An Acad Bras Cienc (2023) 95(4): e20191259 DOI 10.1590/0001-3765202320191259
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Structural and histochemical aspects of the *Gymnotus carapo tegument* (Teleostei: Gymnotiformes)

TANIA BLANCO COHENE, GABRIELA OLEA & CAROLINA FLORES QUINTANA

Abstract: The present study aims to structurally and histochemically characterize the *Gymnotus carapo* tegument. 30 specimens were captured and slaughtered by spinal section with anesthesia. The observation was carried out with a stereoscopic microscope and the body surface was photographed. Fragments of the dorsal, ventral and lateral region were fixed in Bouin's solution for 12 hours and subsequently preserved in 70% alcohol. They were subsequently observed in the scanning electron microscope (SEM). The preparation for SEM was performed following the standardized protocol. Histological preparations were made, and the cuts were colored with H-E, PAS and Coomassie Blue. The images were obtained in an Olympus BX41-ENUTV-4 microscope. From the observations in SEM a plain tegument with pores of different sizes could be evidenced. The scales of the different regions of the body have different ornaments. Microscopically it was composed of a stratified non-keratinized epithelium consisting of two types of morphologically distinct cells: epidermal cells and mucous cells (PAS-Commassie Blue positive). Under the epithelium there is a layer of dense irregular connective tissue with associated chromatophores and more deeply scales. These analyzes are the basis for future studies that will focus on elucidating the events related to integumentary healing in this species.

Key words: Dermis, epidermis, fish, mucous glands.

INTRODUCTION

The tegument of an animal is an exposed tissue on the surface of the body, which is in direct contact with the environment. It acts in numerous functions related to the organism / environment interface and also participates in the protection mechanisms against physical, chemical and biological agents, such as pathogens (Santos 2016). In the case of fishes, it completely covers it, including the fins, and constitutes the largest surface of contact with water. It is a cover that provides both communications with the environment and protection against external agents or mechanical damage and has some healing and regeneration capacity (Damasceno et al. 2012, Manera et al. 2016, Furtado et al. 2019).

The epidermis of teleost consists of a non-cornified stratified flat epithelium. They may or may not be covered by scales of dermal origin. The number of cell layers can vary from two in the larvae to ten or more in adults (Bonilla Lizarazo et al. 2008, Shooraki et al. 2018). In turn, it can vary depending on other factors such as sex, degree of maturation, time of year, and adaptations to the environment (Elliott et al. 2000, 2011, Mistri et al. 2018). Among the different types of cells found in the epidermis are goblet cells, which constitute a type of unicellular exocrine gland common to most groups of

animals. They are the second most important secretory cell category in teleosts skin (Elliott et al. 2011, Concha et al. 2017). The number of goblet cells can change seasonally and during processes such as larval metamorphosis and sexual maturation (Damasceno et al. 2012, Mistri et al. 2018). Another cell group present is the large unicellular glands called Club cells, which are located in the middle and deep layers of the epidermis (Mistri et al. 2018).

The dermis is composed of loose connective tissue in the papillary region, which is located below the epidermis and by dense connective tissue in the reticular region. In addition, it presents pigment cells, macrophages and mast cells. The hypodermis separates the dermis from the underlying muscle layer. (Elliott 2000, Souza et al. 2003, Le Guellec et al. 2004). This layer is it is considered as a variant of connective tissue, specialized in lipid storage, whose main cells are adipocytes, which are found in a mesh of connective tissue reticular, in which it is possible to observe blood capillaries.

Gymnotus carapo is commonly known as "brunette." It is included in the order. Gymnotiformes and has a wide distribution that includes the basins of the Paraná, Paraguay, Uruguay, and Plata rivers. It is a kind of nocturnal habits that during the day remain under camalotes or other floating vegetation. These teleosts can receive and emit electrical pulses that are used for individual communication, courtship, and care of the territory. Tolerates low concentrations of dissolved oxygen and by using the swim bladder can breathe atmospheric air (Casciotta et al. 2005). Studies on the histomorphology of this species are scarce, and it represents a good model of study of the integumentary morphology. Therefore, the objective of the present work is to characterize the integumentary structure of *G. carapo* from the anatomical, histological and histochemical analysis of the tegument.

MATERIALS AND METHODS

Study area

The sampling was carried out biweekly between the months of February to December 2018 in bodies of lentic waters near the city of Corrientes, 10 km in the NE direction (Perichón Area: 27°26'36.6"S 58°45'14.0"W). Phytogeographically, the sampling site is located in the Humid Chaco (Cabrera 1976). The climate is subtropical or mesothermal (Carnevali 1994), with an annual average temperature gradient that ranges from north to south between 21°C and 19,5°C, January being the warmest month (annual average, 27°C) and July the coldest month (annual average, 14°C).

Material capture and processing methodology

A total of 30 individuals of *G. carapo* were manually captured. The animals were transferred to the laboratory in plastic bags and then euthanasia of the specimens with overdose of 2% lidocaine was proceeded, following the protocol established in the Guide for Animal Euthanasia proposed by the IACUC (The Institutional Animal Care and Use Committee) and the teleosts necropsy guide of Noguera et al. (2015), under the norms of the ethics committee of the School of Veterinary Sciences-UNNE.

Morphological analysis (macroscopic)

Fragments of the tegument of the dorsal, ventrolateral, and ventral region of the trunk of the individuals were fixed in Bouin solution and preserved in formalin with pH 7.4 at 10%. The fragments were observed and photographed under stereoscopic magnifying glass.

Morphological analysis (ultrastructural)

The fragments of the tegument were observed in scanning electron microscope (SEM). The preparation of the specimens for SEM was carried out following the standardized protocol of dehydration in solutions of increasing concentration of Acetone (12.5, 25, 50, 75, 100%), critical point drying, and gold plating. The observations were made in a JEOL JSM-5800 LV Microscope belonging to the Scanning Electron Microscopy Service of the General Secretariat of Science and Technology of the National University of the Northeast (UNNE). A macroscopic description was made in which the presence of grooves, folds, crevices or small protrusions was taken into account, as well as the different colors that were observed.

Histological analysis

With the tegument fragments of the dorsal, ventrolateral, and ventral trunk region previously fixed in Bouin and preserved in alcohol 70%, histological preparations were prepared following the conventional technique of dehydration, inclusion in paraffin and staining. Dehydration was performed in increasing concentrations of ethyl alcohol (70, 80, 96 and 100%); the clearance was performed with xylol. The inclusion in xylol-paraffin (50% and 50%) was carried out for 12 hours and three paraffin baths of 2 hours each. Finally, tacos were made by orienting the samples to obtain cross-sectional or longitudinal sections from 5 to 7µ. These were obtained with a manual Spencer Rotary Microtome. The samples were colored with Hematoxylin-Eosin (HE) for general cytological and histological characterization and histochemical reactions of PAS-H (Periodic acid-Schiff-hematoxylin) for the detection of primarily carboxylated acid glycosaminoglycans and Coomassie Blue for the detection of conjugated proteins (Kiernan 1999). The proportions of the glandular types for each body region were estimated qualitatively. The preparations were observed and photographed with an Olympus BX41-ENUTV-4 microscope belonging to the chair of histology and embryology of the School of Veterinary Sciences - UNNE.

RESULTS

At a macroscopic level, the skin of *G. carapo* is smooth, with subepidermal scales, of variable coloration, presenting dark spots of serpentine form in the dorsal andlateral region, towards the ventral region is light in color with fainter spots (Fig. 1a-d). From the observations in SEM, a smooth tegument with pores of different sizes could be evidenced (Fig. 2a-c). The scales of the different regions of the body (dorsal/ventral) have different ornaments, being more prominent and numerous those of the ventral region (Fig. 2d-f).

At the histological level, both the dorsal, ventrolateral and ventral region are composed of a stratified non-cornifiedepithelium, which is supported by a thick layer of connective tissue and a wide muscular tissue (Figs. 3a, 4a). The epithelium is composed of two types of morphologically distinct cells: epidermal cells (Figs. 3b, 4b) and mucous cells (unicellular gland) (Figs. 3c-d, 4c), positive for histochemical staining of PAS (for mucopolysaccharides) and Coomassie blue (for conjugated proteins). Epidermal cells are small when compared to mucous cells. These two types of cells form a heterogeneous stratified epithelium composed of small flattened cells and large globular cells (Fig. 3a). The number of layers varies according to the arrangement and heterogeneity of cell types, between the 3 regions analyzed (dorsal, lateral and ventral) (Figs. 3a, 4a); the mucous cells in the ventral region appear in greater numbers, with a gradual increase from the lateral region

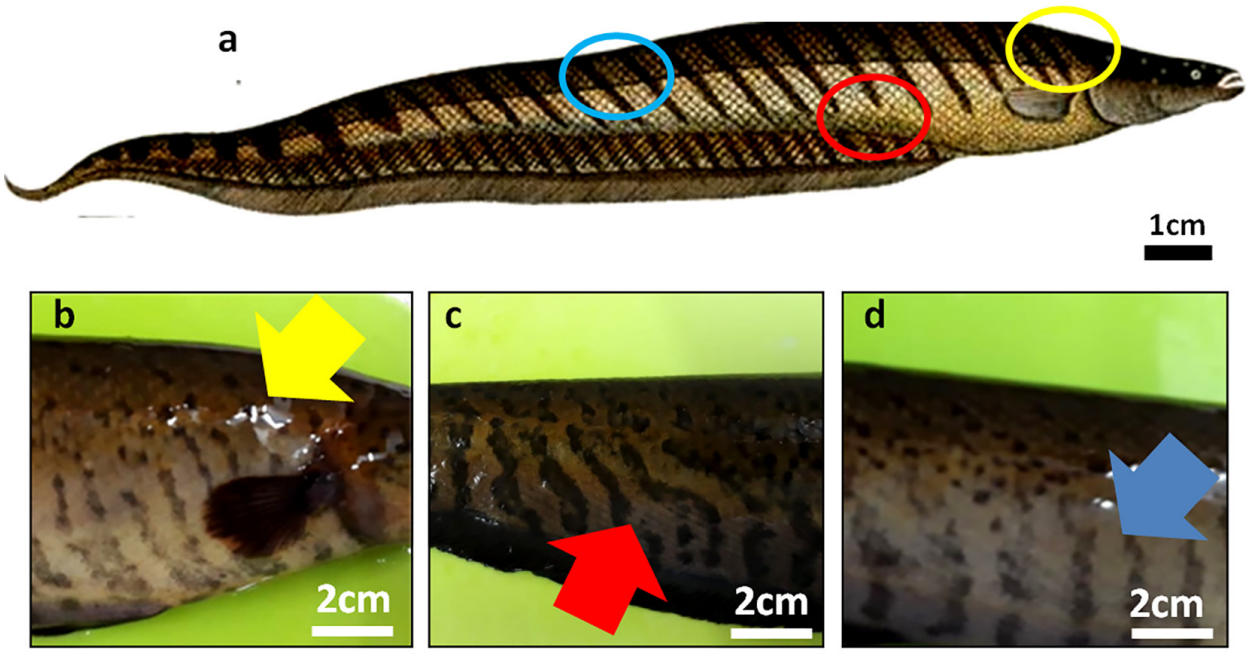

Figure 1. a) Macrophotography of the *Gymnotus carapo* tegument. b) Macroscopic detail of the dorsal region tegument. c) Macroscopic detail of the tegument of the lateral region. d) Macroscopic detail of the tegument of the ventral region. References: circle and yellow arrow: sampled fragment of the dorsal region. Circle and red arrow: sampled fragment of the lateral region. Circle and light blue arrow: sampled fragment of the ventral region.

(Fig. 4b). The cytoplasm of the mucous cells is quite poor in the organelles and rich in PAS + secretion (Figs. 3c, 4c), usually the mucous cells have a regular distribution in the superficial and middle layers of the epidermis. Figures 3c, 4c show the glycoprotein content of these cells with the PAS technique, which is observed in purple-magenta color and granular appearance with a well defined cell contour. Regarding the presence of club cells, their presence has not been evidenced in the species under study.

Beneath the epithelium is the dermis, which is made up of connective tissue, with associated fibroblasts and chromatophores (Figs. 3a-b, 4a-b), under the dermis, a thick layer of skeletal muscle tissue is found. The dermis consists of a layer of loose connective tissue in the papillary region and irregular dense connective tissue in the reticular region; in which the nuclei of the fibroblasts can be observed, it has melanophores (pigment cells), which flank the dermis; This layer was poorly vascularized (Fig. 3b). The hypodermis is located under the dermis and is mainly composed of adipose tissue (Fig. 3e). It is observed that the main cells of this tissue are adipocytes (Fig. 3e).

The scales could be observed in the histological sections of the three regions; they are immersed in the dermis, below the chromatophores layers (Fig. 3f). It is of elasmoid type, and it appears in horizontal and vertical lines forming a regular pattern (Fig. 3g). Its origin, like other bone elements is dermal. Each scale consists of 3 layers; First, the outer layer consisting of a network of collagen fibers (Fig. 3f) and well-mineralized tissue (Fig. 3g, 4d-e) is developed, then the basal layer composed of a partially mineralized tissue called elasmodine is formed and finally, the boundary layer composed of hypermineralized fibers is formed (Fig. 3h).

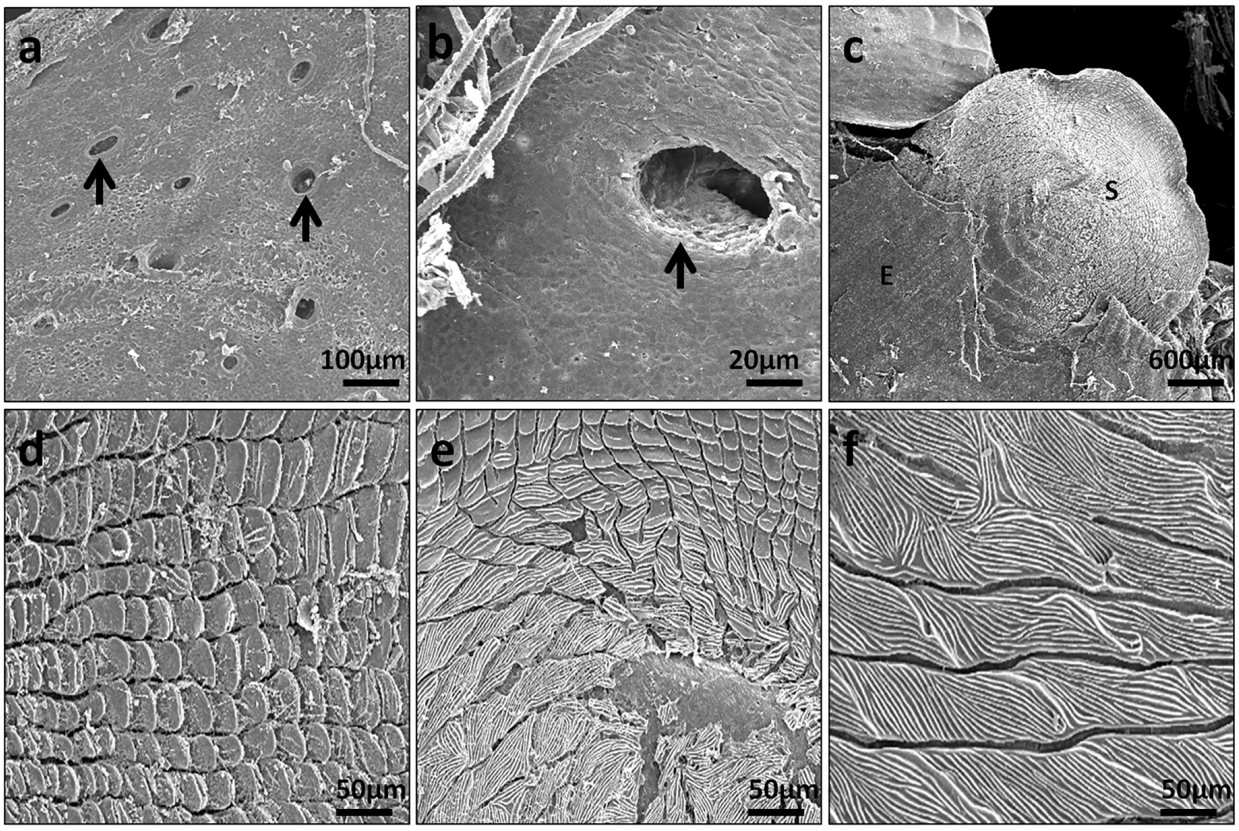

Figure 2. a-b) Micrograph of SEM of the *Gymnotus carapo* tegument, with detail of the pores (b). c) Micrograph of SEM with detail of the tegument and scale. d) Detail of the micrograph of SEM of dorsal scale. e) Detail of the micrograph of SEM of lateral scale. f) Detail of the micrograph of SEM of dorsal scale. References: E: epidermis. S: scale.

Associated with the epidermis, the receptors that make up the lateral line could be evidenced, these are constituted by neuromasts, and each composed of a group of hair cells. The cilia are surrounded by an outstanding dome (Fig. 3i, 4f).

DISCUSSION

The *G. carapo* tegument comprises three main layers: the epidermis, the dermis, and the hypodermis. The main function of the epidermis is protection against environmental risks. In teleosts, mucogenic cells generally provide this function by secreting their surface content. In *Ancistrus dolichopterus,* they are bulky, but their density is remarkably low, and they are confined to the upper layers of the epidermis. In *Bagarius bagarius*, a siluroid fish without scales, mucous cells are few and restricted to epidermal grooves (Garg et al. 2008), in the case of *G carapo*, abundant mucous cells were evidenced in the tegument, with predominance in the ventral region. In *Punctius sophore*, in regions where mucous cells are numerous and well developed, club cells are scarce or absent, while in regions where mucous cells are smaller and present in a small number the cells of the Clubs are numerous and well developed (Mittal 1968). Singh & Mittal (1990) suggested that the low density of mucous cells is compensated by the high density of club cells as an effective defensemechanism, in the tents of India. However, no club cells were observed in the dorsal skin of *G. carapo*. The reduced number

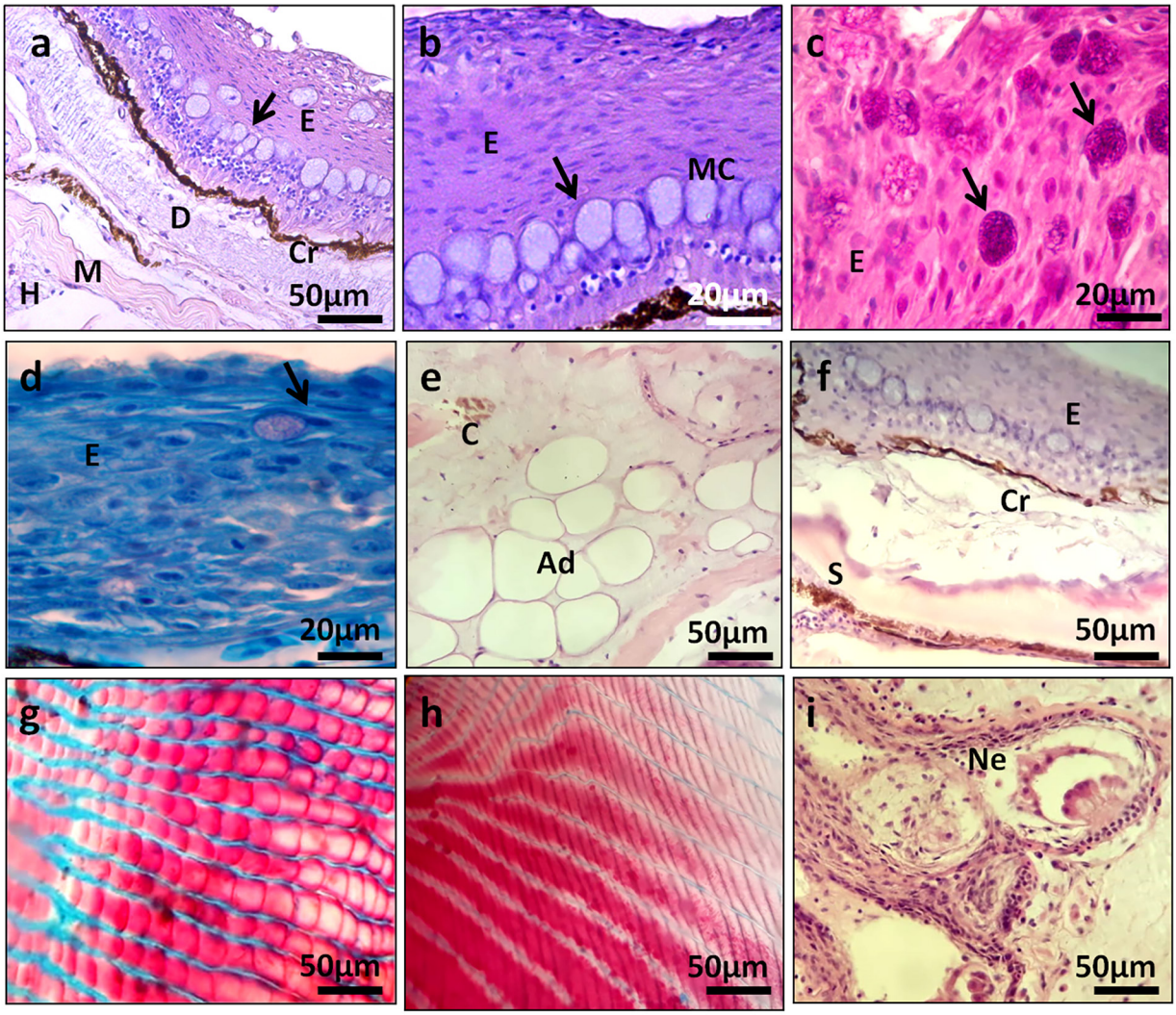

Figure 3. Photomicrography of the *Gymnotus carapo* dorsal tegument. a) Cross section of the tegument, with detail in the 3 regions (epidermis, dermis and hypodermis) (Staining: H-E). b) Detail of the epidermis of *Gymnotus carapo* in cross section. (Staining: H-E) c) Detail of PAS positive mucous cells (Staining: histochemical reaction of PAS). d) Detail of Commassie Blue mucosal cells positive (Staining: histochemical reaction of Commassie Blue). e) Detail of the hypodermis of *Gymnotus carapo* in cross section. (Staining: H-E). f) Histological detail of the scale of *Gymnotus carapo*. (Staining: H-E). g-h) Macroscopic detail of the scale of *Gymnotus carapo*. i) Detail of the neuromastes in cross section. References: E: epidermis. D: dermis. M: muscle layer. H: hypodermis. MC (arrow): mucous cell. C: capillary; Cr: chromatophore. S: scale. Ne: neuromast.

of mucous cells and the absence of club cells indicate that their protective function has been assumed by the thick epidermis. In *Chaca chaca*, cornified structures unlike the mucogenic epidermis are devoid of secretory cells - the mucous goblet cells and the club cells (Mistri et al. 2018). Mittal & Banerjee (1975) reported that where keratinization (or cornification) does occur, secretory cells are not found and suggested that there is an inverse relationship between the keratinization (or cornification) and slime secretion. So the absence of club cells in *G. carapo* is supported by previous reports that "there is an inverse relationship between keratinization and mucogenesis". Cornified or corneal structures are associated to provide

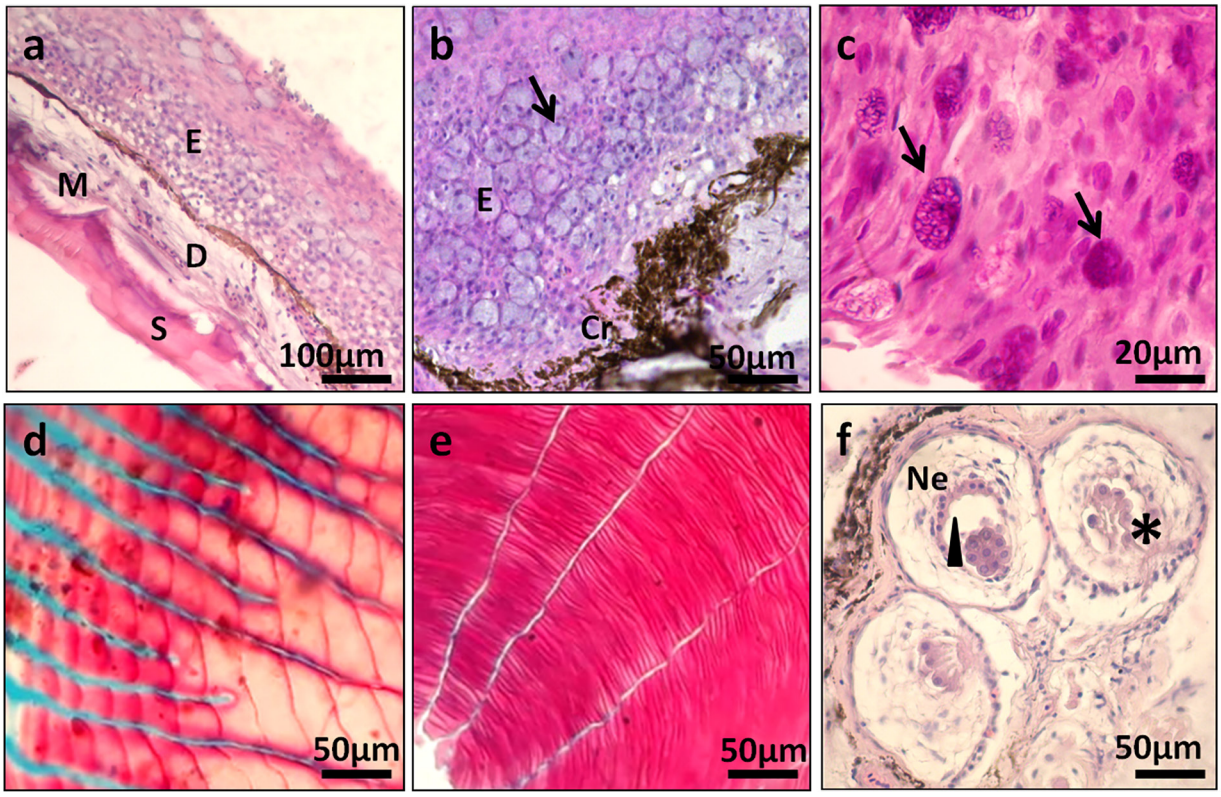

Figure 4. Photomicrography of the *Gymnotus carapo* lateral and ventral tegument. a) Cross section of the lateral tegument (Staining: H-E). b) Detail of the epidermis of the ventral region of Gymnotus carapo in cross section. (Staining: H-E) c) Detail of PAS positive mucous cells of the ventral region (Staining: PAS histochemical reaction). d-e) Gross detail of the scale of the lateral region (D) and ventral region (E) of *Gymnotus carapo*. f) Detail of the neuromastes in cross section. References: E: epidermis. D: dermis. M: muscle layer. MC (arrow): mucous cell. C: capillary; Cr: chromatophore. S: scale. Ne (asterisk): neuromastes.

protection against abrasions and friction stress during excavation.

The glycoprotein content of epidermal secretions in teleosts may vary depending on the species and cell types (Whitear 1986, Roussel & Delmotte 2004, Mistri et al. 2018). The secretion of abundant mucus was reported in teleosts that are buried and live associated with muddy waters (Mittal & Munshi 1971, Mittal & Banerjee 1975). The lubricating role of mucus reduces body friction in water, helps swimming, and also protects the body from abrasion during nest excavation (Mittal & Munshi 1971, Rosen & Cornford 1971, Mittal et al. 1995). Mucus can act as a barrier to several pathogens and prevent its colonization in the epidermis (Mittal et al. 1995).

A continuous layer of melanocytes immediately below the basement membrane, and its random presence in different layers of the dermis and epidermis in teleosts that live in the bottom impart coloration and camouflage functions (Mistri et al. 2018); this could be seen in *G. carapo*. Toledo & Jared (1993) and Olea et al. (2019), for anurans suggested that the chromatophores units provide color patterns and can also function to absorb or reflect radiation (thus contributing to the regulation of body temperature). They can also play similar roles in *G. carapo*, since this teleosts may be exposed to high levels of solar radiation during episodes of environmental hypoxia and the dry season.

As for the *G. carapo* dermis, the histological observations do not show a clear division of the spongy and compact strata. This suggests that the dermis would withstand the pressure exerted by the physical medium (associated with the substrate) acting as a buffer layer. Regarding the vascularization of the dermis described in other teleosts (Berra & Humphrey 2002, Park 2002, Park et al. 2003, Souza et al. 2003, Le Guellec et al. 2004); It can be said that *G. carapo* differs slightly, as it presents little vascularization that can be attributed to a low metabolic level or possibly to the fact that the dermis has a lot of collagen. Another peculiarity of the histological observations was to find pigment cells that correspond to the group of melanophores flanking the dermis. Although a count of these cells was not performed, abundance was evident towards the dorsal and lateral surfaces of the teleosts, which can be associated with the characteristic pigmentation observed microscopically; These cryptic conditions can be attributed to the environment in which they live and to their adaptation to the evasion of predators, as proposed for *Eremophilus mutisii* (Bonilla Lizarazo et al. 2010). The histological results for *G. carapo* skin allow us to assign a protective and supportive function for this teleosts due to the conformation of the skin layers and the cells found in the epidermis.

This study aimed to describe the morphology of the *G. carapo* tegument without focusing on functional aspects; however, we can infer a protective function for the tegument in this species. There are few studies in the literature on scanning electron microscopy and comparative histology of the tegument in teleosts where the tegument and dermal scales are analyzed together. Therefore, the current findings represent a reference point in the epidermal structure of a neotropical species of the Gymnotiform order at morphological and histochemical level. These analyses are the basis for future studies that will focus on elucidating the events related to integumentary healing in this species.

Acknowledgments

This work was funded by research projects accredited by the UNNE Secretariat of Science and Technology: PI18B005 "Structural, histochemical, and healing aspects of the Gymnotus carapo tegument." Director: Flores Quintana, Carolina; and PI16F014 "Study of tegumentary morphology, anatomy, and ecophysiology in vertebrates of northeastern Argentina." Director: Claver, Juan.

REFERENCES

BERRA TM & HUMPHREY JD. 2002. Gross anatomy and histology of the hook and skin of forehead brooding male nurseryfish, Kurtus gulleveri, from northem Australia. Environ Biol Fishes 65: 263-270.

BONILLA LIZARAZO RJ, QUINTERO VIRGUEZ M, GÓMEZ RAMÍREZ E, RODRÍGUEZ CAICEDO D & HURTADO GIRALDO H. 2008. Histología y morfometría de piel del pez *Eremophilus mutisii* (Trychomecteridae, Siluriformes) Rev Biol Trop 56(2): 885-893.

CABRERA AL. 1976. Enciclopedia Argentina de Agricultura y Jardinería: Regiones Fitogeográficas argentinas (Vol II). Buenos Aires: ACME, 85 p.

CARNEVALI R. 1994. Fitogeografía de la provincia de Corrientes. Gobierno de la provincia de Corrientes (Edición del autor). Corrientes: Intituto Nacional de Tecnología Agropecuaria, Argentina, 324 p.

CASCIOTTA JR, ALMIRÓN A & BECHARA JA. 2005. Peces del Iberá: hábitat y diversidad. La Plata: UNDP, Fundación Ecos, UNLP, UNNE, Argentina, p. 181-182.

CONCHA K, OLIVARES P, FONSECA-SALAMANCA F, SANCHEZ R, SERRANO F & PARODI J. 2017. Aditivos Mucogénicos para el Control de *Caligus rogercresseyi* en Salmón del Atlántico (*Salmo salar*). Rev de Investig Vet del Peru 28(3): 477-489.

DAMASCENO EM. 2012. Caracterização Morfológica da Epiderme do Bagre Pimelodella Cf. Vittata (osteichthyes: Ostariophysi: Siluriformes: Heptapteridae) Com Ênfase nas Células de substância de Alarme. Tesis de Maestría, Universidade Federal do Espírito Santo.

ELLIOTT DG. 2000. Integumentary System. In: Ostrander G, The Laboratory Fish. Academic, Washington, DC, USA. 271-300.

ELLIOTT DG. 2011. THE SKIN | Functional morphology of the integumentary system in fishes. Encyclopedia of fish physiology. San Diego: Academic Press (Elsevier), 476-488 p.

FURTADO WE, CARDOSO L, FIGUEREDO AB, MARCHIORI NC & MARTINS ML. 2019. Histological and hematological alterations of silver catfish *Rhamdia quelen* highly parasitized by *Lernaea cyprinacea*. Dis Aquat Org 135(2): 157-168.

GARG TK, DOMINGOS FV, ALMEIDA-VAL VF & VAL AL. 2010. Histochemistry and functional organization of the dorsal skin of *Ancistrus dolichopterus* (Siluriformes: Loricariidae). Neotrop Ichthyl 8(4): 877-884.

KIERNAN J. 1999. Histological and histochemical methods: theory and practice (3rd ed). Oxford: Butterworth Heinemann.

LE GUELLEC D, MORVAN-DUBOIS G & SIRE JY. 2004. Skin development in bony fish with particular emphasis on collagen deposition in the dermis of the zebrafish (*Danio rerio*). J Dev Biol 48: 217-231.

MANERA M, GIARI L, DE PASQUALE JA & DEZFULI BS. 2016. Local connected fractal dimension analysis in gill of fish experimentally exposed to toxicants. Aquat Toxicol 175: 12-19.

MISTRI A, KUMARI U, MITTAL S & MITTAL AK. 2018. Keratinization and mucogenesis in the epidermis of an angler catfish *Chaca chaca* (Siluriformes, Chacidae): A Histochemical and fluorescence microscope investigation. Zoology 131: 10-19.

MITTAL AK. 1968. Studies on the structure of the skin of *Rita rita* (Ham.) (Bagridae Pisces) in relation to its age and regional variations. Indian J Zool 9(2): 61-75.

MITTAL AK & BANERJEE TK. 1980. Keratinization versus mucus secretion in fish epidermis. In: Spearman RIC & Riley PA (Eds), The Skin of Vertebrates (Linn Soc Symp Ser). London: Academic Press, 1-12 p.

MITTAL AK, GARG TK & VERMA M. 1995. Surface architecture of the skin of the Indian catfish, Bagarius bagarius (Hamilton) (Sisoridae; Siluriformes). Jap J Ichthyol 42: 187-191.

MITTAL AK & MUNSHI JSD. 1971. Acomparative study of the structure of the skin of certain air-breathing fresh-water teleosts. J Zool London 163: 515-532.

NOGUERA P, UBEDA C, BRUNO D & SEMENES L. 2015. The Fish Necropsy Manual. Accessed from: https://necropsymanual.net/en/home/.

OLEA GB, CHEIJ EO, CURI LM, CUZZIOL BOCCIONI AP, CÉSPEDEZ JA & LOMBARDO DM. 2019. Histological and immunohistochemical characterization of the integument and parotoids glands *Rhinella bergi* (Anura: Bufonidae): Development and differentiation. Acta Histochem 121(3): 277-283.

PARK JY. 2002. Structure of the skin of an air-breathing mudskipper, *Periophthalmus magnuspinnatus*. J Fish Biol 60(6): 1543-1550.

PARK JY, LEE YJ, KIM IS & KIM SY. 2003. Morphological and Cytochemical study on the skin of Korean eel goby, *Odontamblyopus lacepedii* (Pisces, Gobiidae). Korean J Biol Sci 7(1): 43-47.

ROSEN MW & CORNFORD NE. 1971. Fluid friction of fish slimes. Nature Lond 234: 49-51.

ROUSSEL P & DELMOTTE P. 2004. The diversity of epithelial secreted mucins. Curr Org Chem 8: 413-437.

SANTOS A. 2016. Análise estrutural, citoquímica e morfométrica da epiderme de *Pseudauchenipterus affinis* (Steindachner, 1877) (Siluriformes: Auchenipteridae). Master's thesis, Universidade Federal do Espírito Santo.

SINGH SK & MITTAL AK. 1990. A comparative study of the epidermis of the common carp and the three Indian major carp. J Fish Biol 36(1): 9-19.

SHOORAKI HF, SAADATFAR Z & SHAHSAVANI D. 2018. Skin Development in H. huso Larvae. J Morphol Sci 35(1): 37-43.

SOUZA MLR, DOURADO DM, MACHADO SD, BUCCINI DF, JARDIM MI A, MATIAS R, CORREIA C & FERREIRA IC. 2003. Análise da pele de três espécies de peixes: histologia, morfometria e testes de resistência. Rev Bras Zootec 32: 1551-1559.

TOLEDO RC & JARED C. 1993. Cutaneous adaptations to water balance in amphibians. Comp Biochem Physiol Part A Physiol 105(4): 593-608.

WHITEAR M. 1986. The skin of fishes including cyclostomes. In: Bereiter-Hahn J, Matoltsy AG & Richards KS (Eds), Biology of the integument, Vol. 2 Vertebrates. 8-38 p.

How to cite
BLANCO COHENE T, OLEA G & FLORES QUINTANA C. 2023. Structural and histochemical aspects of the *Gymnotus carapo* tegument (Teleostei: Gymnotiformes). An Acad Bras Cienc 95: e20191259. DOI: 10.1590/0001-3765202320191259.

Manuscript received on October 21, 2019;
accepted for publication on August 2, 2020

TANIA BLANCO COHENE[1,3]
https://orcid.org/0000-0003-2293-0172

GABRIELA OLEA[1,2,3]
https://orcid.org/0000-0002-2816-0263

CAROLINA FLORES QUINTANA[1,2]
https://orcid.org/0000-0001-9646-3293

[1]Universidad Nacional del Nordeste, Facultad de Ciencias Veterinarias, Cátedra de Histología y Embriología, Sargento Cabral, 2139, 3400 Corrientes Capital, Argentina

[2]Universidad Nacional del Chaco Austral, Departamento de Ciencias Básicas, Cátedra de Histología y Embriología, Comandante Fernández, 755, 3700 Pcia.R. Sáez Peña, Chaco, Argentina

[3]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Godoy Cruz, 2290, C1425FQB Buenos Aires, Argentina

Correspondence to: **Carolina Flores Quintana**
E-mail: carofloresq@gmail.com

Author contributions

Blanco Cohene, Tania and Flores Quintana, Carolina designed the experiments, analyzed the data and drafted the manuscript; Blanco Cohene, Tania and Olea Gabriela, performed the experiments.