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MORPHOLOGICAL STUDY OF THE SKIN AND ANNEXES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract: Nile tilapia (*Oreochromis niloticus,* Linnaeus, 1758) skin has histological characteristics that favor wound healing and attest to its use as an occlusive biological dressing, such as the overlapping of intertwined collagen fibers forming a peculiar bond that results in resistance and softness. Aquatic vertebrates have a lateral acoustic system formed by specific epidermal organs, which is compared to the olfactory system in terrestrial vertebrates. The present work aimed to analyze the main anatomical and histological characteristics of the skin and appendages of Nile tilapia. The results obtained are an important contribution to add to other studies, which are not very scarce, when in relation to the morphology of the fish and its skin.
Keywords: Anatomy, fish anatomy, lateral line, tilapiculture, veterinary.

INTRODUCTION

Nile tilapia (Oreochromis niloticus, Linnaeus, 1758) belongs to the kingdom Animalia, phylum Chordata, class Actinopterygii, order Perciformes, family Cichlidae and subfamily Pseudocrenilabrinae. It is a tropical, omnivorous freshwater species. It originated in Africa and was brought to Brazil in 1970 (MACIEL, 2015).

Currently, Brazil is the fourth largest producer of tilapia in the world, and its sale is mostly fillet. However, for 46 kg of fish, 14 kg of fillet and 32 kg of waste are generated. Therefore, with this high rate of waste, the reuse of these by-products is necessary (BOSCOLO; HAYASHI; MEURER, 2001, JACZYNSKI, 2005; ABP, 2019).

Thus, studies that use these residues are essential to seek solutions. When it comes to skin, which is often discarded, only 1% is used in handicrafts. In view of this, studies on its use as a biomaterial in the treatment of burn wounds in humans began in 2015, and report that Nile tilapia skin has prevailing type I collagen and high resistance. And even after sterilization, it maintained its histological and physical properties, thus reinforcing its use in medicine (FRANCO; FRANCO; DOURADO, 2013, ALVES et al., 2015).

Histologically, it is formed by epidermis and dermis. The epidermis consists of thin stratified squamous epithelial tissue, has cylindrical epithelial basal cells distributed in layers. These cells, when moving to the most superficial layer of the skin, acquire a squamous shape and release their contents, resulting in a viscous appearance of the skin. They are actually cells that produce mucus or mucin, a lubrication that guarantees protection for the fish (WHITEAR; ZACCONE, 1984, DOURADO et al., 1996).

The dermis is subdivided into two layers: one formed by loose connective tissue, the most superficial, where blood vessels of varying thicknesses are visible, nerve bundles and cells that synthesize melanin, called melanophores chromatophores, which act resulting in grayish pigmentation blackened skin of Nile tilapia. The other layer, called the deep dermis, is formed by dense connective tissue and has an abundance of collagen fibers organized horizontally, parallel, and also transversely. This arrangement of collagen fibers differs according to the analyzed region of the fish skin (PASOS, 2002).

The skin of Nile tilapia displays a particularity in its dermis: the overlapping of these collagen fibers that intertwine forming a type of connection, with portions of long and organized fibers. This arrangement after skin processes results in high resistance and at the same time softness (DOURADO; SOUZA; SANTOS, 1995).

In addition, fish, cyclostomes and amphibians have specific epidermal organs that constitute the lateral line component of the lateral acoustic system of aquatic vertebrate

organisms (DIJKGRAAF, 1963).

According to Hildebrand (1995), fish commonly manifest bilateral symmetry and can be divided into three regions: head, trunk and tail. And still on the external anatomy (figure 1), described Silva et al., (2015) that, Nile tilapia has a grayish blackish color, has vertical stripes and a laterally flattened body with the presence of cycloid elasmoid scales. Roberts (1993) considers the elasmoid scale to be a synapomorphy in teleost fish, a subclass of the *Actinopterygii* class.

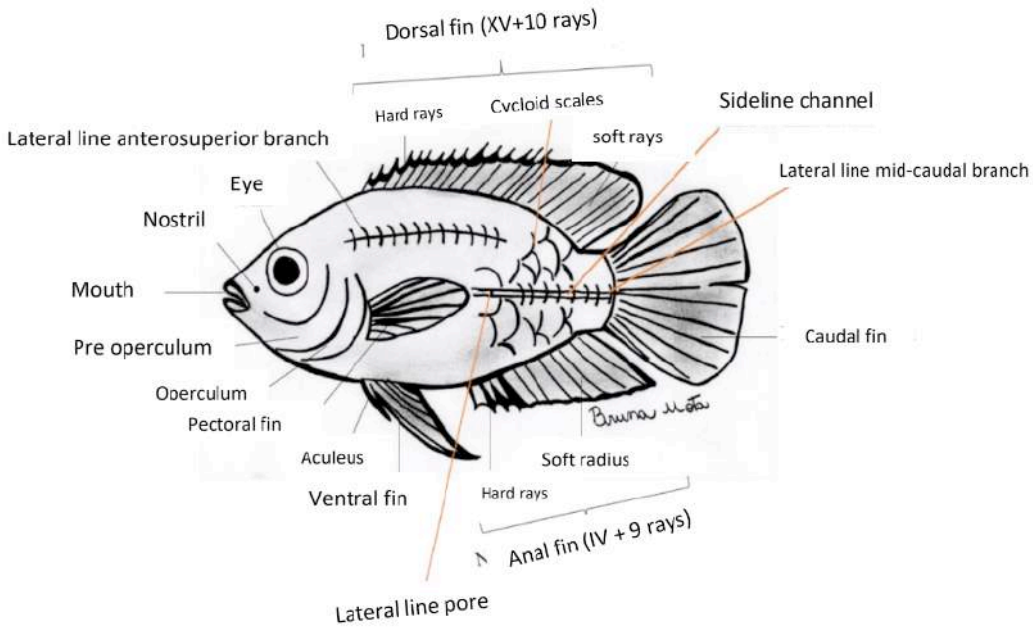

Figure 1: Illustrative representation of the external anatomy of fish from the perciformes group, such as Nile tilapia.

The present work aimed to analyze the main anatomical and histological characteristics of the skin and appendages of Nile tilapia. The results obtained will be an important contribution to add to other studies, not very scarce, when in relation to the morphology of the fish and its skin.

MATERIAL AND METHODS

The project was developed at the Histology Laboratory and the photomicrographs were taken at the Animal Pathology Laboratory of the Centro Universitário da Fundação de Ensino Octavio Bastos – UNIFEOB, located in the city of São João da Boa Vista, SP. For the project, the skins of five tilapias (n=05) and one specimen of whole Nile tilapia were used for macroscopic visualization of the skin and appendages *in situ*. The fish and skins were obtained from fish farms in the region of São João da Boa Vista, SP, from donations. International parameters of bioethics and animal welfare were respected, as recommended by CEUA – Commission for Ethics in the Use of Animals of the Faculty of Veterinary Medicine of São João da Boa Vista – UNIFEOB. For the macroscopic analysis of the fish and skins, basic materials were used, such as anatomical tweezers and a #22 scalpel blade, which helped in sectioning samples from different anatomical regions of the fish: cranial region, medial region, ventral region,

dorsal region and caudal region. For the microscopic analysis, after being fixed in 10% formalin, the skin samples underwent alcohol baths in increasing concentrations (70% - 100%) aiming at tissue dehydration. After this step, it was followed by the clearing, where xylene is used, a solvent that has bleaching potential, according to Junqueira and Carneiro (2013). Then, the samples were immersed in similar Histosec® paraffin to make the histological blocks. Subsequently, using the LEICA® Model 2165 microtome, histological sections were made with a thickness of 5µm; the sections underwent a histological bath, and then the histological slides were mounted with coverslips and Entellan® glue. The ready-made and dried slides were stained with Hematoxylin and Eosin, Masson's Trichrome and Picrosirius Red techniques, were observed and photomicrographed through a LEICA® Model ICC50 microscope, at the Laboratory of Animal Pathology at UNIFEOB. All results obtained were described in accordance with the Veterinary Anatomical Nomina (International Committee on Veterinary Gross Anatomical Nomenclature, 2017) and the Veterinary Histological Nomenclature (International Committee on Veterinary Histological Nomenclature, 2017).

RESULTS

The results obtained were found through the macroscopic analysis and microscopic analysis of the tegument of the Nile tilapia species(*Oreochromis niloticus*), and also of the entire specimen of the species, for a better understanding of the study.

It was possible to observe that the fish have bilateral symmetry and division of three regions: head, trunk and tail. It was seen that the Nile tilapia has a laterally flattened shape, with the presence of scales covering the entire body (Figure 2), showing a grayish-black color with vertical stripes (Figure 3).

In the *perciform* group, the presence of the peripheral lateral line can be seen, divided into two branches: the anterosuperior branch located more dorsally and the lateral branch-caudal line located caudally. It has scales with the entrance of the lateral line channel and distributed pores (figure 4 B) that run along the flank of the fish. The visualized scales are rounded and have smooth contours (figure 4). They are classified as cycloid elasmoid scales.

Microscopically, two layers were observed: the epidermis and the dermis (figure 5 A, B, C and D). Nile tilapia skin is thin, has stratified squamous epithelial tissue, superficial dermis with loose connective tissue. The deep dermis is formed by dense non-modeled connective tissue (figure 5 A, B and C). It has cylindrical epithelial cells. These cells go from the most basal layer to the surface (figure 5 C and D) and acquire a squamous shape. The second layer is the dermis, and it is subdivided into two layers, one formed by loose connective tissue, the most superficial one, where blood vessels of varying thicknesses can be observed (figure 6 A, B, C and figure 8 A and B) and nerve bundles (figure 5 C and D). The second layer of the deeper dermis is formed by dense connective tissue and has an abundance of collagen fibers, organized in a parallel and transverse manner (figure 5 C and D). This arrangement of collagen layers differs according to the region of the skin.

The sensory unit of the lateral acoustic system of the fish is observed, composed of epidermal organs, called superficial neuromasts (figure 8) and neuromasts in subepidermal canals (figure 6 B and C). Comprising the lateral line system of teleost fish are the free organs (superficial neuromasts), the branches and the trunk canal (figure 5 B and C, figure 9). The domes (figure 9 A, B and C) are filled with gelatinous substance and grouped hair cells. These channels are connected through pores located under the scales of the fish (4 B), those

of the Nile tilapia are scales of the cycloid elasmoid type. Scales, which are located close to the epidermis and even the dermis region.

The skin of Nile tilapia displays in its dermis the overlapping of collagen fibers that intertwine forming a peculiar type of connection (figure 10), with portions of long and organized fibers.

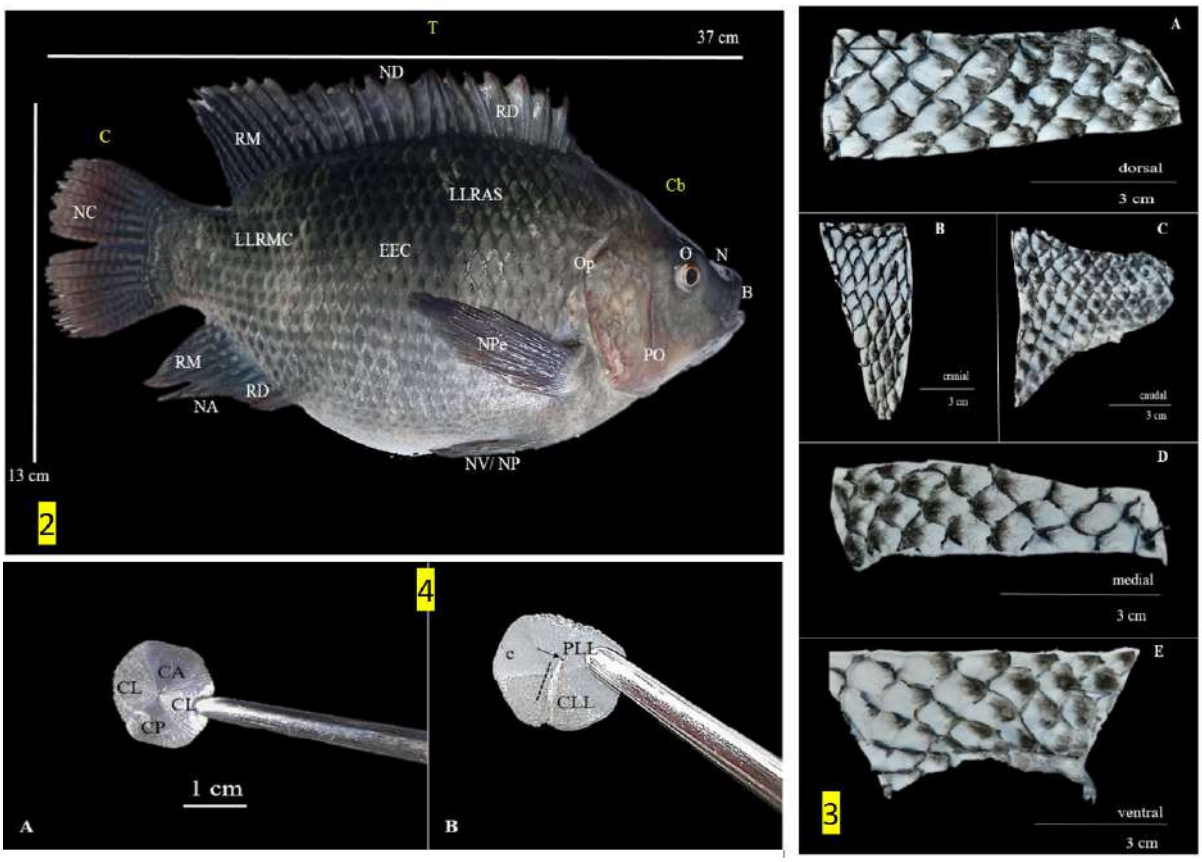

Figure 2: Photograph of a specimen of Nile tilapia (Oreochromis niloticus) demonstrating its external morphology. Observe the head (Cb), trunk (T) and tail (C) In the left side view, in the head region (Cb) there is a pair of eyes without eyelids (O), nostrils (N) and terminal mouth (B), in addition to preoperculum (PO) and operculum (Op). In the trunk region (T), the dorsal fin (ND) is divided into hard rays on the right and soft rays on the left. Note the lateral line of the anterosuperior branch (LLRAS) covered by cycloid elasmoid scales (EEC), still in the trunk region, but more caudally, the lateral line of the median-caudal branch (LLRMC) can be seen below, note the anal fin (NA) divided into soft rays (RM) and hard rays (RD). Next to the operculum, the pectoral fins (NPe) and ventrally the ventral or pelvic fins (NV/ NP) are observed. In the caudal region (C), the homocercal caudal fin. Height bar: 13 cm. Bar length: 37 cm. Photography: Canon EOS Rebel T6. Lens: 15-55mm. Figure 3: Photograph of sectioned skin samples of Nile tilapia (Oreochromis niloticus) from different regions of the fish, used in the project. Observe its grayish blackish coloration. Bar: 3 cm. Photography: Canon EOS Rebel T6. Lens: 15-55mm. Figure 4: Photograph of integumentary attachment: Nile tilapia scales. In A, there is a cycloid elasmoid scale, divided into anterior field (CA), lateral fields (CL) and posterior field (CP). In B, there is a cycloid elasmoid scale, however it is a scale from the lateral line region, so observe the lateral line pore (PLL) indicated by an arrow and the lateral line entrance channel indicated by a dotted arrow (CLL). Bar: 1 cm. Photography: Canon EOS Rebel T6. Lens: 15-55mm.

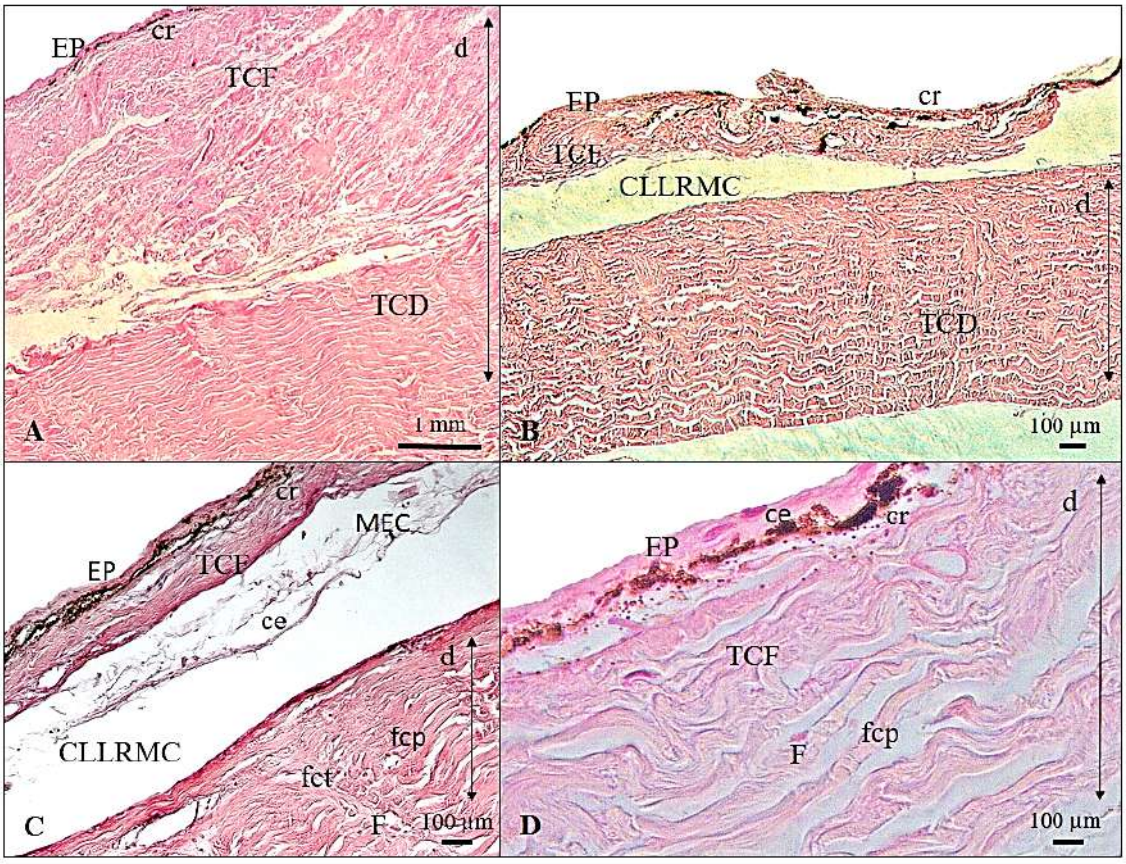

Figure 5: Photomicrograph of Nile tilapia skin. In A, histological section of the medial region of tilapia skin, the two layers, epidermis (EP) and dermis (d), can be seen. In the epidermis, the presence of melanophore chromatophores (cr) is noted. In the dermis (d) superficially just below the epidermis there is notable loose connective tissue (TCF) and in the deep dermis, dense connective tissue (TCD). Bar: 1mm. 10x magnification. In B, histological section of the caudal region of tilapia skin, the two layers, epidermis (EP) and dermis (d), are also observed. There are chromatophores in epidermis (EP). Note a particularity in this region, the lateral line canal of the caudal median branch. Below the canal there is still the presence of dermis (d) and composition of dense connective tissue (TCD). 10x magnification. Bar: 100µm. In C, with a 20x magnification and histological section of the caudal region, the epidermis composed of stratified squamous epithelial tissue (EP) is observed, below there is the presence of an extracellular matrix with amorphous substance, fibers and common cells of the epidermis. Where also passes the canal of the lateral line branch median caudal. In the dermis (d) transverse collagen fibers (fct) and parallel collagen fibers (fcp) can be seen, in addition to discreet fibroblasts between the collagen fibers (F). Bar. Bar: 100µm. In D, 40x magnification, histological section of the medial region, note the absence of the lateral line channel. Observe the epidermis (EP) with numerous melanophore chromatophores (cr) and epidermal cells (ce). Below, in the dermis (d), loose connective tissue and parallel thick collagen fibers (fcp) and the presence of fibroblasts between them (F). Bar: 100µm. Both histological sections are cross-sectional and were stained with Hematoxylin and Eosin.

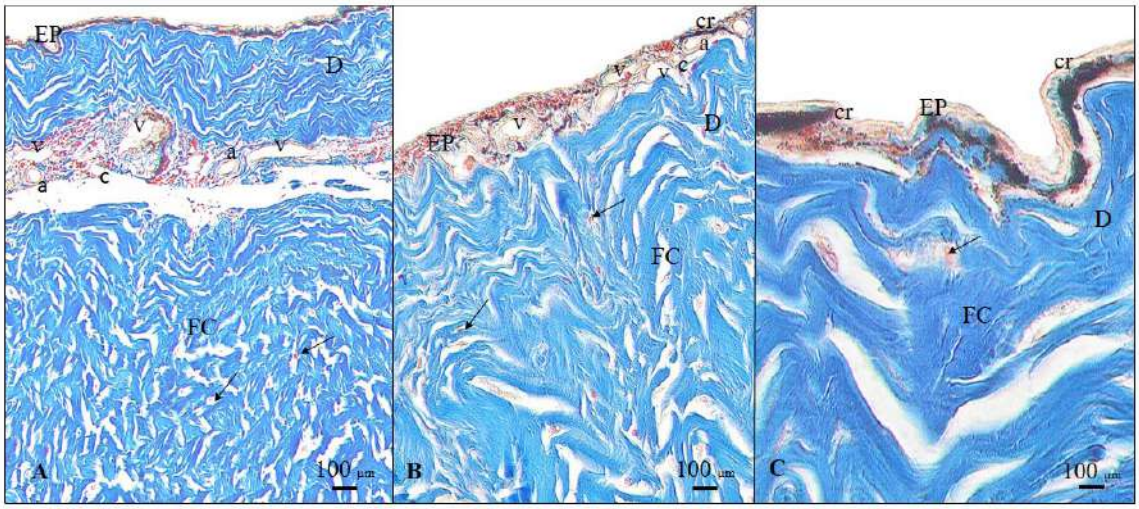

Figure 6: Photomicrograph of the integumentary organ of Nile tilapia. A, B and C stained with Masson's Trichrome to highlight collagen fibers and localized blood vessels. In A, there is the epidermis (EP), dermis (D) with the presence of venules (v), capillaries (c) and arterioles (a). Note the collagen fibers highlighted in blue (FC) and fibroblasts between them (arrows). 10x magnification. Bar: 100µm. In B, there is a histological section of the lateral line region where there is epidermis (EP) with melanophores chromatophores (cr), and adjacent to it the presence of structures such as venules (v), capillaries (c) and arterioles (a). Collagen fibers highlighted in blue (FC) and arrows signaling fibroblasts. Bar: 100µm. 20x magnification. In C, it is a similar histological section, however at 40x magnification. Bar: 100µm.

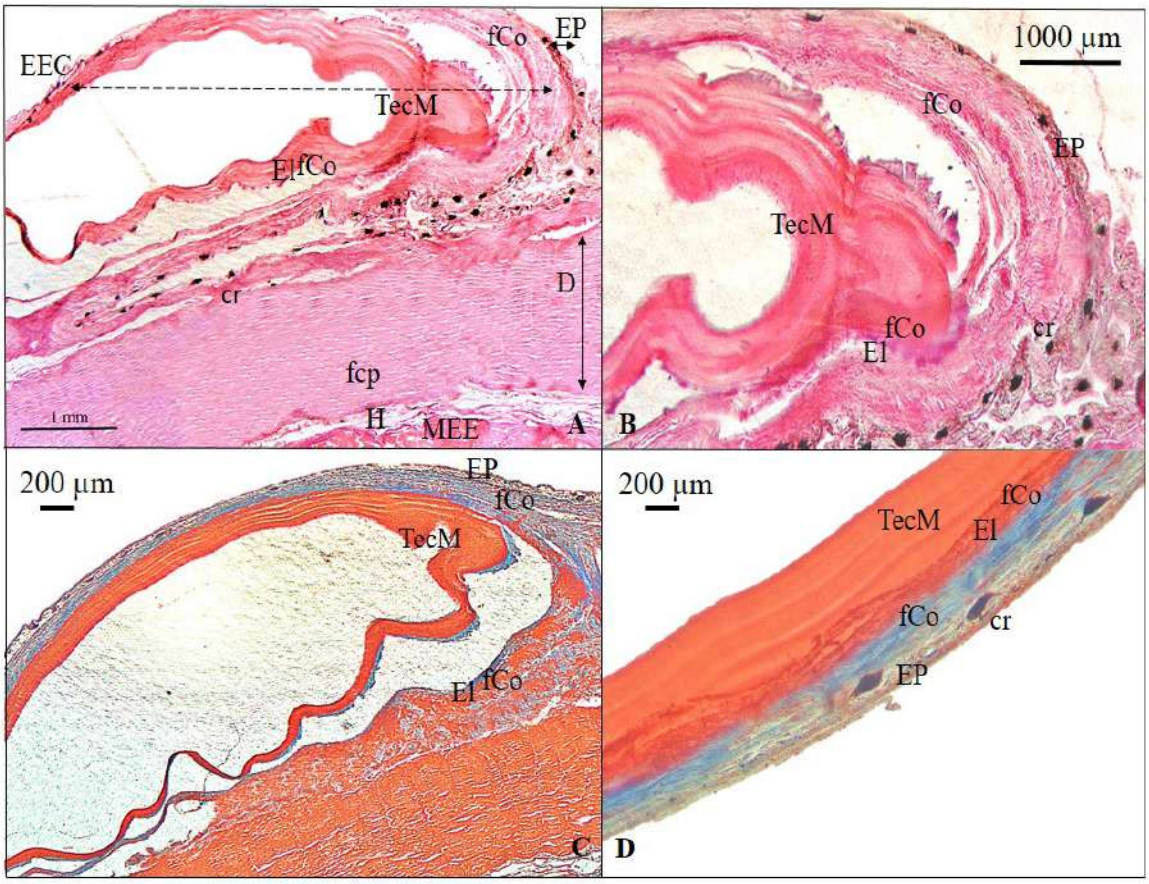

Figure 7: Photomicrograph of tilapia skin and appendage: scale. In A, represented by EEC (double dotted arrow) we have the cycloid elasmoid scale. Formed by three layers, the basal layer consisting of elasmodin and collagen fibrils (El and fCo) followed by another layer with little mineralization also with collagen fibrils (fcO) and finally, the outermost layer forming the mineralized tissue (TecM). In Ep (double horizontal arrow) there is the region of epidermis formed by stratified squamous epithelial tissue with epithelial cells, note the presence of melanophores chromatophores (cr). Below, the dermis (double vertical arrow) formed by dense connective tissue (superficial dermis) and below loose connective tissue (deep dermis). Note the distribution of parallel collagen fibers (fcp) and transverse collagen fibers (fct). Further down is the hypodermis (H) and below this layer is skeletal striated muscle (SEM). Hematoxylin and Eosin staining. 5x magnification. Bar: 1mm. In B, there is the same section, but in 10x magnification, also stained with Hematoxylin and Eosin. Bar: 1000µm. In C, the section is similar to A, but stained with Masson's Trichrome at 5x magnification. Bar: 200µm. In D, we tried to highlight the three layers of the scale, already mentioned in image B, however in the special Masson's Trichrome stain and 20x magnification. All cross cuts.

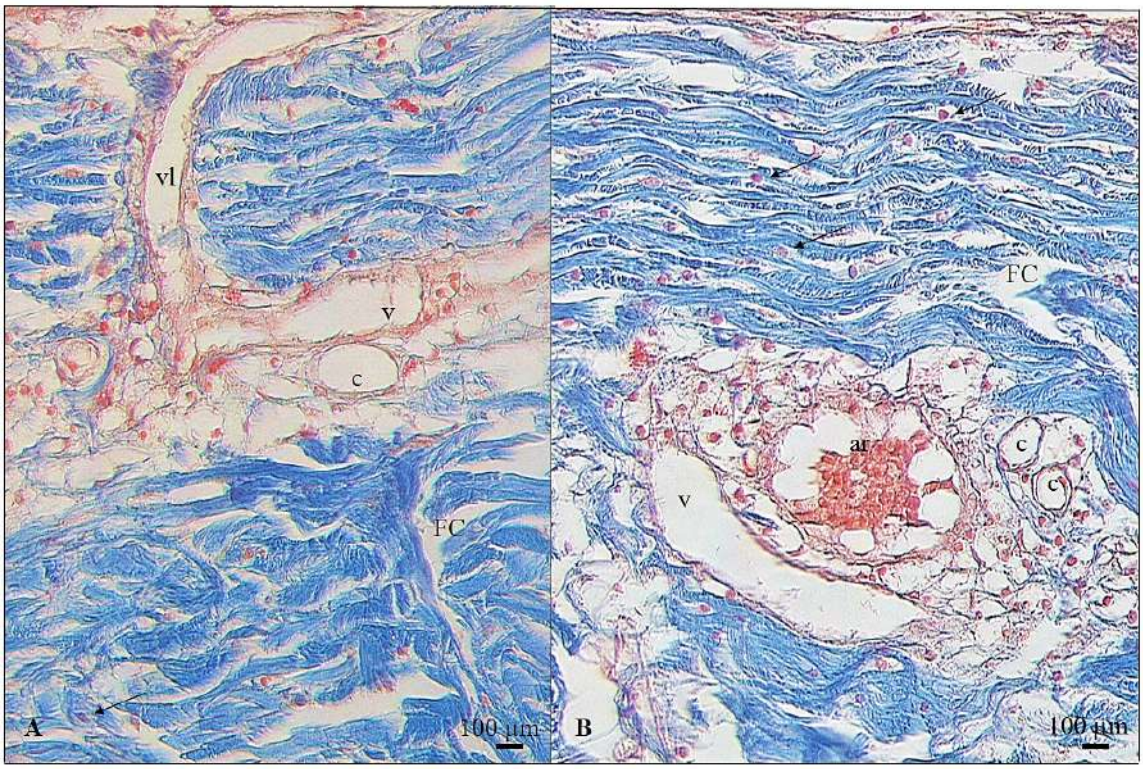

Figure 8: Photomicrograph of the integumentary organ of Nile Tilapia. Stained with Masson's Trichrome. In A, lymphatic vessels (vl) can be seen in the dermis region, venules (v) and capillaries (c). Note the presence of organized collagen fibers and fibroblasts between them (arrow). Bar: 100µm. 40x magnification. In B, note several fibroblasts (arrows) between the collagen fibers (FC). There are arterioles (air), capillaries (c) and venules (v). Bar: 100µm. 40x magnification. Both cross cuts.

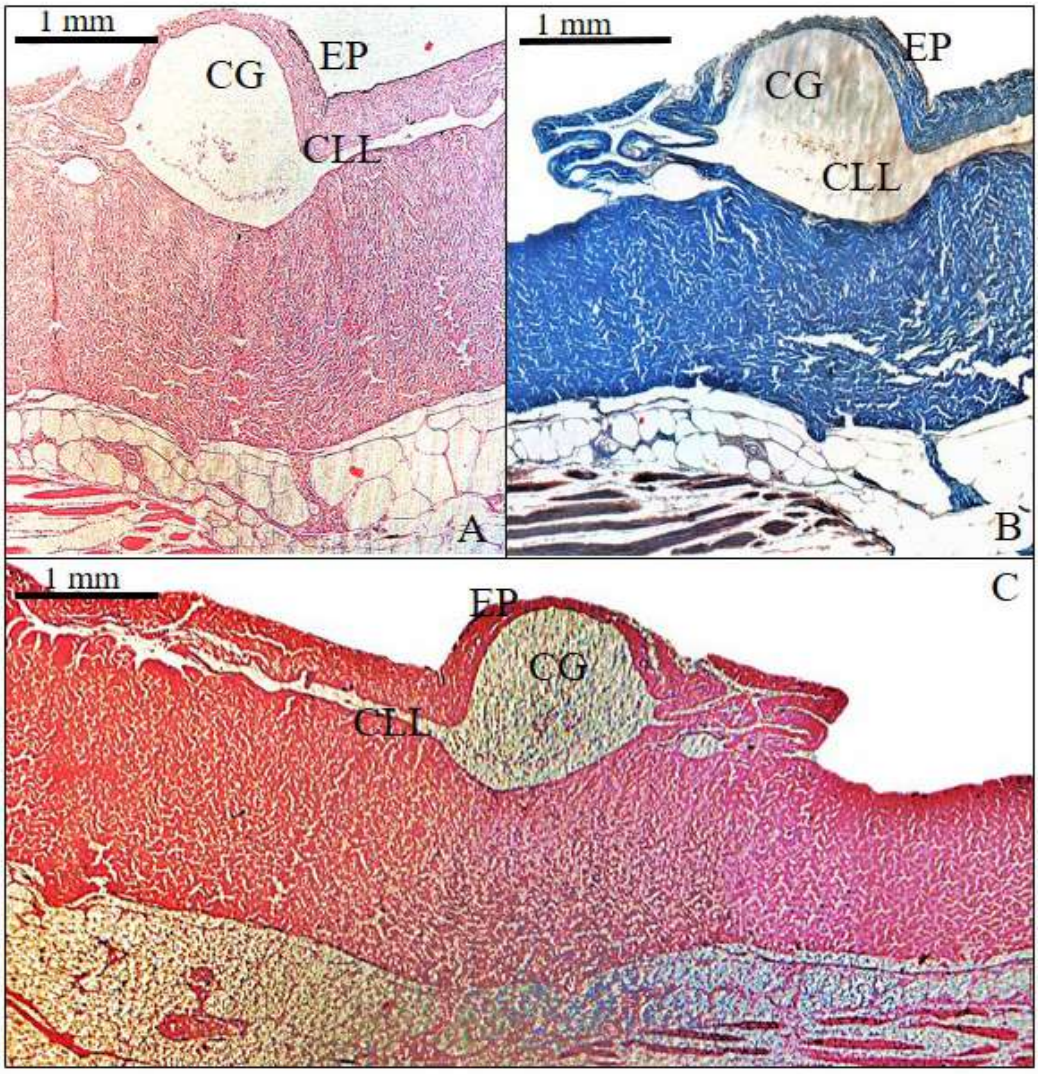

Figure 9: Photomicrograph of the sensory organ of Nile Tilapia fish. Cross-sectional histological sections of sensory organ. In A, stained with Hematoxylin and Eosin, the presence of a neuromast dome (CG) can be seen, in its contour there is connective tissue and, more externally, epithelial tissue (EP). There is a clear view of the peripheral lateral line channels (CLL) where the water flows. Bar: 1mm. 5x magnification. In B, there is the same histological section as in A, but stained with Masson's Trichrome in order to highlight the collagen in blue. Bar: 1mm. 5x magnification. In C, similar histological section of A, also stained with Hematoxylin and Eosin at 2.5x magnification. Bar: 1mm.

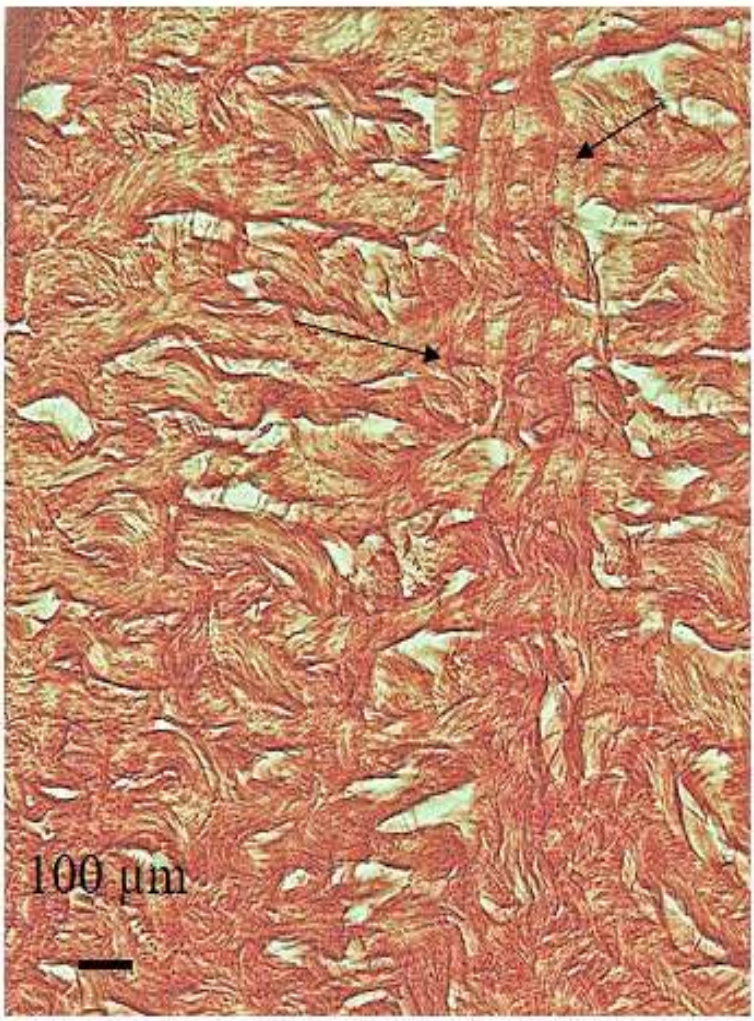

Figure 10: Photomicrograph of collagen fibers from Nile Tilapia dermis. Cross-sectional histological section of tilapia dermis showing intertwined collagen fibers (arrows) forming a peculiar connection, which ensures softness, but also resistance. Bar: 100µm. 40x magnification Color: Picro-sirius Red. Lens: Gr.

DISCUSSION

According to Hildebrand (1995), fish have bilateral symmetry and their body is divided into three regions: head, trunk and tail. Silva et al. (2015) describe that Nile tilapia shows a grayish-black color with vertical stripes, laterally flattened shape, with scales covering the entire body. They are classified as cycloid elasmoid scales according to Vernerey and Barthelat (2014) and Daniels (1996).

Helfman et al., (2009) report that the visualized scales are more flexible compared to other existing types, and it is believed that this is due to their arrangement: they are superimposed on each other. Furthermore, Roberts (1993) considers this type of elasmoid scale, a synapomorphy in teleost fish, a subclass of the class Actinopterygii, which belongs to Nile tilapia.

And according to Pasos (2002), this blackened coloration of Nile tilapia occurs as a result of melanophores chromatophores, cells that synthesize melanin, which act resulting in the pigmentation of this species. Whitear and Zaccone (1984) describe that in the most external region of the fish skin, called the

epidermis, there are mucus-producing cells.

Dourado et. al. (1996), describe epidermis and dermis in Nile tilapia. Thin Nile tilapia skin has stratified squamous epithelial tissue, superficial dermis with loose connective tissue, and deep dermis, formed by dense unshaped connective tissue, in addition to cylindrical epithelial cells. These cylindrical epithelial cells, go from the most basal layer to the surface and acquire a pavement shape, causing the release of their content, the mucus, which guarantees a viscous appearance for the skin, lubricating and guaranteeing protection for the fish, as mentioned by Whitear and Zaccone (1984).

The second layer is the dermis and it is subdivided into two layers, one formed by loose connective tissue, the most superficial, where there are blood vessels of varying thicknesses and nerve bundles. And the second layer of the dermis, deeper, is formed by dense connective tissue and has an abundance of collagen fibers organized, some parallel and others transverse. This arrangement of collagen layers differs according to the region in the skin of the fish, according to Farias (1991), and the amount of cellular strata may vary according to the region of the body studied.

Dourado et al (1997) reported finding large amounts of small, rounded cells in a region close to the lateral line. Fish have a lateral acoustic system consisting of specific epidermal organs, these form the lateral line according to Dijkgraaf (1963).

According to Münz (1969), the sensorial unit of the lateral line is the neuromast, and these can be superficial, those that occur alone in the skin, in fossae or in raised pedestals in the skin, or neuromasts in subepidermal channels that, through their pores of the channel, come into contact with water.

This system is compared to the olfactory system in terrestrial vertebrates, as it is capable of identifying changes in the environment where the animal is inserted, such as perceiving variations in water, depth, prey, predators, members of the species itself for reproduction purposes, for example (ACHE ; YOUNG, 2005; BEHRA et al., 2009).

The lateral line canal has three main branches, one passes above the eye and is called the supraorbital canal; another below the eye, and called the infra-orbital canal, and a third in the mandible, which is called the hyomandibular canal, in union with the free organs (superficial neuromasts), these branches and the trunk canal with their respective neuromasts, constitute the lateral line system of teleost fish. This is constituted by neuroepithelial plaques formed by domes that are full of gelatinous substance and grouped ciliated cells; when this viscous gel moves, it stimulates the cells present, releasing neurotransmitters, signaling to primary afferent neurons that carry the message to the central nervous system, such as fleeing, turning, preying (MOYES; SCHULTE, 2010). These channels are connected through pores located under the scales of the fish, those of Nile tilapia are scales of the cycloid elasmoid type. Scales, which are located close to the epidermis and even the dermis region. It is a structure in thin lamellae, composed of collagen. Formed by three layers: slightly mineralized basal, formed by elasmodin and small collagen fibers, the second layer more mineralized and still with collagen fibrils and the third most external one there is no presence of collagen making it just mineralized, according to Sire and Akimenko (2004) and Daniels (1996).

According to Dourado, Souza and Santos (1995), the skin of Nile tilapia displays in its dermis the overlapping of collagen fibers that intertwine forming a peculiar type of connection, with portions of long and organized fibers. This arrangement, after chemical processes on the skin, results in high

resistance and also softness.

It is concluded, therefore, that the integumentary organ of aquatic vertebrates has several characteristics that differ from terrestrial vertebrates, such as the presence of the lateral line system, formed by sensory organs.

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