



Histology of juvenile skin of *Lepidosiren paradoxa* Fitzinger, 1837 (Sarcopterygii, Dipnoi)

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Abstract: The skin of three juvenile *Lepidosiren paradoxa* specimens was examined. The epidermis was composed of a polystratified epithelium resting on a basement membrane, including mucus-secreting cells, and a cuticle of mucopolysaccharides on the surface. Two types of skin receptors, electroreceptors and mechanoreceptors, were found; the first type was located in the dermoepidermal junction, and the second type was completely intraepiderma. The skin structure of these fish, suggests the possibility of the skin participating in the breath.

Key words: electroreceptors, lungfish, mechanoreceptors, Paraná River basin, pirambóia.

INTRODUCTION

The South American Lungfish (*Lepidosiren paradoxa*) is found in the marshes and slow waters of the Amazon, Paraguay Basin and Paraná River in South America (Vallone 2017). As a remarkable obligate air breather, he is also the only recent member of the family, Lepidosirenidae (Froese and Pauly 2014).

Juvenile lungfish feed on the larvae of insects and snails, while the adults are omnivores, adding algae and shrimp to their diets and crushing them with their highly mineralized dental plates. The usual habitats of the fish disappear during the dry season; during this time, they hide in the mud and make a chamber approximately 30-50 cm beneath

the mud surface, leaving some holes to the surface for air to enter. The skin has important functions during this aestivation, and a layer of mucus is produced to seal in moisture and slow down its metabolism as well as to participate in respiration (Bruton 1998).

The objective of this work is to describe the skin of *L. paradoxa* juveniles, as well as to demonstrate characteristics of adaptation of the species to the environment with the presence of electro and mechanoreceptors, which are necessary for their survival.

MATERIALS AND METHODS

Three *L. paradoxa* juveniles were captured by fishermen in a sector with mud and scarce water where they were buried in Esteros del Ibera in the province of Corrientes, Argentina. The lengths

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of the specimens were 110, 107 and 125 mm. The specimens were anaesthetized with 0.5% benzocaine (Sigma-Aldrich) for approximately 30 minutes according to De Moraes et al. (2005). Transverse skin sections were obtained from the dorsal and dorsocephalic region, dorsolateral and abdominal region, fixed in 10% formalin, and embedded in Paraplast. The histological sections were stained with haematoxylin and eosin, periodic acid Schiff (PAS), Kuhn blue and Cajal gold sublimate modified for sections embedded in Paraplast and identified histological structures according to Mokhtar (2017) (Naoumenko and Feigin 1961).

RESULTS

In the histological sections, we observed the classic layers that form the skin of fish: the epidermis, the stratum spongiosum and stratum compactum of the dermis, the hypodermis and the subcutaneous muscle (Figure 1).

The epidermis was composed of a polystratified epithelium of between 4 and 5 layers of cells, and the entire epidermis rested on a basement membrane (BM) (Figure 2).

The epidermis was composed of several cell types. The basal cells are totipotent cells that generate all other cells, and these cells can become irregular. Mucus-secreting cells were present in the Malpighian stratum. As juvenile animals were observed, mucus-secreting cells showed high cellular activity; these cells were hyperchromatic with several mitotic cells (Figure 3). On the surface, a PAS-positive cuticle was observed, possibly composed of mucopolysaccharides (Figure 4). The epidermis rested on a BM that separated it from the stratum spongiosum of the dermis in which there was abundant melanin belonging to melanophores. Melanin was also observed scattered throughout the thickness of the epidermis. Melanophores stained with Cajal gold sublimate showed

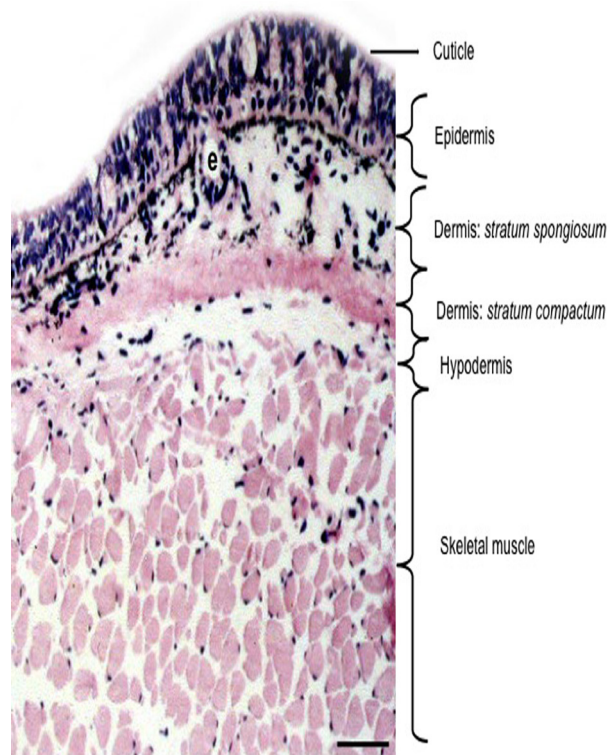


Figure 1 - Skin of dorsolateral region of *L. paradoxa* with its differentiated skin layers. A tubule of an electroreceptor is visible in the dermoepidermal junction (e). H-E. Bar: 200 μ m.

irregular prolongations (Figure 5). In the stratum compactum of the dermis, we observed abundant collagen and elastic fibres and vascular structures. The hypodermis was very thin, with a few isolated vascular capillaries

Mechanoreceptors were located in the epidermis, and electroreceptors were observed within the dermis near the epidermis. The mechanoreceptors comprised a group of columnar cells derived from the epithelium, with a central neuroepithelial cell from which stereocilia exited to the surface; modified columnar epithelial cells surrounded and supported the mass of the sensory cells (Figure 6).

Electroreceptors were present in the epidermis and superficial areas of the dermis and consisted of a double layer of cuboidal cells forming a hollow bulb located below the collagen of the dermoepidermal junction (Figure 7). The cells lining the bulb had

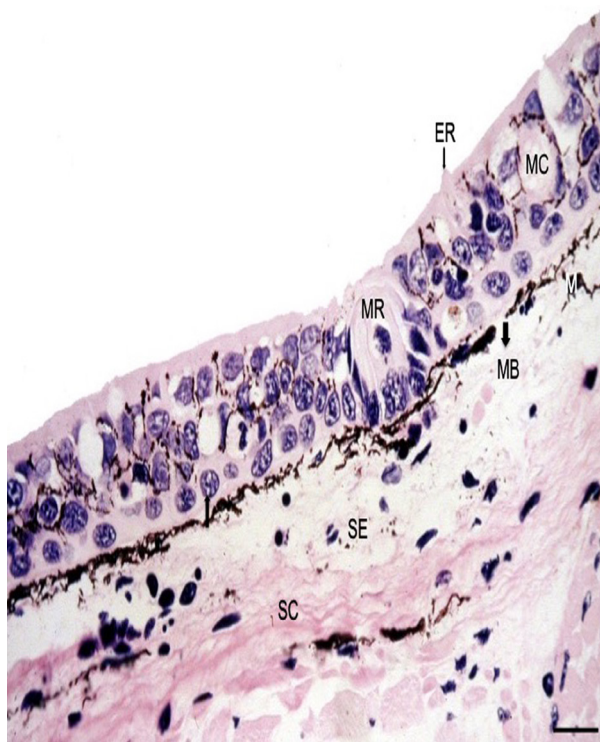


Figure 2 - Skin of dorsocephalic region of *L. paradoxa*. Epidermal basal cells (arrow) with an underlying basement membrane (BM arrow) and mucus-secreting (MC) cells. A mechanoreceptor (MR) is visible at the external end of the electroreceptor (ER). The stratum spongiosum and compactum (SE-SC) of the dermis with melanophores (M) are also visible. H-E. Bar: 100 μ m.

tiny projections from the surface, and some of the cells showed evidence of cell division, which was uncommon elsewhere in the epidermal and dermal tissues. A narrow duct, lined by a single layer of epithelial cells, extended from the base to the surface of the epithelium where it opened like a small circular hole surrounded by small papillae.

DISCUSSION

The structure of the skin of *L. paradoxa* and other lungfish has been described by several authors (Coelho and Sawaya 1972, Imaki and Chavin 1975a, b). The importance of skin structure in the physiology of these fish is significant; the skin of all teleosts is the primary protective barrier against external pathogens, and mucus production plays an

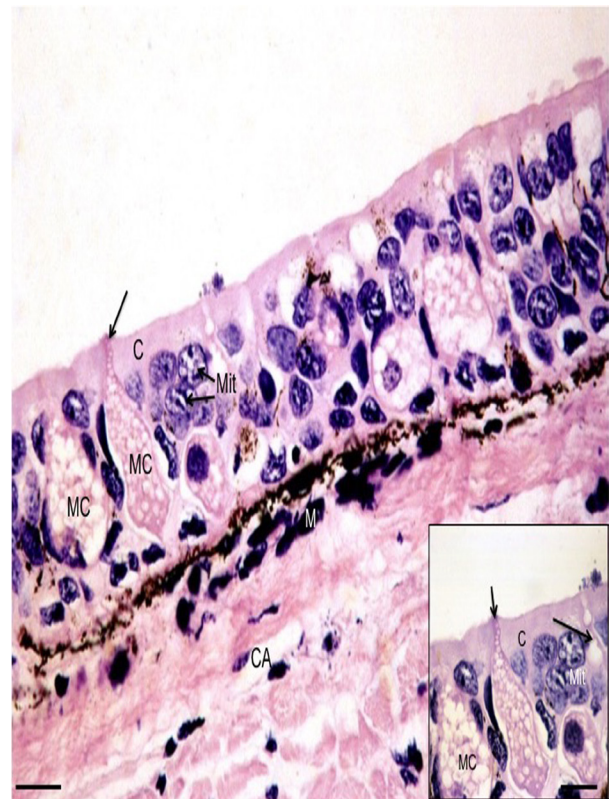


Figure 3 - Skin of dorsocephalic region of *L. paradoxa*. Epidermis with abundant mucus-secreting (MC) cells. The mucus is released at the surface, forming the cuticle (C). Several mitotic (Mit) cells are visible. In the dermis, melanophores (M) and capillaries (CA) are visible. H-E. Bar: 50 μ m. Insert: mucus-secreting (MC) cells releasing mucus to the outside (short arrow), forming the cuticle (C). There is a tubule of an electroreceptor (long arrow), and mitosis (Mit) can be observed. H-E. Bar: 20 μ m.

important role in the non-specific immune response (Heimroth et al. 2018, Xu et al. 2013). Moreover, since *L. paradoxa* is a lungfish, the mechanisms and tissues involved in the respiratory process are diverse, and the skin plays a very important role; in this sense, lungfish skin is similar to that of amphibians (Hughes 1976).

The skin of the snout and lips in the living *Neoceratodus forsteri* is soft, and free of scales at all stages of the life cycle. In external view, the skin of the snout is pitted, with perforations of various sizes, for goblet cells and sense organs, and apart

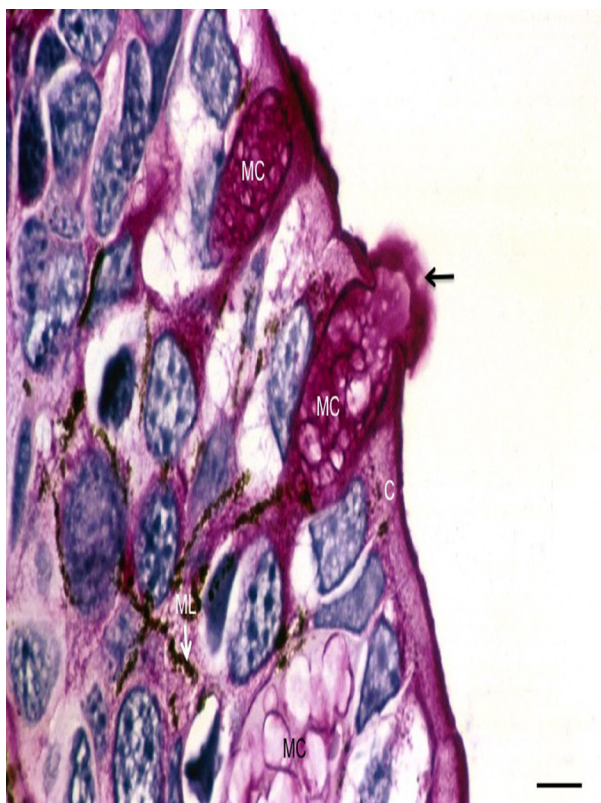


Figure 4 - Skin of dorsal region of *L. paradoxo*. Epidermis with PAS-positive mucus-secreting (MC) cells. One of these cells is releasing the mucus at the surface (arrow), contributing to the formation of the cuticle (C). Melanina (ML). PAS. Bar: 20 μ m.

from this, individual epithelial cells are visible in surface view (Kemp 2012).

The epidermis in these juvenile animals showed intense cellular activity, such that almost all of their cells had hyperchromatic nuclei with abundant mitosis. Such cellular activity has not been previously reported, even in studies on the juvenile skin of both *L. paradoxo* and *Neoceratodus forsteri* (Imaki and Chavin 1975a, b). This high cellular activity is expected in all vertebrates since the epidermis belongs to the class of labile tissues with a high rate of cell renewal. In addition, in the case of juvenile animals, the average life span of cells is shorter (Gartner 2017). Johansen and Lenfant (1967) have observed blood capillaries in the epidermis, while we observed capillaries in the dermis only. The epithelium structure does not

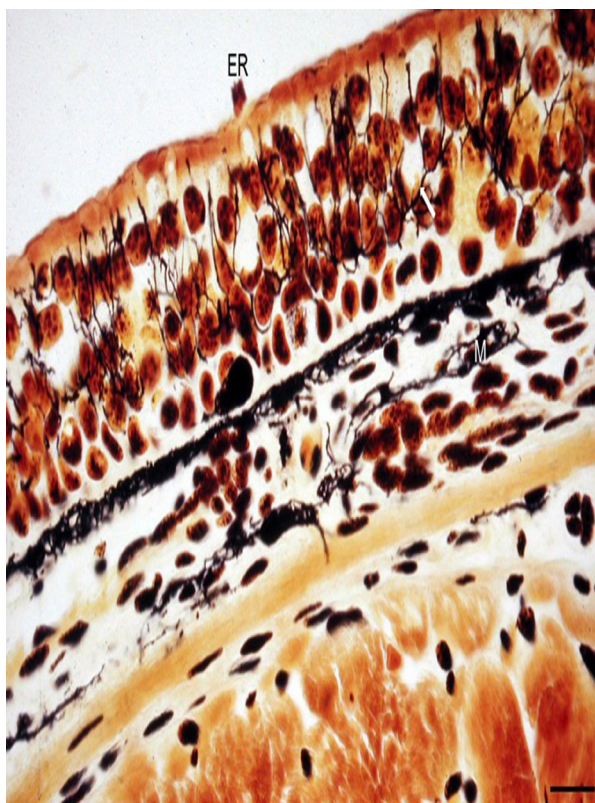


Figure 5 - Skin of abdominal region. of *L. paradoxo* Epidermis with marked cellular activity. Melanin can be observed in the epidermis (arrow) at the surface of an electroreceptor (ER). In the dermis, melanophores with "dendritic" extensions (M) can be observed. Cajal gold sublimate. Bar: 50 μ m.

contain capillaries; instead, it is nourished by the blood capillaries of subepithelial connective tissue, in this case, the dermis (Gartner 2017).

PAS-positive mucus-secreting cells were predominantly found in the epidermis. These cells were loaded with mucus, which they released at the surface, generating a mucosal surface over the entire body of the fish. Although the mucus should help with swimming, it may be more helpful when the animals exit the caves that they make in the mud; when the mud dries, the mucus provides lubrication to allow mobility. In addition, mucus is rich in lysozyme that acts as a broad spectrum bactericide (Romano 2010).

The mechanoreceptors are part of the acoustic-lateral system. These receptors collect mechanical signals in the water and have both afferent and

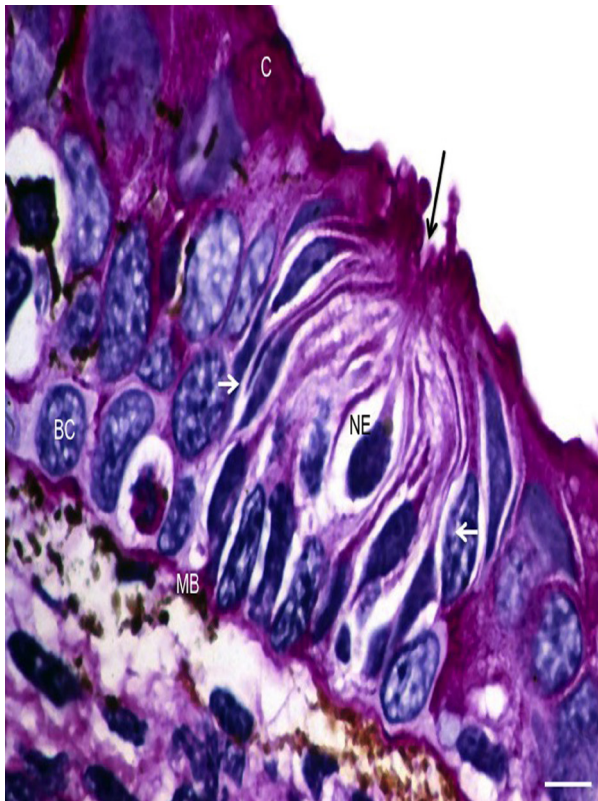


Figure 6 - Skin of dorsocephalic region of *L. paradoxa*. A mechanoreceptor that occupies the entire thickness of the epidermis. The basal part rests on the basal membrane (MB). The neuroepithelial central cell (NE) is surrounded by support cells (short arrows) and the basal cells of the Malpighian stratum (BC), and the neuropil reaches the surface (long arrow) through the cuticle (C). PAS. Bar: 20 μ m.

fferent innervation. The electroreceptors are small, confined to the superficial layers of the dermis or epidermis, and exhibit single innervation (Northcutt 1986). These receptors are sensitive to weak electrical currents in the water possibly emitted by prey animals (Roth 1973, Roth and Tschardt 1976, Bullock et al. 1983, Kramer 1996, Collin and Whitehead 2004).

The skin is recognized as a secondary respiratory tissue in several species (Urbina and Meredith 2014), and in lungfish, epidermal mucus is directly related to respiratory physiology (Heimroth et al. 2018).

This study provides a characterization of the skin of a sarcopterygian fish, the juvenile

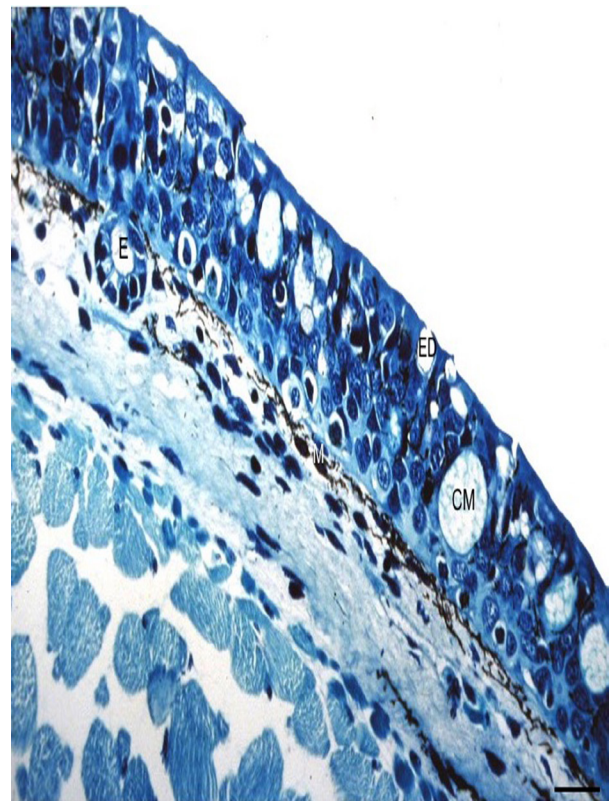


Figure 7 - Skin of dorsal region of *L. paradoxa*. At the dermoepidermal border, a tubule of an electroreceptor with a double layer of cuboidal cells (e) is visible; in the epidermis, mucus-secreting (MC) cells are visible, along with the duct of an electroreceptor (ED). Kuhn blue. Bar: 100 μ m.

South American lungfish. In the present study, the structure of the skin of these fish, together with the identification of electroreceptors and mechanoreceptors, was elucidated.

According to Zena et al. (2017), the skin of *Lepidosiren paradoxa* actively exchanges CO₂ through the skin. Therefore, studying the structure of this organ that seems important in the appearance of terrestrial respiration is essential.

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AUTHOR CONTRIBUTIONS

Luis Alberto Romano in the animal acquisition, necropsy and slide analysis. Virgínia Fonseca Pedrosa in the histological processing, necropsy and follow-up analysis. Andrea Idelette Hernández López and Juan Rafael Buitrago in the histological processing and slide staining.

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