Original article

The esophagus of the crocodilian *Caiman latirostris* (Reptilia, Crocodylia): histological, histochemical and immunohistochemical study

Machado-Santos, C.[1]*, Zeca, SG.[2], Abidu-Figueiredo, M.[3], Ribeiro, ICA.[4] and Sales, A.[5]

[1]Biomorphology Department, ICS, Federal University of Bahia – UFBA,
Av. Reitor Miguel Calmon, s/n, Vale do Canela, CEP 40110-250, Salvador, BA, Brazil
[2]Institute of Veterinary, Rio de Janeiro Federal Rural University – UFRRJ, Seropédica, RJ, Brazil
[3]Animal Biology Department, Rio de Janeiro Federal Rural University – UFRRJ, Seropédica, RJ, Brazil
[4]Institute of Veterinary, Federal Fluminense University – UFF, Niteroi, RJ, Brazil
[5]Animal Biology Department, Rio de Janeiro Federal Rural University – UFRRJ, Seropédica, RJ, Brazil
*E-mail: claricemachado@yahoo.com.br

Abstract

The purpose of this study was to analyze the esophageal morphology of *Caiman latirostris* using histochemical and immunohistochemical techniques. The mucosae layer is composed of a pseudostratified columnar epithelium with goblet cells that occasionally form intra-epithelial glands. The goblet cells showed different affinity for the histochemical techniques. The lamina propria is composed of loose connective tissue with blood vessels. The smooth muscle fibers that compose the muscularis mucosa are thick and form a clear demarcation between the mucosa layer and adjacent submucosa. There are no glands in the esophageal submucosa. The muscle layer is composed a circular internal layer and a longitudinal external one. The adventitia layer is composed of loose connective tissue and blood vessels. The serotonin immunoreactive (IR) cells were not preferentially distributed in any region of the epithelium, ranging from the base of the intra-epithelial gland to the connection to the lumen. Somatostatina, glucagon and CCK IR cells were not detected.

Keyworks: reptiles, esophageal morphology, mucins, endocrine cells.

1 Introduction

The histological study of the structures of different classes of vertebrates is fundamental to understand their physiology and habits. Among these classes are the reptiles (Reptilia), whose strategic position on the phylogenetic scale permits a better understanding of the evolution of living organisms in their transition from the aquatic to terrestrial environments (VITT and PIANKA, 2005). The crocodile family is positioned at a very important location in the phylogenetic tree (BUCHAN, LANCE and POLAK, 1983). Broad-snouted caimans (*Caiman latirostris* Daudin 1802-Crocodylia: Alligatoridae) are widely distributed in South American aquatic ecosystems (YANOSKY, 1990; VERDADE, 1995). They are at the top of the food web and are extremely important to ecological balance (STOKER, BELDOMENICO, BOSQUIAZZO et al., 2008).

In reptiles, the histological and histochemical aspects of the esophagus have been described in turtles (MADRID, BALLESTA, PASTOR et al., 1989; PEREIRA, FONSECA, MENIN et al., 2005; PEREZ-TOMAS, BALLESTA, MADRID et al., 2005), lizards (IMAI, SHIBATA and IZUMI, 1992) and snakes (FERRI and MEDEIROS, 1975; IMAI, SHIBATA and MORIGUCHI, 1991; IMAI, SHIBATA, MORIGUCHI et al., 1991). In the Crocodylia order, Jin, Rodrigues, Silva et al. (1985) described the anatomy and histology of the esophagus of *Caiman crocodilus yacare*. Uriona, Farmer, Dazel et al. (2005) in a study of the structure and function of the esophagus of the *Alligator mississippiensis,* showed that the alligator is a useful model for studying regulation of the lower esophageal sphincter (LES) and Secor (2003) reported that reptilians are a model to advance understanding of the regulation of the esophagus and stomach.

Gastrointestinal endocrine cells dispersed in the epithelia and glands of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the gastrointestinal tract (BELL, 1979). Intensive work has centered on the gastrointestinal endocrinology of crocodilians because their phylogenetic position is of particular interest to comparative endocrinologists (BUCHAN, LANCE and POLAK, 1983; LANCE, HAMILTON, ROUSE et al., 1984; YAMADA, CAMPOS, KITAMURA et al., 1986; YAMADA, RODRIGUES, KITAMURA et al. 1991). However, there are only a few studies of the esophageal mucosa of reptiles using immunohistochemical techniques. Among these are studies of *Takydromus wolteri* (LEE and KU, 2004), *Trachemys scripta elegans* (KU, LEE, LEE et al., 2001) and *Egernia kingii* (ARENA, RICHARDSON and YAMADA, 1990).

This paper describes the histological structure of the esophagus in the crocodile *Caiman latirostris*, based on histochemical and immunohistochemical techniques, in order to increase current knowledge in basic morphology of the tissues in the reptilian and thus to contribute to the research of wild animals.

2 Material and methods

2.1 Animals and tissue preparations

Ten adult specimens of *Caiman latirostris,* raised for meat, were obtained from Bonsucesso Farm (Nossa Senhora do Amparo, Barra Mansa, RJ, Brazil). They were slaughtered in the Acquanature abattoir (Itaguaí, RJ, Brazil). The breeder is registered with the Brazilian federal environmental agency (IBAMA) and has authorization from the local and government, Rio de Janeiro state environmental agency (FEEMA) and Regional Board of Veterinary Medicine. The animals were submitted to hypothermia and slaughtered by hypovolemia. The tissues were sampled from the proximal (pharyngeal or cranial), middle and distal (gastric or caudal) esophagus and fixed by immersion in Bouin's solution for 6 hours. Then the samples were processed and embedded in paraffin using routine protocols. Five-micrometer thick sections were cut by microtome for histology and immunohistochemistry examination. The sections were stained with hematoxylin and eosin and by Mallory's trichrome method to show general histological observations. Also, the characteristics of the mucins secreted by the epithelial goblet cells were studied using a series of histochemical tests, as described below:

1) The periodic acid Schiff (PAS) method, with or without prior digestion with a-amylase (SPICER, HORN and LEPPI, 1967), was performed on the neutral mucins;
2) Alcian blue (AB) at pH 0.4 and pH 2.5 (STEEDMAN, 1950; LEV and SPICER, 1964) was used to stain the acid mucins;
3) Alcian blue at pH 2.5 and periodic acid Schiff (AB–PAS) (BEN and LI, 2001) staining were used to differentiate acidic and neutral mucins; and
4) Aldehyde fuchsin (AF) with AB at pH 2.5 (SPICER and MEYER, 1960) was also used to determine the mucins with sulphate and carboxyl groups.

2.2 Immunohistochemistry

The sections were dewaxed and rehydrated by routine protocols. They were incubated with methanol containing 3% H2O2 for 15 minutes to block any endogenous peroxidase activity. The sections were washed in three drops of PBS, incubated in a humid chamber at 37 °C for 30 minutes with 1% goat serum and then incubated overnight at 4 °C with rabbit polyclonal anti-serotonin (S 5545 - Sigma-Aldrich, Inc.) diluted to 1:6000, polyclonal rabbit anti-somatostatin (SOM) (A 0566 - Dakocytomation) diluted to 1:300, rabbit polyclonal anti-cholecystokinin (CCK) (C 2581 - Sigma-Aldrich, Inc.) diluted to 1:6000, mouse monoclonal anti-glucagon (GLUC) (G 2654 - Sigma-Aldrich, Inc.) diluted at 1:2000, and monoclonal anti-smooth muscle α-actin (Cat. no. 08-0106, Zymed, California, USA) diluted to 1:600. The sections were then incubated with biotinylated "Universal" secondary antibody diluted to 1:200 (PK 7200, Vector Laboratories, Inc., U.K.) for 30 minutes, then with ABC, diluted at 1:200, for 30 minutes (both from PK 6200, Vector Lab. Inc.). The sections were again washed in three drops of PBS and revealed by treatment with 3,3'-diaminobenzidine tetrahydrochloride (003222 - DakoCytomation, California, USA) prepared according to the kit instructions, washed in distilled water, dehydrated in an increasing concentration series of ethanol solutions and mounted using Entellan (Merck). The negative controls were processed by replacing the anti-serotonin (5-HT), anti-somatostatina, anti-cholecystokinin, anti-glucagon and anti-smooth muscle α actin antibody with PBS.

3 Results

3.1 Histological and mucin histochemical findings

The esophageal portion of the digestive tract in *C. latirostris* is composed of the four layers: the mucosa, submucosa, muscularis and the adventitia. The esophageal surface is crossed by many longitudinal folds (Figure 1), which was found in all esophagus regions.

The mucosal lining is composed of a pseudostratified columnar epithelium with goblet cells (Figure 2). Along there epithelium there was intra-epithelial glands mainly containing mucous cells (Figure 2). In the cranial region there were cilia in the epithelium, becoming sparse in the medial region and absent in the posterior region.

The goblet cells, which are uniformly numerous throughout the esophagus, showed different affinity for the histochemical techniques. They were strongly stained by the PAS and after digestion in a-amylase showed slightly less intensity than the PAS reaction (Figure 3). AB at pH 0.4 and 2.5 stained the goblet cells lightly and after double staining with AB-PAS, a small proportion of cells exhibited blue staining, while most cells showed red or intense purple staining (Figure 4). The goblet cells stained with AF-AB at pH 2.5 appeared purple and blue (Figure 5).

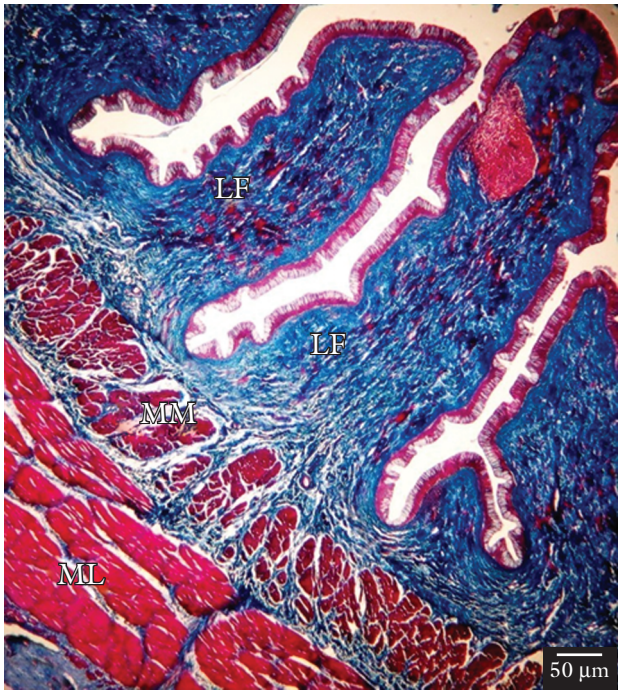


Figure 1. Photomicrographs of a cross section of the esophagus of *Caiman latirotris.* Note the surface with many longitudinal folds (LF), thick muscularis mucosae (MM) and muscle layer (ML). Mallory Trichrome. Bar = 50 µm.

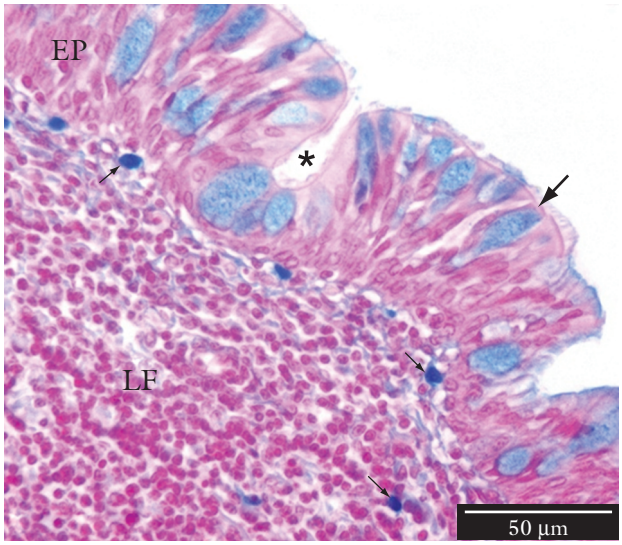


Figure 2. The upper portion of esophagus lined by pseudostratified ciliated columnar epithelium (EP) with goblet cells (thick arrow). Note the intra-epithelial gland (*), lymph follicle (LF) and strongly Alcian blue positive cells. Alcian blue at pH 2.5. Bar = 50 µm.

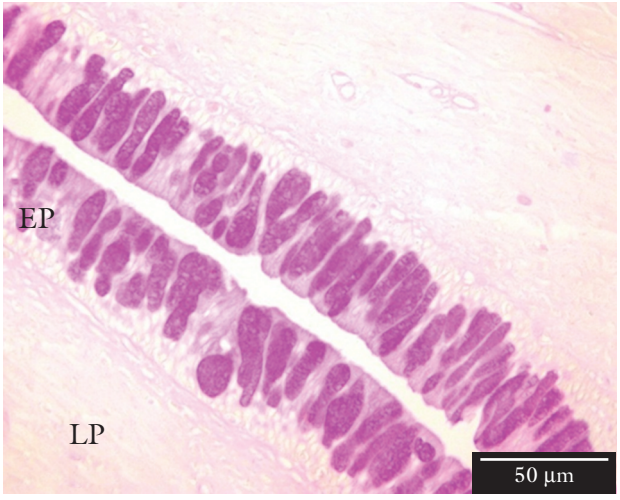


Figure 3. Note PAS positive cells in the epithelium (EP). Lamina propria (LP). Periodic acid Schiff (PAS) with digestion with a-amylase. Bar = 50 µm.

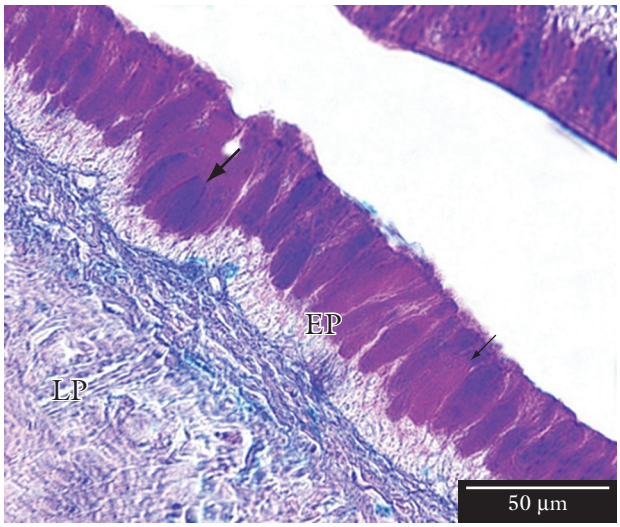


Figure 4. The middle portion of the esophagus. Note the intra-epithelial gland showing blue-stained cells (thick arrow) and cells with strong purple staining (thin arrow) in the epithelium (EP) Lamina propria (LP). PAS - AB at pH 2.5. Bar = 50 µm.

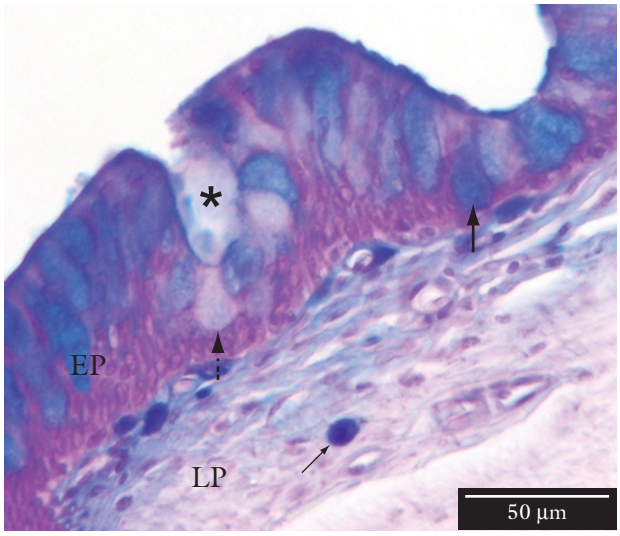


Figure 5. The epithelium (EP) with goblet cells showing positive reaction (solid arrow) and absence of reaction (empty arrow) in the intra-epithelial gland (*). Note the positive reaction in cells of the lamina propria (LP). AF-AB at pH 2.5. Bar = 50 µm.

The lamina propria is composed of loose connective tissue with blood vessels. In this layer, near the epithelium, we observed cells strongly positive to AB pH 2.5 and AF-AB pH 2.5 (Figure 5). There are large numbers of lymph follicles, close to the lumen, all over esophageal mucosa. The transition between the lamina propria of the mucosa and the submucosa could be clearly identified by a thick muscularis mucosae composed of smooth muscle fibers. We did not observe esophageal glands.

The submucosa is a narrow layer compressed between the muscularis mucosae and the muscular layer, consisting of loose connective tissue, blood vessels and nerves. There are no glands in the esophageal submucosa.

The muscle layer of the esophagus is composed of two layers: inner circular and outer longitudinal (Figure 6). In the cranial portion of esophagus the muscle layer consists of only striated muscles. The middle portion is composed of striated muscles in the inner circular layer and smooth fibers in the longitudinal muscle layer. The caudal portion of esophagus consists only of striated fibers.

The adventitia layer is the most external, composed of loose connective tissue and blood vessels (Figure 6).

3.2 Immunohistochemistry

We did not see any positive labeling in any of the negative control sections.

3.2.1 Anti-smooth muscle α–actin

The immunohistochemistry of the mucosa layer revealed a positive reaction in the smooth muscle cells of the blood vessel walls in the lamina propria and in the muscularis mucosae (Figure 7a). In the middle portion there was a positive reaction only in the outer longitudinal muscle layer. There was also a positive reaction in the smooth muscle cells of the blood vessel walls in the adventitia (Figure 7b).

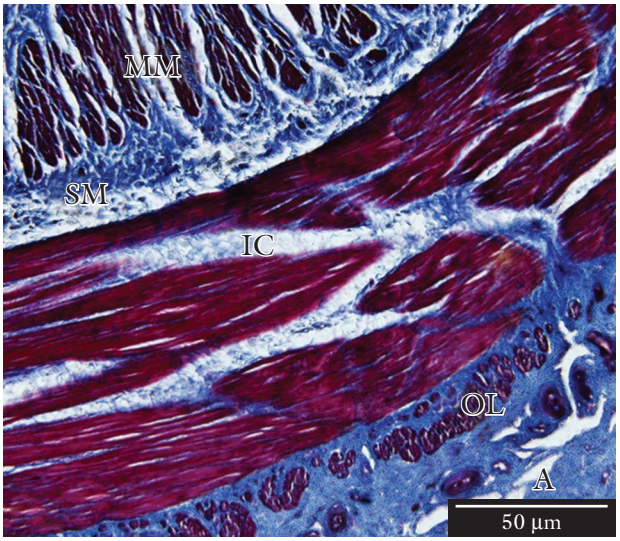


Figure 6. Muscularis mucosae (MM), submucosa (SM), muscle layer - inner circular (IC) and outer longitudinal (OL) and adventitia (A). Mallory Trichrome. Bar = 50 µm.

3.2.2 Endocrine cells

The serotonin (5-HT) immunoreactive (IR) cells are not preferentially distributed in any region of the epithelium, being found from the base of the intra-epitelial glands (Figure 8a) to the connection to the lumen. They are relatively abundant and either of the open type, making luminal contact via an apical cytoplasmic process (Figure 8b), or the closed type (Figure 8c). Somatostatina, glucagon and CCK IR cells were not detected.

4 Discussion

As observed in some fish (REIFEL and TRAVILL, 2005), amphibians (GALLEGO-HUIDOBRO, PASTOR and CALVO, 2005) and reptiles, such as the crocodile *C. crocodilus yacare* (JIN, RODRIGUES, SILVA et al., 1985), the esophagus of *C. latirostris* has longitudinal folds in the esophageal mucosa. In the Japanese lizard the esophagus lumen of the cranial portion has no fold. However, the middle and caudal portions are formed of very complicated folds (IMAI, SHIBATA and IZUMI et al., 1992). According to Jin, Rodrigues, Silva et al. (1985), the folds are totally distensible, to enable expansion of the esophagus, allowing it to store food that is ingested.

The esophageal epithelium in *C. latirostris* is the same as observed in other reptiles, such as *C. crocodilus yacare* (JIN, RODRIGUES, SILVA et al., 1985), *Alligator mississippiensis* (URIONA, FARMER, DAZEL et al., 2005), rock snake (IMAI, SHIBATA and MORIGUCHI, 1991), *Xenodon merremii* snake (FERRI and MEDEIROS, 1975), skink *Egernia kingii* (ARENA, RICHARDSON and YAMADA, 1990) and lizard *Tupinambis teguixin* (ZAMITH, 1952). However, in the turtle *Testudo graeca*

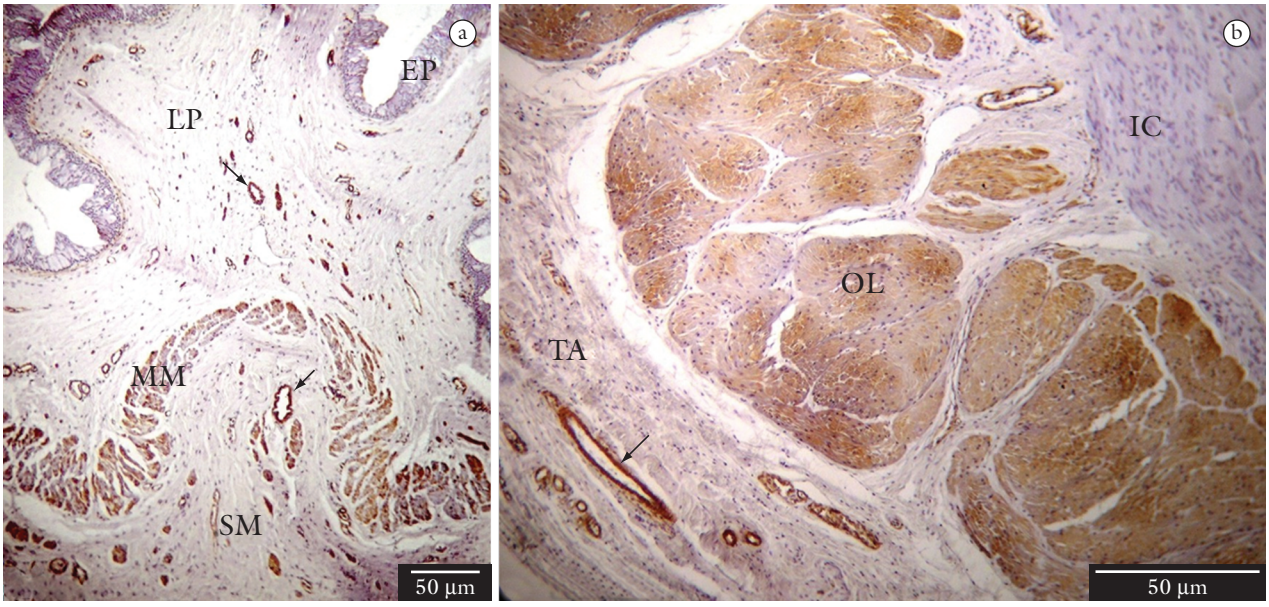
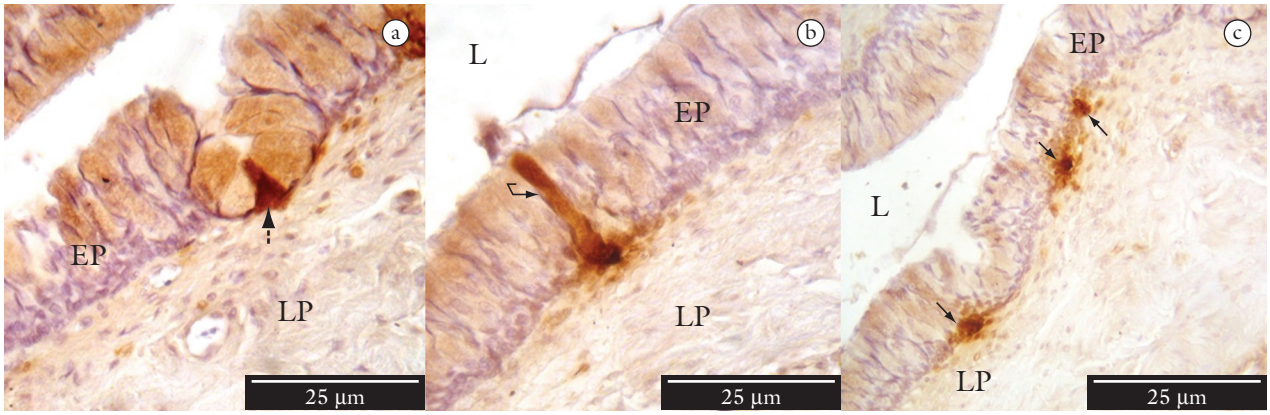


Figure 7. Photomicrographs of a cross-section of the esophagus of *Caiman latirotris* with anti-smooth muscle α-actin. a) Note the positive reaction in the smooth muscle cells of the blood vessel walls (arrows) in the lamina propria (LP), submucosa (SM) and muscularis mucosae (MM). b) The middle portion shows a positive reaction only in the outer longitudinal muscle layer (OL). Positive reaction in blood vessel walls (arrow) in the adventitia (TA). (IC) inner circular muscle layer. Bar = 50 µm.



Figures 8. Serotonin-immunoreactive cells. a) Note the positive reaction in the intra-epithelial gland (arrow). b) Note the presence of an apical cytoplasmic process (arrow) directed toward the lumen (L). c) The absence of apical process - the closed type (arrows). Epithelium (EP), lamina propria (LP). Bar = 25 µm.

the stratified squamous epithelium is found in the proximal one-third of the esophagus and in the one-half to distal one-third it is covered by a single layer of columnar mucous secreting cells (MADRID, BALLESTA, PASTOR et al., 1989). In the Japanese lizard and gecko, the mucosal lining of the esophagus consists of simple columnar cells (IMAI, SHIBATA, MORIGUCHI et al., 1991).

According to Luppa (1977) and Przystalski (1980), the esophageal reptilian epithelium mainly has two kinds of cells: ciliated cells and goblet cells. The goblet cells in *C. latirotris* were strongly PAS-positive and stained lightly AB at pH 0.4 and 2.5; suggesting that, in addition to this neutral mucins, the major components of the esophageal mucus, these cells also synthesize sulfated and carboxylated mucins. Double staining with AB-PAS detected a small proportion of cells exhibited blue staining, thus indicating the occurrence of acid mucins. Conversely, the majority of cells showed red or an intense purple staining, thus indicating the occurrence of neutral and a mixture of neutral and acidic mucins The mucous cells are stained purple with AF-AB pH 2.5, indicating the presence of sulphated mucins, while those that stained blue are carboxylated mucins (CAO and WANG, 2009). These mucins have different functions, and the sulphated related to increased mucus viscosity and protection against invasion of bacteria (ROBERTON and WRIGHT, 1997; TIBBETS, 1997) and carboxylated for lubrication (CAO and WANG, 2009).

In the Japanese lizard and gecko (IMAI, SHIBATA, MORIGUCHI et al., 1991b), the goblet cells reacted strongly to PAS and moderately to AB (pH 2.5 and 0.5). According to Madrid, Ballesta, Pastor et al. (1989), in *Testudo graeca,* the mucous cells of the surface epithelium contain sulphosialo-mucosubstances with a variable proportion of sulfate groups, while in *Lacerta lepida* the esophageal mucous cells contain either-sialo-mucosubstances or sulphosialo-mucosubstances (AB/PAS positive) and in *Mauremys caspica* they only contain acidic mucins (PAS positive). The sulphated mucins secreted by goblet cells in the reptilian esophagus play a cytoprotective role against refluxed gastric contents, and acid mucins may also have protective properties against the glycosidases present in the saliva (MADRID, BALLESTA, PASTOR et al., 1989).

In the lamina propria of *C. latirotris,* we observed large numbers of lymph follicles. These infiltrations can also be observed in the esophagus of the red-eared turtle (BIANCHI, GIANNESSI, DOLFI et al., 1993). In the esophagus of this turtle the areas of lymphoid infiltration are quite large and are generally located at the bottom of the mucosal plicae. In marine turtles, there are lymph follicles in the lamina propria, as was found in amphibians; and there is lymphocyte migration in the epithelium in some species of snakes, the same as observed in mammal's tonsils (PEREIRA, FONSECA, MENIN et al., 2005).

We did not observe any esophageal glands in *C. latirotris.* The same has been reported for other crocodiles, such as *A. mississippiensis* (URIONA, FARMER, DAZEL et al., 2005) and *C. crocodilus yacare* (JIN, RODRIGUES, SILVA et al. 1985). Imai, Shibata, Moriguchi et al. (1991) reported observing bottle-shaped glands distributed in the lamina propria mucosae of the caudal portion of the esophagus of the Japanese lizard and gecko and suggested that these glands have a phylogenic relation with those of birds. In Chelonia, the presence of esophageal glands depends on each species. In both *Chelonia imbricata* and *Testudo graeca* the mucosal layer has glands, while in *L. lepida* and *M. caspica* it does not (MADRID, BALLESTA, PASTOR et al., 1989). Imai, Shibata and Moriguchi (1991) reported the absence of esophageal glands in the rock snake but found pepsinogen granules, as observed in the in the epithelium of the esophageal glands of the Japanese lizard and gecko. These granules are secreted directly into the esophageal lumen.

The distributions of striated and smooth muscles in the muscular layer of the esophagus are known to vary widely among animal species (IZUMI, MATSUYAMA, YAMAMOTO et al., 2002). In the crocodilians *A. mississippiensis* (URIONA, FARMER, DAZEL et al., 2005) and *C. crocodilus yacare* (JIN, RODRIGUES, SILVA et al., 1985), the esophagus is composed entirely of smooth muscles (URIONA, FARMER, DAZEL et al., 2005). This same composition has been reported to exist in amphibians, birds and other reptiles (INGELFINGER, 1958). In *C. latirotris,* the composition of muscular layer is similar to that described in humans and pigs (SHIINA,

SHIMIZU, IZUMI et al., 2005; BANKS, 1995), where the first (cranial) portion of esophagus is composed of striated muscle fibers, and the middle portion is composed of striated muscles in the inner circular layer and smooth fibers in the longitudinal muscle layer. However, the caudal portion of the *C. latirotris* esophagus is composed only of striated muscle fibers, unlike in mammals, where it is made up exclusively of smooth muscles.

Serotonin (5-HT) consists of monoamines and is widely distributed in the nervous system and gastroenteropancreatic endocrine cells. El-Salhy, Wilander and Lundqvist (1985) reported that 5- HT IR cells were found throughout the gastrointestinal tract of all species and established in the alimentary tract at the early stage of vertebrate evolution, and that this amine has an important function in reptiles because the relative abundance of these cells can exceed that observed in mammals. In *C. latirotris,* we only found 5-HT IR cells. These IR cells have been identified in the esophagus of *E. kingii* (ARENA, RICHARDSON and YAMADA, 1990), *Trachemys scripta elegans* (KU, LEE, LEE et al., 2001) and *Testudo graeca* (PEREZ-TOMAS, BALLESTA, PASTOR et al., 1989). However, other endocrine cells IR to chromogranin, somatostatin and vasoactive inhibitory peptide have been identified in reptilian esophagus (KU, LEE, LEE et al., 2001; ARENA, RICHARDSON and YAMADA, 1990; PEREZ-TOMAS, BALLESTA, PASTOR et al., 1989).

The histological structure observed in *C. latirostris* is similar to that found in other crocodilians, such as the presence of longitudinal folds, the pseudostratified columnar epithelium with goblet cells and the absence of esophageal glands. However, there are some variations, like the presence of smooth muscle cells only in the middle portion of the esophagus.

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References

ARENA, PC., RICHARDSON, KC. and YAMADA, J. An immunohistochemical study of endocrine cells of the alimentary tract of the King's skink (*Egernia kingii*). *Journal of Anatomy*, vol. 170, p. 73-85, 1990. PMid:2254171. PMCid:1257064.

BANKS, WJ. *Applied veterinary histology*. 3th ed. Mosby International, 1995.

BELL, FR. The relevance of the new knowledge of gastrointestinal hormones to veterinary science. *Veterinary Research Communications*, vol. 2, p. 305-14, 1979.

BEN, CE. and LI, SG. Histochemistry carbohydrate. In SHI, GS. ed. *Beijing*: the peoples hygiene press. 2001. p. 160-161.

BIANCHI, F., GIANNESSI, F., DOLFI, A. and LUPETTI, M. Properties of the esophageal epithelium in relation to organizative patterns of lymphoid infiltrations in the red-eared turtle. *Tissue and Cell.*, vol. 25, no. 3, p. 389-397, 1993. http://dx.doi.org/10.1016/0040-8166(93)90080-5

BUCHAN, AMJ., LANCE, V. and POLAK, JM. Regulatory peptides in the gastrointestinal tract of *Alligator mississippiensis*: an immunocytochemical study. *Cell and Tissue Research*, vol. 231, p. 439-449, 1983. PMid:6133628.

CAO, XJ. and WANG, WM. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anatomia, Histologia, Embryologia*, vol. 38, p. 254-261, 2009. PMid:19426437. http://dx.doi.org/10.1111/j.1439-0264.2009.00932.x

EL-SALHY, M., WILANDER, E. and LUNDQVIST, M. Comparative studies of serotonin-like immunoreactive cells in the digestive tract of vertebrates. *Biomedical Research*, vol. 6, p. 371-375, 1985.

FERRI, S. and MEDEIROS, OL. Morphological study of the esophagus of *Xenodon merremii* wagler, 1924 (Ophidia). *Journal of Herpetology*, vol. 9, no. 3, p. 299-302, 1975.

GALLEGO-HUIDOBRO, J., PASTOR, LM. and CALVO, A. Histology of the esophagus of the adult frog *Rana perezi* (Anura: Ranidae). *Journal of Morphology*, vol. 212, no. 3, p. 191-200, 2005.

IMAI, M., SHIBATA, T. and MORIGUCHI, K. Pepsinogen granules in the esophageal epithelium of the rock snake. *Okajimas Folia Anatomica Japonica*, vol. 68, no. 4, p. 231-234, 1991. PMid:1780120.

IMAI, M., SHIBATA, T., MORIGUCHI, K. and HAYAMA, H. Glands distributed in the lamina propria mucosae of the esophagus in the gecko and Japanese lizard. *Okajimas Folia Anatomica Japonica*, vol. 68, no. 5, p. 289-293, 1991. PMid:1806847.

IMAI, M., SHIBATA, T. and IZUMI, T. Histological and histochemical investigations on Japanese lizard esophagus. *Okajimas Folia Anatomica Japonica*, vol. 69, no. 1, p. 25-34, 1992. PMid:1620528.

INGELFINGER, FJ. Esophageal motility. *Physiological Reviews*, vol. 38, p. 533-584, 1958. PMid:13590930

IZUMI, N., MATSUYAMA, H., YAMAMOTO, Y., ATOJI, Y., SUZUKI, Y., UNNO, T. and TAKEWAKI, T. Morphological and morphometrical characteristics of the esophageal intrinsic nervous system in the golden hamster. *European Journal of Morphology*, vol. 40, no. 3, p. 137-144, 2002. PMid:14566606. http://dx.doi.org/10.1076/ejom.40.3.137.16684

JIN, SM., RODRIGUES, CAT., SILVA, MP. and SANTOS, FR. Observações morfológicas do esôfago do *Caiman crocodilus yacare* crocodilia-repitilia (Daudin,1802). *Revista Brasileira de Ciências Morfológicas*, vol. 2, no 1, p. 49-58, 1985.

KU, SK., LEE, HS., LEE, JH. and PARK, KD. An immunohistochemical study on the endocrine cells in the alimentary tract of the red-eared slider (*Trachemys scripta elegans*). *Anatomia, Histologia, Embryologia*, vol. 30, p. 33-39, 2001. PMid:11284161. http://dx.doi.org/10.1046/j.1439-0264.2001.00284.x

LANCE, V., HAMILTON, JW., ROUSE, JB., KIMMEL, JR. and POLLOCK, HG. Isolation and characterization of reptilian insulin, glucagon, and pancreatic polypeptide: complete amino acid sequence of alligator (*Alligator mississippiensis*) insulin and pancreatic polypeptide. *General and Comparative Endocrinology*, vol. 55, p. 112-124, 1984. http://dx.doi.org/10.1016/0016-6480(84)90135-7

LEE, HS. and KU, SK. An immunohistochemical study of endocrine cells in the alimentary tract of the grass lizard, *Takydromus wolteri Fischer* (Laceridae). *Acta Histochemica*, vol. 106, no. 2, p. 171-178, 2004. PMid:15147638. http://dx.doi.org/10.1016/j.acthis.2003.10.008

LEV, R. and SPICER, SS. Specific staining of sulphated groups with alcian blue at low pH. *Journal of Histochemistry & Cytochemistry*, vol. 12, p. 309, 1964. http://dx.doi.org/10.1177/12.4.309

LUPPA, H. Histology of the digestive tract. In GANS C. and GANS, KA., ed. *Biology of the reptilia*. 6th ed. New York: Academic Press, 1977. p. 159-223.

MADRID, J.F., BALLESTA, J., PASTOR, L.M., PEREZ-TOMAS, R. and HERNANDEZ, F. 1989. Distribution of mucins in the mucosa of the digestive tract of reptiles: a histochemical study. *Acta Histochemica*, vol. 85, no. 2, p. 117-129.

PEREIRA, JG., FONSECA, CC., MENIN, E. and NEVES, MTD. Estudo histológico e histoquímico do esôfago do muçuã *Kinosternon scorpioides* Linnaeus, 1766 (Reptilia, Chelonia, Kinosternidae). *Arquivos de Ciências Veterinárias e Zoologia*, vol. 8, no. 1, p. 3-10. 2005.

PEREZ-TOMAS, R., BALLESTA, J., MADRID, JF., PASTOR, LM. and HERNANDEZ, F. Histochemical and ultrastructural study of the digestive tract of the tortoise *Testudo graeca* (Testudines). *Journal of Morphology*, vol. 204, no. 3, p. 235-245, 2005. http://dx.doi.org/10.1002/jmor.1052040302

PEREZ-TOMAS, R., BALLESTA, J., PASTOR, LM., MADRID, JF. and PALAR, JM. Comparative immunohistochemical study of the gastroenteropancreatic endocrine system of three reptiles. *General and Comparative Endocrinology*, vol. 76, no. 2, p. 171-191, 1989. PMid:2574125.

PRZYSTALSKI, A. The dimensions of the mucosa and the structure of the alimentary canal in some reptiles. *Acta Biologica Cracoviensia*, vol. 23, p. 1-33, 1980.

REIFEL, CW. and TRAVILL, AA. Structure and carbohydrate histochemistry of the esophagus in ten teleostean species. *Journal of Morphology*, vol. 152, no. 3, p. 303-313, 2005.

ROBERTON, A.M. and WRIGHT, D.P. Bacterial glycosulphatases and sulphomucin degradation. *Canadian Journal of Gastroenterology*, vol. 11, p. 361-366, 1997. PMid:9218863.

SECOR, S.M. Gastric function and its contribution to the postprandial metabolic response of the Burmese *Python molurus*. *Journal of Experimental Biology*, vol. 206, p. 1621-1630, 2003. PMid:12682094. http://dx.doi.org/10.1242/jeb.00300

SHIINA, T., SHIMIZU, Y., IZUMI, N., SUZUKI, Y., ASANO, M., ATOJI, Y., NIKAMI, H. and TAKEWAKI, T. 2005. A comparative histological study on the distribution of striated and smooth muscles and glands in the esophagus of wild birds and mammals. *Journal of Veterinary Medical Science*, vol. 67, p. 115-117. PMid:15699607. http://dx.doi.org/10.1292/jvms.67.115

SPICER, S.S. and MEYER, DB., 1960. Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsin–alcian blue staining. *American Journal of Clinical Pathology*, vol. 33, p. 453-460.

SPICER, SS., HORN, RG. and LEPPI, TJ. Histochemistry of connective tissue mucopolysaccharides. In WAGNER BM. and SMITH, DE., ed. *The connective tissue*. Baltimore: Williams and Wilkins, 1967. p. 251-303.

STEEDMAN, HF. Alcian blue 8G: a new stain for mucin. *Quarterly Journal of Microscopical Science*, vol. 91, p. 477-479, 1950.

STOKER, C., BELDOMENICO, PM., BOSQUIAZZO, VL., ZAYAS, MA., REY, F., RODRIGUEZ, H., MUNOZ-DE-TORO, M. and LUQUE, EH. Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in *Caiman latirostris*. *General and Comparative Endocrinology*, vol. 156, p. 603-612, 2008. PMid:18384790. http://dx.doi.org/10.1016/j.ygcen.2008.02.011

TIBBETS, IR. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepsis krepptii*. *Journal of Fish Biology*, vol. 50, p. 809-820, 1997.

URIONA, T.J., FARMER, C.G., DAZEL, Y.J., CLAYTON, F. and MOORE, J. Structure and function of the esophagus of the American alligator (*Alligator mississippiensis*). *Journal of Experimental Biology*., vol. 208, no. 16, p. 3047-3053, 2005. PMid:16081603. http://dx.doi.org/10.1242/jeb.01746

VERDADE, LM. Biologia reprodutiva do Jacaré-de-papo-amarelo (*Caiman latirostris*) em São Paulo, Brasil. In LARRIERA A. and VERDADE, LM., ed. *La conservación y el manejo de caimanes y cocodrillos de america latina*. Santo Tomé, Santa Fe Argentina: Fundación Banco Bica, 1995. p. 57-79.

VITT, LJ. and PIANKA, ER. Deep history impacts present-day ecology and biodiversity. *PANS*, vol. 102, no. 22, p. 7877-7881, 2005.

YAMADA, J., CAMPOS, VJM., KITAMURA, N., PACHECO, AC., YAMASHITA, T. and YANAIHARA, N. An Immunohistochemical study of the endocrine cells in pancreas of *Caiman latirostris* (Alligatorinae), with special reference to pancreatic motilin cells. *Biomedical Research*, vol. 7, no. 199-208, 1986.

YAMADA, J., RODRIGUES, MAM., KITAMURA, N., PAI, VD., YAMASHITA, T., MOTIZUKI, T. and YANAIHARA, N. Motilin-immunoreactive cells in the duodenum, pyloric stomach and pancreas of caimans (*Caiman latirostris* and *Caiman crocodilus*, Alligatorinae): a further comparison using region specific motilin antisera. *Archives of Histology and Cytology*, vol. 54, p. 359-364, 1991. PMid:1954044. http://dx.doi.org/10.1679/aohc.54.359

YANOSKY, AA. Histoire naturelle du Caiman à museau large (*Caiman latirostris*), un Alligatorinè mal connu. *Revue Francaise d'Aquariologie Herpetologie*, vol. 17, p. 19-31, 1990.

ZAMITH, APL. Contribuição para o conhecimento da estrutura da mucosa do esôfago dos vertebrados. *Anais da Escola Superior de Agricultura Luiz de Queiroz*, vol. 179, p. 359-434, 1952.

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