonal cell shape epithelium (H). (B) The enlarged area of the supraorbital groove showed the neuromast in pores (arrowhead) and on the papillae (arrow). (C) Neuromast in the epidermal protrusion with long hair bundles (arrow) and short hair bundles (arrowhead). (D) Neuromast in pores on the lateral line.

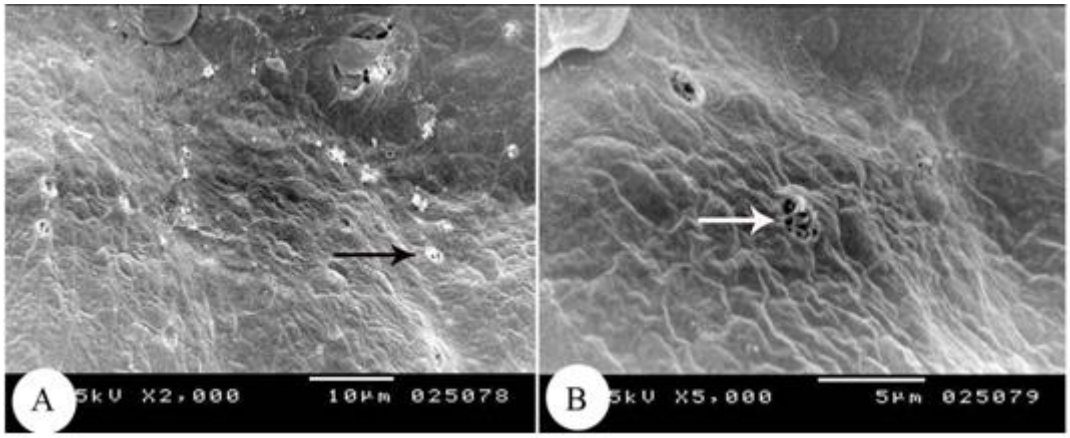


Fig. 15. SEM at the skin of the head region of the 15-day-old larva numerous pores containing neuromasts on the lateral line.

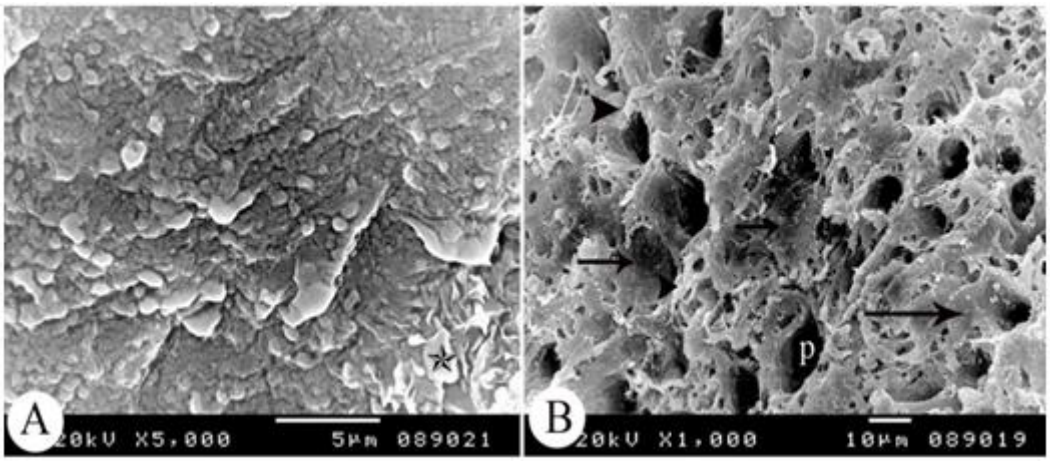


Fig.16. SEM at the skin of the head region of the adult catfish. (A) the surface epithelium showed many projections (star). (B) The broken epidermis indicated a large club cells (arrow), melanocytes with cytoplasmic processes (arrowhead) and pores (p).

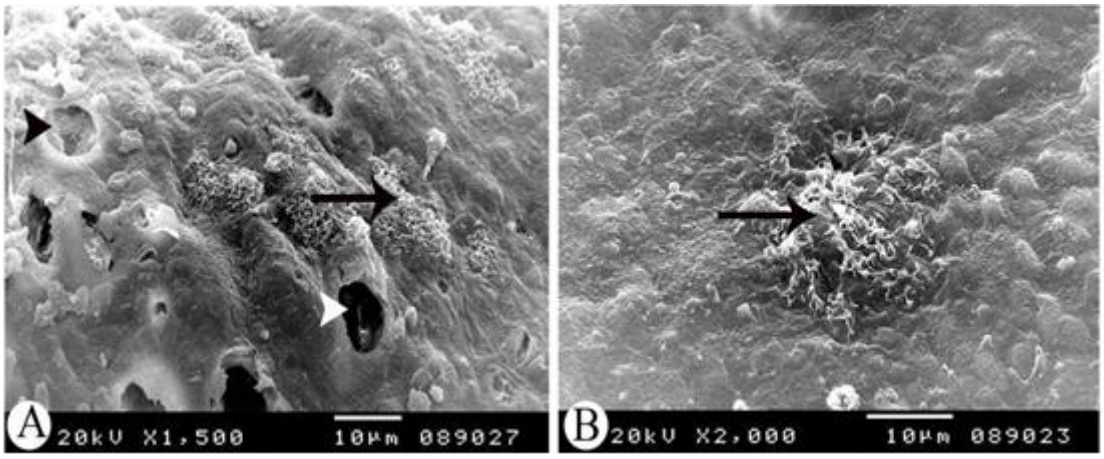


Fig. 17. SEM at the lateral line of the adult catfish. (A) The surface showed many projections and grooves, neuromasts (arrow) and pores contained neuromast (arrow head). (B) Neuromast with different sizes hair bundles (arrow).

### 3.3. TEM findings

By TEM, the surface cells were oval to cuboidal in shape, their cytoplasm occupied by many mitochondria, free ribosomes, rough endoplasmic reticulum and lysosomes. The free surface cells covered by many microridges. Desmosomes distinguished between the neighboring epidermal surface cells. In addition, the interdigitations found between the epidermal surface cells and mucus cells. Their nuclei were large irregular in shape and centrally found, they had euchromatin and indented nuclear membrane (Figs.18-19).

The club cells cytoplasm had scanty organelles, two types of club cells identified. The first type with no vesicular secretion was the present type. The second type with fibrillar cytoplasm rarely found, large to small vacuoles found in the periphery of the cytoplasm.

The plasma membrane displayed invaginations throughout its length, making the cell surface irregular and associated with the epidermal cells (Figs.20-21). The melanocytes found between the club cells that had irregular shape. They had branching processes extended between the neighboring cells. Their cytoplasm occupied with melanin granules that were of various electron density (Fig. 21).

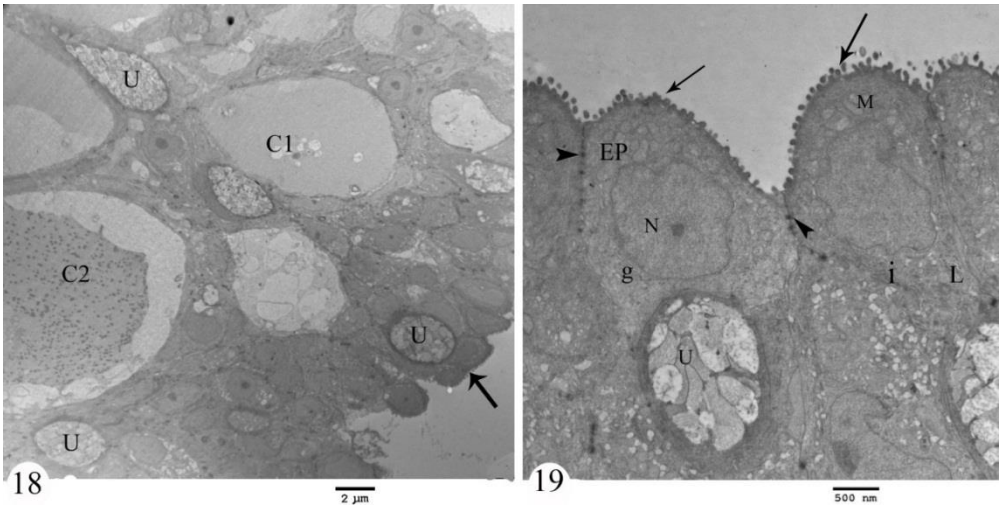


Fig. 18. Transmission electron micrograph of the epidermis of the adult catfish showing the epidermal surface cells (arrow), club cell type1 (C1), club cell type 2 (C2) and mucus cell (U). X 500.

Fig. 19. Higher magnification TEM of the previous photo depicting the epidermal surface cells (EP), mitochondria (M), rough endoplasmic reticulum (g), lysosomes (L), desmosomes (arrow head), interdigitations (i), nucleus (N), microridges (arrow) and mucus cell (U). X 2500.

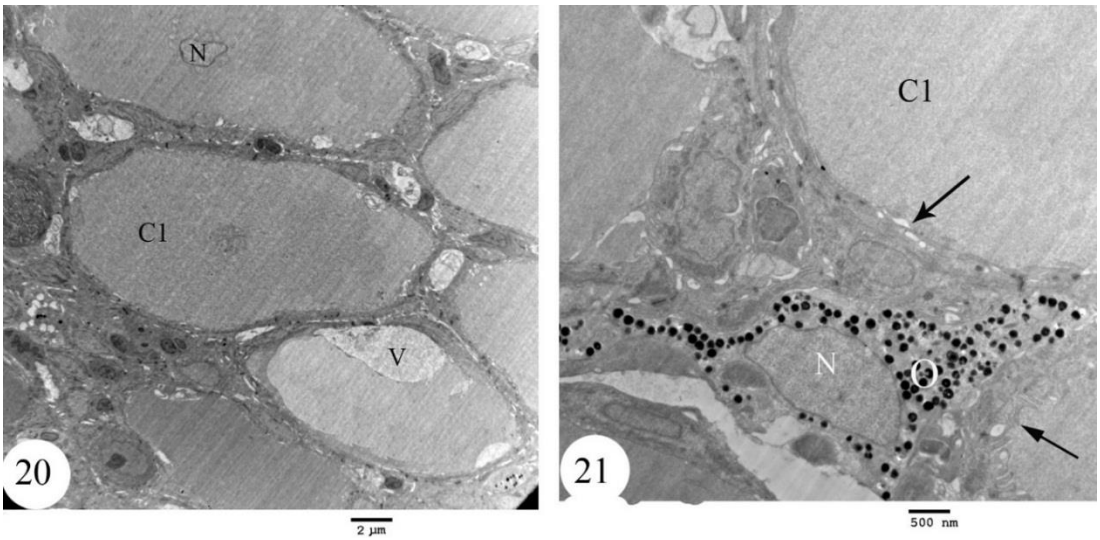


Fig. 20. Transmission electron micrograph of the club cell type1 (C1) showing vacuoles (V) and nucleus (N). X500.

Fig. 21. Another TEM of the club cell type1 (C1) showing invagination of the cell membrane (arrow), melanocytes (O) and nucleus (N). X2000.

## 4. DISCUSSION

The present study is the first description of the detailed skin at the larval and adult stages of the African catfish by immunohistochemistry, SEM and TEM.

The present study showed the epidermis of the adult catfish contained three layers basal cuboidal or columnar, middle polyhedral cells and surface flattened cells simillar results recorded by (El Shafey and Easa, 1979; El Zoghby et al., 2016) in *cyprinus carpio*. While, Bullock and Roberts (1974) noted that the teleost fish epidermis copmprised of five stratum, basalis, spinosum, granulosum, lucidum and corneum.

Our results recorded the free surface cells covered by many microridges like in ictalurids and nematogenys species the surface cells carried villus like microridges (Arratia and Gayet, 1995). Yamada (1968) conceded the microridges on the epidermal surface an adaptive way to stress produced by osmatic pressure gradient between the cells and water, phyiscal forces between water it self-and rocks. While, Reutter (1978) suggested the microridges act as cutaneous respiration, also it had a role in holding mucous secretion to the skin surface.

Our investigation revealed the club cells (alarm cells) were oval, rounded to elongated in shape with acidophilic cytoplasm and some cells were binucleated. In the Korean bullhead *(Pseudobagrus brevicorpus*), the club cells were globular to elongated in shape (Park et al., 2010). While, in African sharptooth catfish *(Clarias gariepinus)* it was elongated in shape (Cernuda-Cernuda and García-Fernández, 1996). By TEM, the club cells plasma membrane had invaginations throughout its length unlike that noted by Henrikson and Matoltsy (1967) in *corydorasaeneus* (*Siluriformes*) and *carassius auratus* (*Cypriniformes*), that confer cell adhesion, essential to the epithelium that usually subjected to the pressure and friction (Damasceno et al., 2012). The club cells related to the production, storage and release of the alarm substance, leading to intra or interspecific alarm reaction in phylogenetically close species (Smith, 1992; Smith, 1997). In ostariophysi, the alarm reaction triggered when individuals threatened or preyed on his injured epidermis. This event causes disruption of the club cells cytoplasmic membrane, resulting in exposition and releasing of cytoplasmic content into the water (Wisenden, 2000). Langerhans like cells showed positive reaction with CD 1a and it found between the surface cells. The cellular immune response predominate in the epidermis, langerhans cells hold their suprabasal position, scanning their environment for antigens or danger signals (Ángeles Esteban, 2012).

In our study, two types of neuromast detected; superficial and canal neuromast embedded in the dermis. The neurmast increased in number and distinguished into long and short hair bundles, as noted by Ghysen and Dambly-Chaudiere (2004) in zebra fish. The lateral line is sensory that allows fish to sense objects and motion in their local environment (Wark and Peichel, 2010). The lateral line always contains mechanoreceptors and often, but not always, electroreceptors (Webb, 1989). The lateral line divided into two subsystems; mechanoreceptive neuromasts and electroreceptive ampullary and tuberous organs. The hair cells are basic transducers for sound, vibration and in determining position in vertebrates (Kornblum et al., 1990). The support cells encircle the hair cells and secrete a gelatinous-like material "cupula'' which covers the whole neuromast. The cupula enables the organ to communicate with the exterior (Cernuda-Cernuda and García-Fernández, 1996).

In conclusion, the skin of the African catfish showed some pecularities through the larval and adult stages than other fish spescies, as the layers number of the epiderms. The mucous cells, neuromast, club cells and the free surface microridges were unique to catfish.

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