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Original Article

Histological structure of the skin in five fish species of Periophthalmus waltoni (Gobiidae) *Silurus triostegus* (Siluridae), *Heteropneustes fossilis* (Heteropneustidae), *Mastacembelus mastacembelus* (Mastacembelidae) and *Coptodon zilli* (Cichlidae)

Raghad I. Abed[1], Azal N. Al-Nusear[*2], Saad M.S. Abdulsamad[3]

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Basrah, Basrah, Iraq.
[2]Department of Anatomy and Histology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.
[3]Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq.

Abstract: The five fish species viz. *Periophthalmus waltoni*, *Silurus triostegus*, *Heteropneustes fossilis*, *Mastacembelus mastacembelus*, and *Coptodon zilli* were collected in the Shatt Al-Arab River from February to June 2019. The samples were transferred to the laboratory to measure their total lengths and weights. To examine the skin histology and structure in these fishes, a skin sample was taken from the dorsal part close to the lateral line. The results showed that the skin of fish contains two layers: the first is the epidermis, which includes the epithelium. The thickness of the tissue in fish varied from 3 to 20 layers. In this layer, there are mucous cells that were spared and varied in their abundance from one species to another. The dermis includes two layers of stratum spongiosum and stratum compactum. These two layers consist of pigment cells that spread directly under the epidermis in a dark brown color. In *C. zilli* and *H. fossilis*, a third layer was found under stratum compactum as hypodermis; the highest thickness of the dermis was recorded in *C. zilli*.

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Introduction

There are many functions in fish skin e.g. it can be used for secretion, sense, maintenance of osmotic pressure, and a defense against microorganisms (Elliott, 2000; Dauod et al., 2009). The skin shows differences between fish species e.g. in having scales (Singh and Mittal, 1990). The skin is a secretory organ in which the cellular components can produce a wide range of biological factors (Damasceno et al., 2012). The function of goblet cells is to conceal mucus which helps the body keep its surfaces moist and protected (Gona, 1979; Yang et al., 2019). Club cells produce alarm substances that initiate their reaction. Pigments were produced from melanocytes to give the fish special coloration (Takeuchi, 1967). The skin is also considered a tool for coetaneous sense organs to detect food and predators. Amongst all these, there are lateral line systems and taste buds, which have electro-receptive organs and neuromasts (Jakubowski, 1974).

The fish skin includes 3 main strata: epidermis, dermis, and hypodermis. The cells that are involved in the epidermis have many different cell types, i.e., cuboidal or columnar cells in the base while in the middle there were polyhedral or superficial (flat) cells (Park, 2002a; Faílde et al., 2014). In some fishes, the epidermis is extensively comprised of five layers (Bullock and Roberts, 1974). Club and some mucous cells were found in Palembang puffer fish and catfish exactly in the intermediate layer (Hertwig et al., 1992; Park et al., 2003).

Iraqi water is rich in terms of biodiversity, especially the Shatt Al-Arab River draining to the Persian Gulf (Mohamed and Mutla, 2008). This study aimed to investigate the skin histological structure of five fish species viz. *Periophthalmus waltoni* (Gobiidae). *Silurus triostegus* (Siluridae), *Heteropneustes fossilis* (Heteropneustidae), *Mastacembelus mastacembelus* (Mastacembelidae), and *Coptodon zilli* (Cichlidae) collected from the Shatt Al-Arab River.

*Correspondence: Azal N. Al-Nusear
E-mail: azel.bader@uobasrah.edu.iq

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Table 1. Thickness of dermis and epidermis (μ) with standard deviation (±) of five studied fish species.

Species	Dermis (μ)	Epidermis (μ)
S. triostegus	39.285±11.338	32.857±11.852
H. fossilis	29.285±13.047	14.285±7.867
M. mastacembelus	40.349±11.112	44.223±12.338
P. waltoni	44.340±9.231	33.655±6.123
C. zilli	66.434±12.198	22.232±8.832

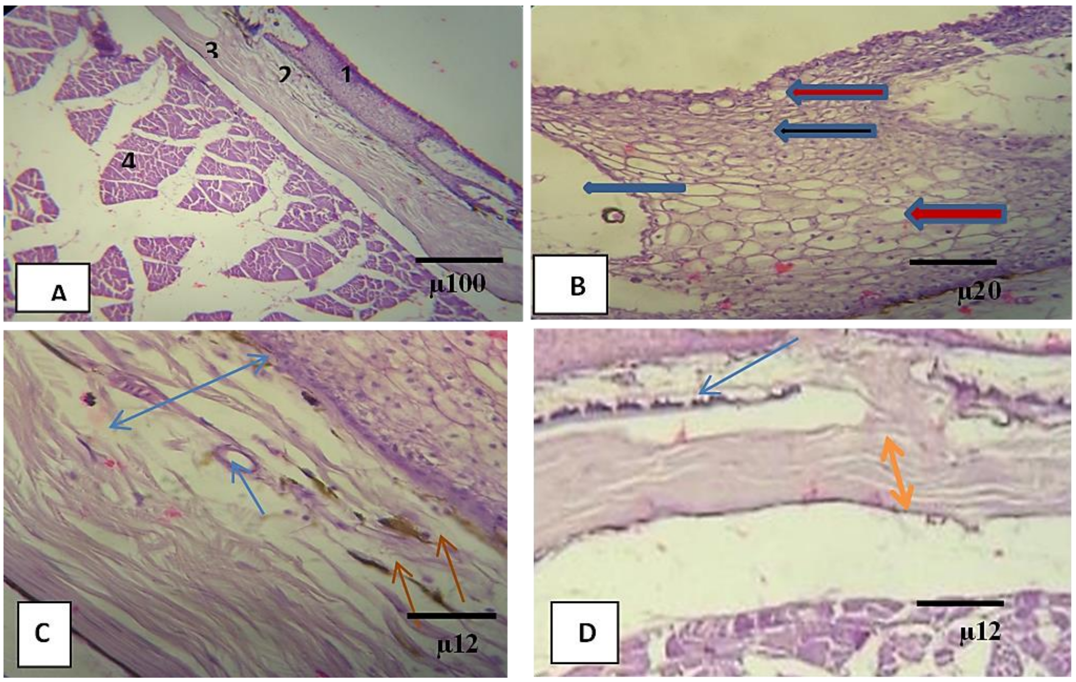

Figure 1. Cross-section of *Mastacembelus mastacembelus* skin. (A) 1: Epidermis, 2: Stratum spongiosum 3: Stratum compactum 4: Skeletal muscles, (B) non-keratinizing stratified squamous epithelium (black arrow), mucus cell (red arrow), scale position (blue arrow), (C) blood vessel (blue arrow), pigment cell (red arrow), stratum spongiosum (blue double-headed arrow), and (D) stratum compactum (orange double-headed arrow). scale: blue arrow (H&E).

Materials and Methods

Specimens of five species, including *P. waltoni* (Gobiidae; 6.1±2.2 cm TL), Wels catfish, *S. triostegus* (Siluridae: 31.6±1.94 cm TL), Fossil catfish *H. fossilis* (Heteropneustidae; 12.6±1.59 cm TL), *M. mastic-embelus* (Mastacembelidae; 25.1±1.3 cm TL), and *C. zilli* (Cichlidae; 10.1±2.3 cm T:) were from the Shatt Al-Arab River at Basrah from February to June 2019. Live animals were transported to the college laboratory. The total length of fish was measured using a vernier caliper, then they were fixed into 10% buffered formalin. A piece of skin (2x1.5 cm) from five species was removed from the dorsal region of the body near the lateral line, washed with tap water to remove the fixation solution, dehydrated progressively with ethanol, cleared with xylene and finally embedded in paraffin wax. The section of skin tissue was cut by microtome into 5 micrometers thickness. With hematoxylin and eosin, the sections were stained, examined under a light microscope, and photographed with a digital camera installed on the microscope (Bancroft and Gamble, 2008).

Results

In five examined fish species, the skin was composed of two layers: an epidermis and a dermis. Moreover, in some species, there is a third layer of subcutis or hypodermis. Some differences in the composition and thickness of layers were observed between species (Table 1). In *M. mastacembelus*, the epithelium has a non-keratinizing stratified squamous epithelium with 20-22 layers thickness. The mean thickness was

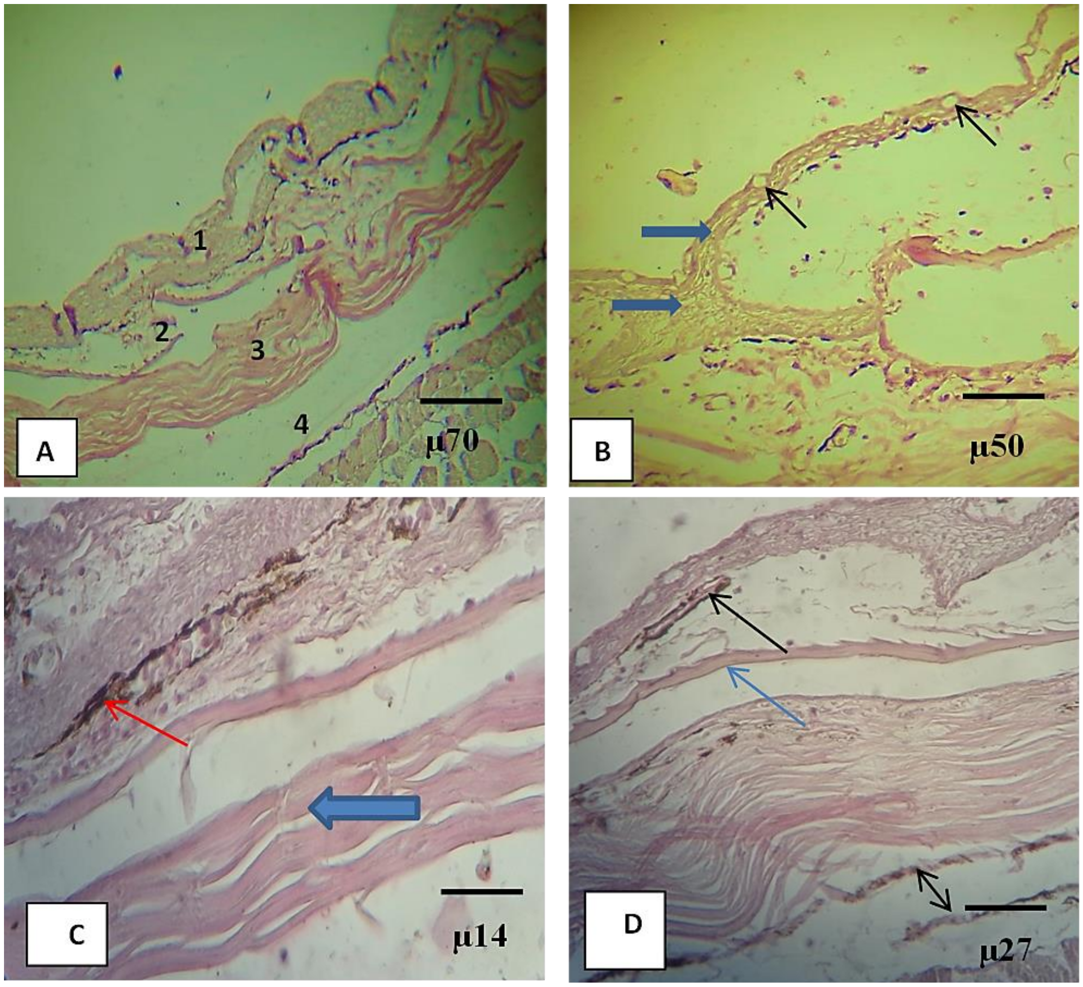

Figure 2. Cross section of *Periophthalmus waltoni* skin A: 1: Epidermis, 2: Stratum spongiosum, 3: Stratum compactum, 4: Subcutis. B: Epithelium (blue arrow), mucus cell (black arrow). C: Pigment cells (red arrow), collagen fibers (blue arrow). D: Pigment cells (black arrow), the scale (blue arrow), subcutis layer (black double headed arrow) (H&E).

44.223±12.338 μ; the shape of cells is rounded or flatted with a rounded purple, centered nucleus (Fig. 1). There were spaces between epithelium where the scales were positioned. The mucus cells diffuse among epithelial cells that are responsible for concealing primarily glycoproteins (mucus). The dermis with 40.349±11.112 μ thickness is divided into stratum spongiosum and stratum compactum. The stratum spongiosum contains loose connective tissue, collagen, reticulum fibers, nerves, capillaries, fibroblasts, and pigment cells (mainly melanophores). These cells were mainly distributed between the dermis (especially in the stratum compactum) and were deeply stained by H&E. The stratum compactum was more developed and formed by collagen.

Periophthalmus waltoni skin (Fig. 2) is composed of an epidermis possessing basal, middle, and outer layers. The superficial layer contained flattened cells and rounded white mucus cells. Pit organs appeared in dark purple and blood capillaries (very fined); their thickness was 33.65±6.123 μ. The was a basement membrane (thin basement) between the dermis, consisting of loose connective tissue and dense connective tissue. The stratum spongiosum contains collagen fiber bundles with blood capillaries and pigment cells with a dark brown color with the presence of blood capillaries and scales. The compactum includes a thick bundle of collagen fibers. The last layer of subcutis is located between the compactum and muscle bundles. There is a white space of fat cells among the nerves and blood vessels in this layer.

The three layers of the skin in *C. zilli* were the epidermis, dermis, and hypodermis, and the last one

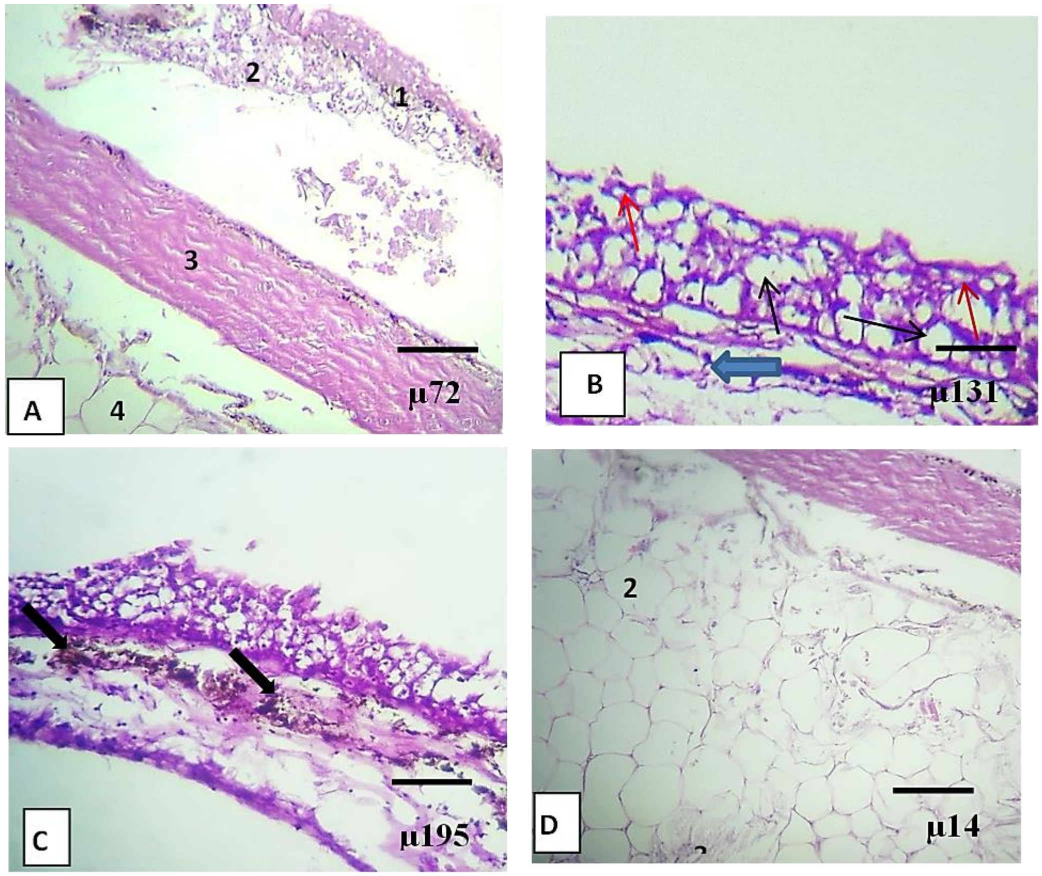

Figure 3. Cross section of *Coptodon zilli* skin. (A) 1: Dermis, 2: Stratum spongiosum, 3: Stratum compactum, and 4: Subcutis, (B) Epithelial cell (red arrow), mucus cell (black arrow) fibers (blue arrow), (C) pigment cells (black arrow), and (D) 1: Collagen fibers, 2: Fatty cell, and 3: Connective tissue (H&E).

was the thickest. The low epithelium contained rounded to flatted cells with dark purple nuclei, and lots of mucus cells (Fig. 3). Under the dermis, the two layers detected the first with loose connective tissue and plenty of melanocytes, described as stratum spongiosum and its beneath as stratum compactum with collagen fibers. The hypodermis contained fatty tissue with connective tissue.

In *H. fossilis*, the epithelial is the thinnest one compared to other studied species in this study which include squamous cells with 2-3 layers (Fig. 4). Beneath the above layer, there is the dermis consisting of nerve fibers, collagen fibers, blood vessels with different size and black color of melanocytes that give the appointed color to the fishes. in some sections, there was a disposed tissue under the dermis.

Siluris triostegus skin has a very high level of epithelial with different shapes of cells (rounded, club, oval, and longitudinal) and a thickness of 39.285±11.338 µ. The mucus cell was extinguished with a white color. The pigment cells, blood vessels, and nervous bundle all can be detected in the stratum spongium (Fig. 5).

Discussions

The thickness of epithelium in this study varied in five species, with 2-3 layers in *H. fossilis* and with many layers in others. In most fish species, the epidermis may vary in thickness, depending on some conditions like the region of the body, age, and environmental conditions where they inhabit (Elliott, 2011). In the studied fishes, the dermis is divided into two layers; the superficial loose connective tissue layer and it's beneath a layer of collagen fibers (arranged as bundles) in 3 species of *M. mastacembelus*, *P. waltoni*, and *C. zilli*, this result is agreed with findings of Le Guellec et al. (2004) and Failde et al. (2014).

In *S. triostegus* and *H. fossilis*, there were no boundaries between the two strata. Mittal and Munshi (1971) reported that in *Heteropneustes* (the

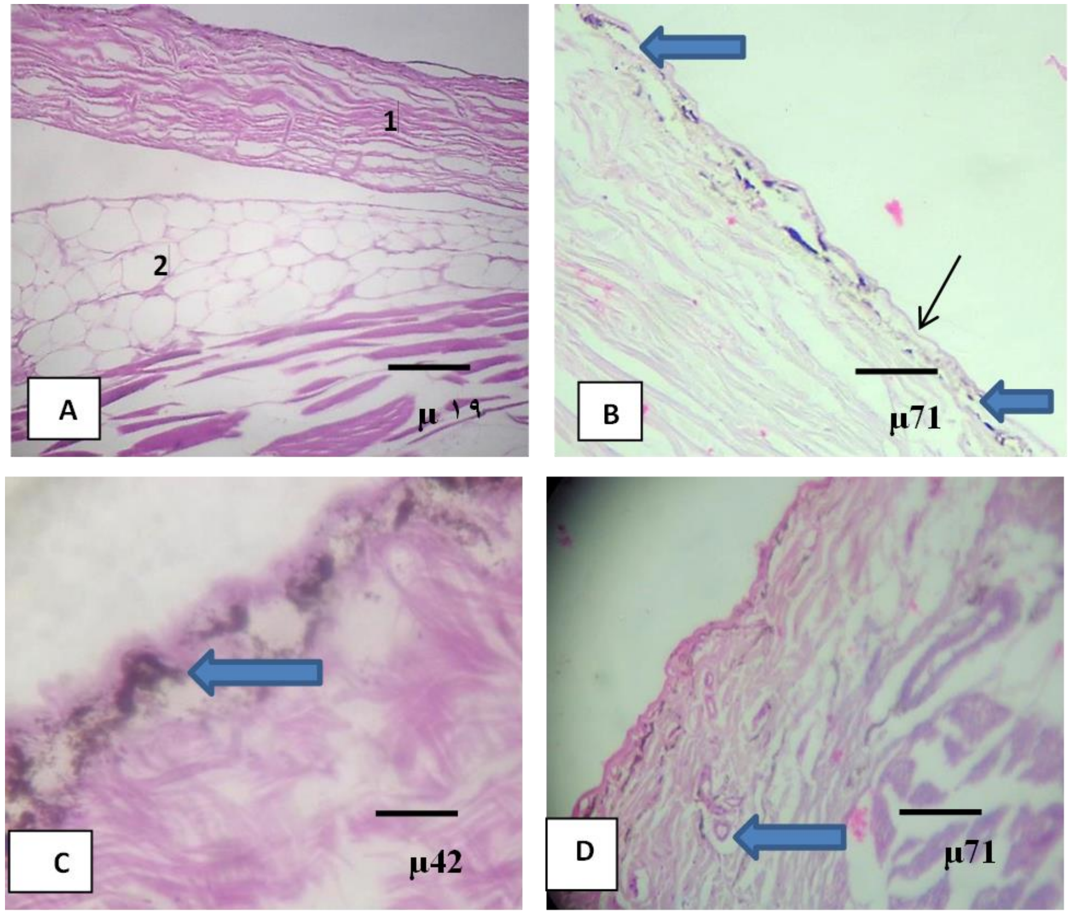

Figure 4. Cross section of skin in *Heteropneustes fossilis*. A: 1: dermis 2: hypodermis. B: mucus cell (blue arrow), epithelial cell (black arrow). C: pigment cells diffused under epithelium (blue arrow). D: blood vessels found between connective tissue in dermis (H&E).

integument in this species has no scales) there is no clear-cut differentiation between the laxum and compactum. The last layer beneath the basal membrane supplied capillaries and nerve fibers.

Zaccone et al. (2001) mentioned the difference in cell layers in epidermis which varies from 2 to 10 or more due to the age of fishes. In open water or pelagic fish species, the thickest epidermis was noted frequently in the dorsal areas of the body, while in the benthic fishes, the epidermis of ventral surfaces is always thick. The formation of the epidermis, the thickness, and the types of cells could probably be influenced by many factors among them the following: environmental condition, contain of cells, pathogens and stress, degree of maturation, and gender of fish, etc.

All 5 species showed dermis with pigment cells and melanophores (dendrites). These results agreed with the reports of Park (2002b) and Burton and Burton (2017). The color of the integument is dependent primarily on the presence of chromatophores in the skin. In different fishes from higher teleost to primitive cyclostomes, these cells were found in the dermis, or probably in both the stratum spongiosum and hypodermis, or in stratum spongiosum (Elliott, 2000).

The results of this study revealed that each species has mucus cells which differ in quantity and distribution. Club cells vary in shape from spherical to flask-shaped with fate cytoplasm and rounded nucleus (Archer, 1979; Rai et al., 2012). The shape of mucus cells had different shapes as reported in other studies (El Zoghby et al., 2017). Many differences in the skin structure of the five studied species because of the environmental conditions of their habitats and habits. Mohamed et al. (2010) pointed out that the ability of fish to adapt to environmental conditions in which they live in a way is compatible with the ability of the

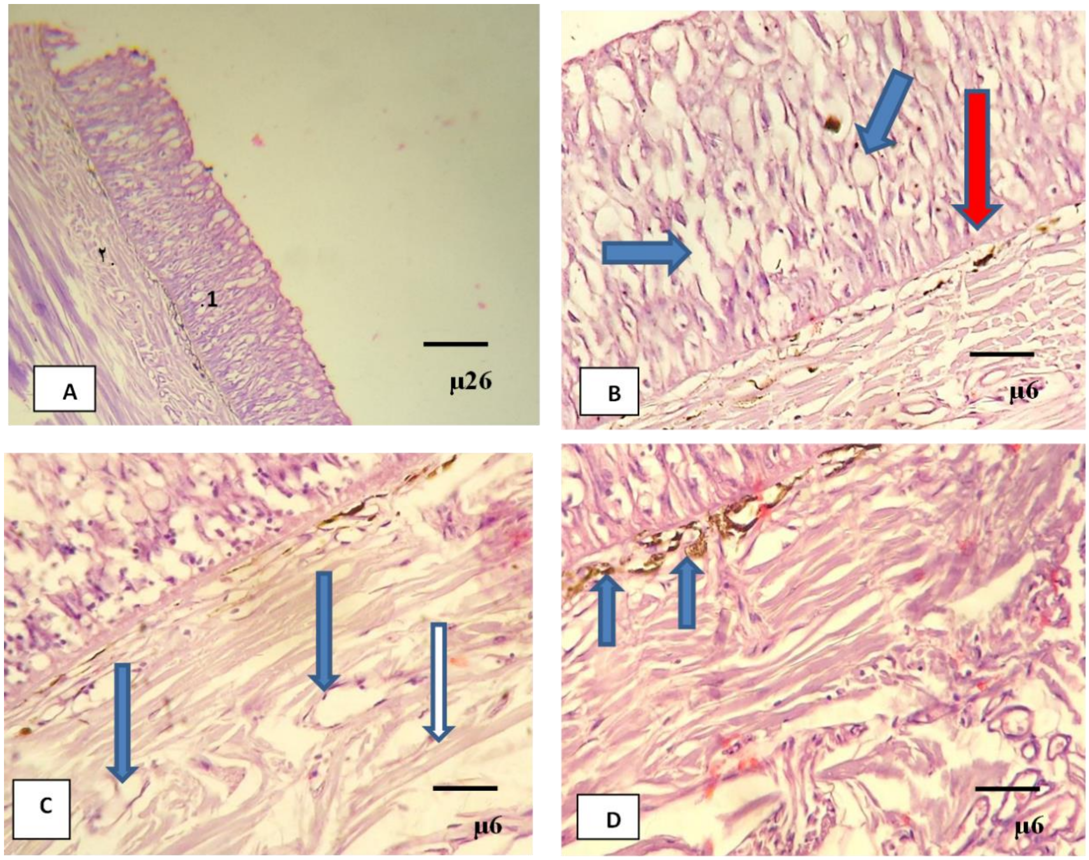

Figure 5. Cross section of skin of Silurus triostegus A: the two layers 1: epidermis 2: dermis B: the epithelium with multilayers and different cells mucus cells (blue arrow) basal layer (red arrow). C: section dermis with collagen fibers (blue arrow), blood vessels (white arrow) D: the shape of dark brown pigment cells (blue arrow) (H&E).

species and the continuous availability of food sources.

The thickness of skin and the distribution of taste buds differ in the five studied species. These variations depend on their physiological state, the environment in which the species live, the resistance against the pathogenic factors, and the fish-feeding nature (Farrell, 2011). Burton and Burton (2017) mentioned that adjacent to the surrounding water, could be expected that skin be resistant to thermal changes. The epidermis and mucus, production can respond very quickly to changes (thermal or chemical stress, or other changes). In anguillid eels the skin is used for respiration and it is an example of the use of the general cutaneous surface when it is out of water for air-breathing. The skin may also be used as a gas-exchange surface in some fish when submerged (Steffensen et al., 1981).

Histological sections showed a third layer of adipose tissue in two species. This layer is used for energy production, and because the time of sampling was the second to the fifth month, perhaps this layer is one of the remnants of this fatty tissue that the fish store. The composition of muscle lipids is firstly dependent on the fish's feeding habits, which differ from one species to another, habitat, and from month to month depending on changes in the organism's composition along the year (Azadi and Naseer 1996; Haliloglu et al., 2004; Jahkar et al., 2012).

Conclusion

The results showed that the skin of fishes contained two layers and *M. mastacembelus* has the thickest epidermis with 44.223±12.338 µ. Mucous cells were also spread in this layer and varied in abundance in different species. The dermis with the two layers consists of blood vessels, connective tissue, and collagen fibers. In *C. zilli* and *H. fossilis*, a third layer was found under stratum compactum as hypodermis; the highest thickness of the dermis was recorded in

C. zilli.

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