Histochemistry and functional organization of the dorsal skin of

Ancistrus dolichopterus (Siluriformes: Loricariidae)

Tarun K. Garg[1,2], Fabíola X. Valdez Domingos[1], Vera Maria F. Almeida-Val[1], and Adalberto L. Val[1]

The structural organization and histo-cytochemical features of dorsal skin of *Ancistrus dolichopterus* (acari bodo) are the main focus of this work. The epidermis, dermis and subcutis are the principal layers of the skin. The epidermis mainly consists of epithelial and mucous cells. Interspersed between them are lymphocytes, pigment cells, eosinophilic granular cells (EGC), and the taste buds as sensory structures. The high number of EGCs is implicated in general and specific immunological defense from pathogenic bacteria and multicellular parasites. The epithelial cells and mucous cells contain glycoproteins with oxidizable *vicinal* diols, carboxyl groups and O-sulphate esters and their high secretory activity is correlated with the bottom dwelling habit of this species. A thick *stratum laxum* contains overlapping osteoderms bearing denticles, and the *stratum compactum* make the integument thicker to help the fish in negative buoyancy for maneuvering near the bottom and protection. The entire body surface is covered by conical, backwardly directed denticles. These are composed of a dentine cone, surrounding a pulp cavity with the top covered by mineralized cap, and are the true homologues of teeth. These structures provide effective protection from abrasion and enemies. These structural peculiarities and histochemical features indicate additional physiological role of the skin of *A. dolichopterus*.

A organização estrutural e aspectos histo-citiquímicos da pele dorsal de *Ancistrus dolichopterus* (acari-bodó) são os principais alvos do presente estudo. A epiderme, a derme e a hipoderme são as principais camadas da pele. A epiderme consiste principalmente de células epiteliais e mucosas. Intercalados entre elas estão os linfócitos, as células pigmentares, as células granulares eosinofílicas (CGE), e as papilas gustativas como estruturas sensoriais. Um grande número de CGEs está relacionado em geral com a defesa imunológica específica de bactérias patogênicas e parasitas multicelulares. As células epiteliais e as células mucosas contem glicoproteínas com grupos diol oxidáveis, grupos carboxilas e ésteres O-sulfatados sendo que sua alta atividade secretória está correlacionada com o comportamento bentônico, de fundo, dessa espécie. Um espesso *stratum laxum* contém osteodermos sobrepostos, parecidos com dentículos, e o *stratum compactum* que torna o tegumento mais espesso, contribuindo com a flutuação negativa necessária ao movimento perto do fundo e proteção. Toda a superfície do corpo é coberta por dentículos cônicos retrodirecionados. Esses dentículos são compostos por um cone de dentina, envolvendo uma cavidade pulpar e com o ápice coberto por uma capa mineralizada, verdadeiros homólogos dos dentes. Essas estruturas oferecem efetiva proteção contra abrasão e oponentes. Essas peculiaridades estruturais e aspectos histoquímicos sugerem a existência de uma função fisiológica adicional para a pele de *A. dolichopterus*.

Key words: Epidermis, Skin structure, Denticle, Siluriform, Amazon fish.

[1]Laboratório de Ecofisiologia e Evolução Molecular, Instituto Nacional de Pesquisas da Amazônia (INPA). Av. André Araújo, 2936, 69060-001 Manaus, AM, Brazil. dalval@inpa.gov.br (corresponding ahthor)
[2]Department of Zoology, K. N. Government Post-Graduate College, Gyanpur, Bhadohi - 221 304, India.

Introduction

Amazonian fishes constitute the most diverse ichthyofauna on the earth; more than 3,000 fish species have been already described in the Amazon, including representatives of almost all freshwater fish groups (de Pinna, 2006). These fishes colonize nearly all types of aquatic habitats in the immense basin (Val & Almeida-Val, 1995). For that they developed a suite of morpho-physiological adaptations that includes the latest frontier between internal and external media, the skin. In fishes, the skin is involved on the protection against outside physical and chemical agents as well as pathogens. The basic structure is similar among different groups, but specific morphological and cytological features can be observed. The comparative anatomy of fish skin, especially the epidermis, has been investigated by light and electron microscopy (for reviews, see Imaki & Chavin, 1984; Whitear, 1986). Bhatti (1938) made an elaborate study of the dermal skeleton when he studied the integument of Siluroidea. Sire & Meunier (1993) described only the superficial ornamentation and structure of the dermal plates in a few armored Siluriformes.

The siluriforms comprise about 27% of the neotropical fish species (Reis *et al.*, 2003). Among them the largest family of neotropical catfishes, the Loricariidae. The "acari bodo" *Ancistrus dolichopterus* (Kner, 1854), is a member of the largest subfamily, of Loricariidae, the Ancistrinae, which presents 217 species (Reis *et al.*, 2003). *Ancistrus dolichopterus* is a bristle-nosed or bushy-faced catfish, sluggish, hardy fish that inhabits fresh to brackish waters: it is a bottom dweller, generally clinging to rocks, logs or other objects through suckermouth (Burgess, 1989). As the skin is in constant contact with the environment and is also an important barrier to pathogens, the present investigation reports the structural organization and histo-cytochemical features in relation to the functional significance of the dorsal skin of *Ancistrus dolichopterus*.

Material and Methods

Live specimens (n = 20) of *Ancistrus dolichopterus* (reference specimen INPA-25626; length 12.5 ± 1.0 cm; weight 45.7 ± 4.8 g) were collected from Solimões River (Catalão) at Manaus, Brazil. The fish were maintained for four weeks in 500 l aerated outdoor tanks in the laboratory and were fed with pelleted food on alternate days. The fish were acclimated to the natural diurnal light-dark cycle of 14-10 h.

Fish were cold anaesthetized following Mittal & Whitear (1978). Skin pieces, from the dorsum side close to the dorsal fin, were excised, rinsed in physiological saline and fixed in aqueous Bouin's fluid, alcoholic Bouin's fluid and 10% neutral formalin. After fixation the samples were treated with 5% formic acid for 24 h and were then washed with running tap water for 2 h. Tissues were dehydrated in graded series of ethyl alcohol, cleared in cedar wood oil and embedded in paraffin wax.

Paraffin sections were cut at 6 mm and were stained with Ehrlich's haematoxylin-eosin (HE), Verhoeff's haematoxylin (VHE) and Papanicolaou stain (PS) following Bancroft and Stevens (1982) for evaluation of general organization. To assess carbohydrate histochemistry, slides were stained with Schiff without prior oxidation to demonstrate free aldehydes; with periodic acid-Schiff (PAS) with or without prior acetylation to localize glycoproteins (or mucopolysaccharides) with oxidizable *vicinal* diols and/or glycogen; with deacetylation-PAS to hydrolyze the acetyl esters and to unblock the reactive hydroxyl groups; with ptyalin (saliva) digestion followed by PAS, and Best carmine to distinguish the glycoproteins with oxidizable *vicinal* diols from glycogen contents; with alcian blue (AB) at pH 2.5 to localize glycoproteins with carboxyl groups and/or with O-sulphate esters; with prior methylation-AB pH 2.5 to block the glycoproteins with carboxyl groups and/or with O-sulfate esters; with prior methylation-saponification-AB pH 2.5 to discriminate between the glycoproteins with carboxyl group or O-sulfate esters; with AB at pH 1.0 to identify the glycoproteins with O-sulfate esters only; with combined AB-PAS to differentiate glycoproteins with oxidizable *vicinal* diols and/or glycogen, and glycoproteins with carboxyl groups and/or with O-sulphate esters; with methylation-AB pH 2.5-PAS to block the carboxyl groups and/or with O-sulphate esters with simultaneous observation of glycoproteins with oxidizable *vicinal* diols and with prior methylation-saponification-AB pH2.5-PAS to demonstrate the glycoproteins with oxidizable *vicinal* diols and/or glycogen and to specify these with carboxyl groups or O-sulfate esters following Bancroft & Stevens (1982) and Pearse (1985).

Stage micrometer and eyepiece measuring disc (Carl Zeiss, Jena) were used to count the number of eosinophilic granular cells and mucous cells per mm length of the epidermis, to measure the height and width of mucous cells and to measure the thickness of the epidermis and skin in cross sections.

For each estimation, samples of 10 randomly selected sites from each animal were analyzed. The results are expressed as mean $\pm$ SD throughout.

Results

The skin of *Ancistrus dolichopterus* consists of a multilayered epidermis, dermis, and subcutis (Fig. 1A). The average thickness of the skin is 638.5 ± 157.5 mm. Furthermore, the osteoderms recessed into the skin with their embedded denticles which are distributed over the entire surface of the body (Figs. 1B, 1C).

Epidermis

Measurements of epidermis, four to six cell layers thick, are summarized in the Table 1. The main structural component of the epidermis is epithelial cells, interspersed in between glandular–mucous cells and migrating lymphocytes and eosinophilic granular cells. Pigment cells are also discernible. Club cells and sensory structures such as the ampullary organs (=pit organs), which are the characteristic features of catfish skin, could not be located in the dorsal skin.

Table 1. Average thickness, density and dimensions of dorsal skin and its cellular components in *Ancistrus dolichopterus*. Values are given as mean ± SD, n = 3 with 10 replicates per fish.

Thickness (in μm ± SD)		Density (per mm length ± SD)		Dimension (in μm ± SD)	
		Eosinophilic granular cells	Mucous cells	Mucous cells	
Skin	Epidermis			Height	Width
638.5±57.5	21.83±7.36	13.96±2.17	3.06±0.86	16.25±3.33	13.66±2.60

Epithelial cells. The basal layer of epithelial cells, arranged in a single layer on a thin non-cellular basement membrane, are usually low columnar in shape with centrally placed rounded nuclei (Fig. 1D), sometimes appearing flattened with flat nuclei. The middle layer epithelial cells, which are arranged less compactly in two to four layers, are, in general, polygonal with centrally placed, rounded nuclei. Epithelial cells, which are below mucous or eosinophilic granular cells, appear flattened with flat nuclei. In the superficial layer, epithelial cells present a flattened aspect with flat nuclei (Fig. 1D). Occasionally, exfoliated epithelial cells isolated or in groups, are also seen on the surface.

Epithelial cells, in general, presented healthy appearing nuclei with distinct chromatin material and nucleoli. They stained dark blue in HE and PS, and blue black in VHE. The cytoplasm stains homogeneously light pink in HE, PS and VHE. Although different histochemical procedures were employed, epithelial cells in the basal layer and lower middle layer remained unstained. Cells in the superficial and outer middle layer stain lightly magenta with PAS, greenish blue with AB (pH 2.5 and 1.0) and purple with AB-PAS (Fig. 1E). These reactions indicate the presence of glycoproteins with oxidizable *vicinal* diols and glycoproteins with carboxyl groups and/or with O-sulphate esters in low concentrations at these sites. A gradual increase on the intensity of the reactions was detected towards the surface. In *A. dolichopterus*, a high level of mucogenicity has been demonstrated and this derives from the active involvement of superficial and middle layers of epithelial cells and their voluminous mucous secretion, as indicated by strong mucopolysaccharides reaction (Table 2).

Mucous cells. The density of mucous cells in *Ancistrus dolichopterus* is remarkably low. Their number, height and width are summarized in Table 1. Sparsely distributed mucous cells are restricted to the outer epidermal layers. These cells are rounded or oval, attain voluminous size, and open on the surface to void their secretions. As the secretory contents accumulate within intracellular vesicles, the remaining cytoplasm, including the flattened nucleus, is pushed basally, towards the periphery, resulting in a strong basophilic unit (Fig. 1E). The flocculent secretory contents are basophilic and stain deep blue with HE and PS. Mucous cells contents stain magenta with PAS with or without prior ptylin treatment, greenish blue with AB (pH 2.5 and 1.0), purple with AB-PAS (Fig. 1E) and methylation-saponification-PAS-AB pH 2.5, and magenta with methylation-PAS-AB pH 2.5, indicating the presence of glycoproteins with oxidizable *vicinal* diols, carboxyl groups, and O-sulphate esters.

Lymphocytes. Rounded or irregular lymphocytes, usually distributed in the intercellular spaces of the deeper epidermal layers of other fishes (Mittal & Munshi, 1971), were observed sparsely in this fish and stain deep blue in HE, PS and black in VHE.

Pigment cells (melanocytes). Showed well developed, highly branched, and are distributed randomly throughout the deeper layers of the epidermis and in the dermis. These cells are most evident as an almost continuous layer just beneath the basement membrane (Fig. 1D). The pigment cells are filled with dark brown or black coarse granules.

Eosinophilic granular cells. A large number of very well developed eosinophilic granular cells (EGC), spherical or ovoid, are present in the upper, and occasionally in the lower layers of the epidermis. Average density of these cells is summarized in Table 1. These cytoplasmic granules stain bright red in HE, pinkish-red in Verhoeff's elastin stain, orange-red in Papanicolaou's stain and deep red with alizarin red S. The nucleus is eccentrally placed, usually basally, and is flattened with distinct chromatin material and a nucleolus (Fig. 1D). In contrast to several other previous reports these cells stain deep magenta with PAS and AB-PAS (Fig. 1E). However, these cells could not be located in the deeper layers of the dermis and the subcutis.

Taste buds. Very few taste buds could be located on the skin surface. These are pear-shaped structures consisting of spindle shaped neuro-epithelial cells and supporting cells each positioned above a well-developed dermal papilla (Fig. 1F). Long taste hairs arise on the free surface from these neuroepithelial cells.

Dermis

The dermis is about 15-20 times as thick as the epidermis and hence is the largest component of the dorsal skin of *Ancistrus*. It consists of two distinct layers - the outer *stratum laxum* and the inner *stratum compactum* (Fig. 1A).

***Stratum laxum*.** Adjacent to the basement membrane (Fig. 1A) and composed of comparatively loosely arranged connective tissue fibers, mainly collagen, and richly supplied with fine blood capillaries, nerves and pigment cells. The anteriorly-directed elliptical osteoderms are embedded in the *stratum laxum* and overlap partly the next osteoderm posteriorly. Two horizontal osteoderms are attached with attachment fibers (Fig. 1C). The osteoderms are hollow and have cup shaped projections towards the epidermis for firm anchoring of the denticles (Fig. 1C). An amorphous acellular

Table 2. Summary of histochemical reactions of the carbohydrate moieties in different cell types of *Ancistrus dolichopterus* epidermis at the dorsal part of the body. Symbols: GB, greenish blue; M, magenta; Pur, purple; R, red; -, negative reaction; ±, weak; +, moderate; ++, strong; +++, very strong reaction.

Histochemical technique	Epithelial Cells			Mucous Cells	Eosinophilic Granular Cells	Basement Membrane
	Superficial Layer	Middle Layer	Basal Layer			
No treatment/Schiff	-	-	-	-	-	-
Periodic acid/Schiff (PAS)	M^+	$M^?$	-	M^{+++}	M^{+++}	M^+
Acetylation/PAS	-	-	-	-	-	-
Deacetylation/PAS	M^+	$M^?$	-	M^{+++}	M^{+++}	M^+
Saliva (Ptyalin)/PAS	M^+	$M^?$	-	M^{+++}	M^{+++}	M^+
Best carmine	-	-	-	-	-	-
Alcian Blue (AB) pH 2.5	GB^+	GB^+	-	GB^{+++}	-	GB^+
Methylation/AB pH 2.5	-	-	-	-	-	-
Methylation/saponification/AB pH 2.5	GB^+	GB^+	-	GB^{+++}	-	GB^+
PAS/AV pH 2.5	Pur^+	Pur^+	-	Pur^{+++}	M^{+++}	Pur^+
Methylation/PAS/AB pH 2.5	M^+	$M^?$	-	M^{+++}	M^{+++}	M^+
Methylation/Saponification/PAS/AB pH 2.5	Pur^+	Pur^+	-	Pur^{+++}	M^{+++}	Pur^+
AB pH 1.0	GB^+	GB^+	-	GB^{+++}	-	GB^+

material may be recognized where the osteoderms are lodged. These areas are stained light pink in HE, greenish blue with AB at pH 1.0 and 2.5, magenta with PAS, and purple with AB-PAS. These histochemical reactions indicate that glycoproteins with oxidizable *vicinal* diols, carboxyl groups and with O-sulphate esters are present in low concentrations.

Stratum compactum. Comparatively thin (Fig. 1A), this tissue consists of layers of coarse collagen fibre bundles arranged parallel to the skin surface with a few fine elastic fiber bundles, which stain black with Verhoeff's elastin stain. Many fibrocytes are scattered among the collagen fibers. Branches from the main blood vessels, pigment cells and nerves in the subcutis run through this layer and supply the capillaries in the *stratum laxum*. In the inner surface of the *stratum compactum*, a layer of small-branched pigment cells is discernible. The staining pattern of this layer is similar to that of the *stratum laxum*.

Subcutis

This is the innermost region of the skin and lies in between the *stratum compactum* and the muscles. It is composed of loose connective tissues and is richly infiltrated with fat cells that in routine HE preparations appear as empty spaces. Many blood vessels and nerves may be found in this region.

Denticles. Dermal denticles (denticles) in the skin of *A. dolichopterus* are bilaterally symmetrical structures. They comprise various non cellular and cellular components and both epidermal and dermal elements contribute to their structure. They have caudally pointed crown and embedded broad basal tissue, which latter is narrower as the pedestal. These denticles are well seated with the narrow pedestal basal parts inserted into the cup shaped structures of the elevated mineralized osteoderms to which they are firmly anchored by the attachment fibers. Enameloid and dentin are the components of the crown (Fig. 1B, C). A thin layer of enameloid covers the dentin. The dentine layer is thicker, and thickens

by continuous deposition of dentin centripetally towards the center of the pulp cavity. Odontoblasts are present along the dentine. These cells extend odontoblastic processes to form dentine tubules. The pulp cavity is supplied with the cells and some connective tissues (Fig. 1C). The dentine stains red with HE, magenta with PAS and AB-PAS (Fig. 1B). The attachment fibers appear pinkish with HE, magenta with PAS and AB-PAS and remain unstained with AB at pH 2.5 and pH 1.0.

Discussion

The skin of *A. dolichopterus* comprises three principal layers: the epidermis, dermis and subcutis. The epidermis of *A. dolichopterus* is thicker than the skin of other teleosts (Banerjee & Mittal, 1975; Park *et al*., 2003; Lizarazo *et al*., 2008) ranging two to three time fold. The primary function of the epidermis is protection against environmental hazards. In fish, mucogenic cells generally provide this function by secreting their contents on the surface, but siluroids generally present a low number of mucous cells in epidermis. In *A. dolichopterus* they are voluminous, but their density is remarkably low and they are confined to the upper layers of the epidermis. In *Bagarius bagarius*, a scaleless siluroid fish, mucous cells are few and restricted to epidermal furrows; keratinized epithelial cells and epidermal plaques provide protection (Mittal & Munshi, 1970; Mittal & Whitear, 1979; Mittal *et al*., 1995). *Rita rita* presents small mucous cells at a low density, but in contrast presents numerous club cells that are arranged in several layers (Mittal, 1968). In *Barbus sophor* (=*Punctius sophore*), in regions where mucous cells are numerous and are well developed, club cells are either sparse or absent, whereas in regions where mucous cells are smaller and present in a small number, the club cells are numerous and well developed (Mittal *et al*., 1976). Singh & Mittal (1990) suggested that the low density of mucous cells is compensated by the high density of club cells as an effective defense mechanism, in carps of India. However,
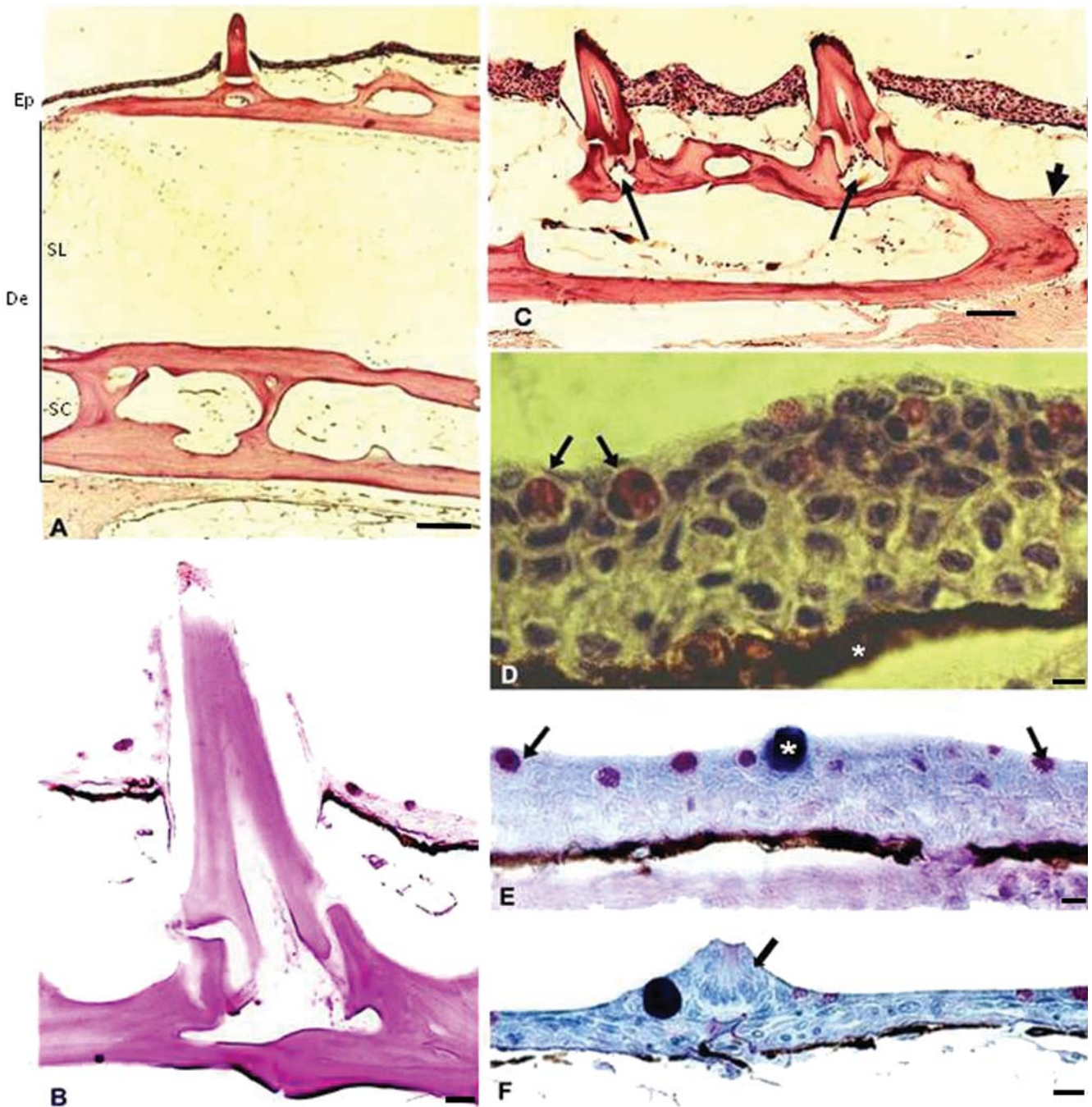
Fig. 1. Skin of *Ancistrus dolichopterus*. **A**) Epidermis (Ep) and thick dermis (De) of *A. dolichopterus* showing *stratum laxum* (SL) and s*tratum compactum* (SC), note also the hollow osteoderm and the denticle (HE). Scale bar = 100 mm. **B**) Denticle at higher magnification. (PAS) Scale bar = 10 mm. **C**) Denticles with pointed crown and broad basal parts. The narrow pedestal basal part fit in the cup shaped structure of dermal osteoderm (arrows). The dentine surrounds the pulp cavity, filled with cells and connective tissue, and covered by a thin enamel layer. The osteoderm is connected with the denticles with attachment fibers (small arrows). (HE) Scale bar = 20 mm. **D**) Epidermis showing epithelial cells distributed in different layers, low columnar cells in basal, polygonal in the middle and flat cells in the superficial layer. Spherical eosinophilic granular cells (arrows) with granular cytoplasmic contents and eccentric nucleus. Note the pigment cells with dark brown coarse granules (*) in a continuous layer below the basement membrane (HE). Scale bar = 10 mm. **E**) General glycoproteins in the epidermis. The mucous cell (*) and EGCs (arrows) stain very strong while epithelial cells located in superficial and outer middle layers stain moderately (AB/PAS). Scale bar = 10 mm. **F**) A pear shaped taste bud (arrow) (AB/PAS) Scale bar = 10 mm.

club cells were not observed in the dorsal skin of *Ancistrus*. The reduced number of mucous cells and absence of club cells indicate that their protective function has been taken up by the thick epidermis and also by the osteoderms and the denticles impregnated in its dermis. Mucous cells of *Ancistrus* present a voluminous aspect which seems to compensate its low number resulting in a high mucogenicity that was observed trhough histochemistry.

Glycoprotein contents of epidermal secretions in fish may vary according to the species and cell types (Whitear, 1986). In *A. dolicopterus*, a high level of mucogenicity may be an adaptation to its peculiar bottom-scooping habitats. This species frequently deal with organic debris in the bottom when searching for food, and must keep the skin surface clean then the mucus can precipitate mud held in suspension (Hora, 1934). The secretion of copious mucus was reported in fishes which burrow and live associated with muddy waters (Mittal & Munshi, 1971; Mittal & Banerjee, 1975). The lubricant role of mucus reduces body friction in water, helping on swimming and also protecting the body from abrasion during burrowing and nest digging (Mittal & Munshi, 1971; Rosen & Cornford, 1971; Mittal *et al.*, 1994). The detection of glycoproteins with oxidizable *vicinal* diols and O-sulphate esters in epithelial and mucous cells in the epidermis indicates its role on lubrication of skin surface in *A. dolichopterus*. Mucus can act as barrier to various pathogens and prevent their colonization in the epidermis (Lewis, 1970; Mittal *et al.*, 1994). Furthrmore, the sulphate groups provide an acidification of glycoproteins which is effective to prevent bacterial and viral invasion (Lewis, 1970; Mittal *et al.*, 1994).

The marked histological and biochemical similarity between fish eosinophils and mammalian mast cells has been noted by Ellis (1977, 1985) and reviewed by Powell *et al.* (1993b). Eosinophils release toxic proteins and oxygen radicals onto the body surface of multicellular parasites in areas of inflammation. Degranulation of eosinophils induces a fast generalized inflammation and infiltration by wondering leucocytes as well as destruction of invading parasites. A large population of eosinophilic granular cells is present in the upper epidermal layers of *A. dolichopterus*. There is no clear-cut distinction among various categories of granulocytes in the dorsal part of the skin. But according with their size, structure and staining these were considered as eosinophilic cells (Rombout *et al.*, 1989; Powell *et al.*, 1990, 1993a, 1993b; Cross & Matthews, 1991). The presence of these cells plays an important role on the protection of *A. dolichopterus* and other catfish species which can be exposed to a large number of skin parasites in their peculiar habitat (Thatcher, 1991).

Eosinophils in fish can be implicated in both specific immunological and general defense processes (Powell *et al.*, 1990). They show degranulation after exposure pathogenic bacteria (Ellis, 1985; Powell *et al.*, 1993a) and other stressors as UV light (Roberts & Bullock, 1981) and substance P (Powell

et al., 1993b). The granulocytes were motile, chemotactic to histamine and to the degranulation products of neighboring granulocytes, and their population increased by handling stress within the epidermis (Barnett *et al.*, 1996). Granulocytes contribute to wound healing in carp *Cyprinus carpio* (Iger & Abraham, 1990) and have been reported to be part of the trout immune response to parasitic infections (Sharp *et al.*, 1989). The presence of metazoan parasites in species of sedentary catfishes, astroblepids, callichthyids, hypophthalmids, and loricariids, including *Ancistrus* from tropical (Amazon) and sub-tropical South American streams has been documented (Freihofer & Neil, 1967; Kohn & Cohen, 1998; Thatcher, 1998). Thus, the presence of EGCs in appreciable amount in the epidermis of *Ancistrus* can be seen as a system that destroys such multicellular parasites. This indicates a relationship between this epidermal feature and ectoparasite protection in fish.

A continuous layer of melanocytes immediately beneath the basement membrane, and their random presence in different layers of the dermis and epidermis in bottom dwelling fish impart coloration and camouflaging functions. Toledo & Jared (1993), while working with amphibian skin, suggested that the dermal chromatophore units provide patterns of coloration and may also function to absorb or reflect radiations (thereby contributing to regulation of body temperature). They may play similar roles in *Ancistrus* too, since this air-breathing fish can be exposed to high solar radiation levels during episodes of environmental hypoxia and dry season in the Amazon (Val & Almeida-Val, 1995).

Taste buds are characteristic features of fish skin and assist them in the recognition and location of food. In many ostariophysans (*e.g.*, cyprinids and catfish) external taste buds cover the entire body surface and aggregate in areas that frequently contact and locate food, such as lips, barbells or elongated fins (Kotrschal, 1996). A low number of taste buds in the dorsal skin was expected in *A. dolichopterus* and can be seen as an adaptive response because these algal grazers feed by night and bear long branched-tentacles on the snout, which may have sufficient taste buds. However, it needs detailed investigation.

In *A. dolichopterus* the *stratum compactum* of the dorsal part of the skin is very thin. But the *stratum laxum*, unlike that of other siluroid fishes, *Clarias batrachus* (Banerjee & Mital, 1975), *R. rita* (Mittal, 1968), and *B. bagarius* (Mittal & Munshi, 1970) is very thick, provided with the osteoderms, and is distinct from the *stratum compactum*. The thickness of this layer is increased in fishes that present well developed scales and comparatively thinner in species that have less developed scales. In scaly fishes, the scales are lodged in the characteristic connective tissue pockets in the *stratum laxum*. Thus a well developed *stratum laxum* is found in fishes having highly developed scales or osteoderms may be explained. In scaleless fishes, there is no need of such structural modifications and then *stratum laxum* remains insignificant. Banerjee & Mittal (1975)

suggested an inverse relationship between the thickness of the *stratum laxum* and the *stratum compactum* in fishes. This corroborates our view regarding *Ancistrus*. The thick integument together with denticles and osteoderms helps to keep them at the bottom. The compact dermis of the *Ancistrus* thus evidently serves two functions: it offers effective protection and, because of its weight, facilitates precise maneuvering near the bottom.

A subcutis is present in all the fishes except *Amia calva* (Rabl, 1931). But Bhatti (1938) did not describe this layer while working with the integument of a number of siluroids. As observed in *A. dolichopterus*, subcutis is also poorly developed in *B. bagarius* (Mittal & Munshi, 1970), thin and inconspicuous in *Mestacembelus* and *Amphinuous* (Mittal & Munshi, 1971) but well differentiated in *Heteropneustes* and *Clarias* (Mittal & Munshi, 1971; Banerjee & Mittal, 1975), respectively.

Denticles cover the head and body of *Ancistrus*. These sharp, conical, backwardly-directed denticles comprise a dentine cone covered by a hypermineralized cap that surrounds the pulp cavity which is supplied with the cells and connective tissue, are similar to the placoid scales and are the true homologous to true teeth. Bhatti (1938) suggested these denticles to be homologous with true teeth. In *Corydoras aeneus* (Sire & Huysseune, 1996) and *Denticeps clupeoides* (Sire *et al.*, 1998) the denticles present in the skin are true odontodes. This structural similarity between the denticles and the elasmobranch placoid scales shows that a morphogenetic/histogenetic potential has been present in vertebrate skin even since the early times of Agnathan evolution and that such structures were expressed convergently in various groups during fish evolution. The resemblance of the cell types in the epidermis of elasmobranches as *Raja* to those known from teleost fishes, also support this idea (Whitear & Moate, 1998).

A conspicuous feature such as loricariid denticles raises the question of their biological significance. They provide effective protection from abrasion and deter enemies. These crevice dwellers use their spines to jam themselves into crevices when any attempt is made to remove them, and the movable hooked spines on the cheek skin of *Ancistrus* are also used in this way. Thus dermal bony plates and denticles of *Ancistrus* are noteworthy ostariophysan innovations that facilitate the invasion of new habitats or niches.

Acknowedgements

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