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Morphophysiology of the green - turtle salt gland *Chelonia mydas* (Linnaeus, 1758)

Morfofisiologia da glândula de sal de tartaruga verde *Chelonia mydas* (Linnaeus, 1758)

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ABSTRACT
In the group of reptiles there are many marine species whose foods have high concentration of salts getting with the blood plasma very concentrated. Turtles, lizards and sea birds have salt glands that, through active transport, excrete excess ingested salt. The objective of this study was to describe the morphology of green - turtle salt glands (*Cheloniamydas*). The salt gland was removed and analyzed macroscopically and microscopically by light microscopy and scanning electron microscopy. Macroscopically, the salt glands are paired in the hind laterally region of the eyes, with a reddish-brown color and lobulated appearance. In the most apical region of the gland its shape is concave adhered to the eye and in the basal region is triangular. Microscopically it was observed that the lobes are separated by blood vessels and connective tissue. The glands have simple tubular glandular tissue that emerge into a central duct. Morphological findings suggest that this gland helps blood osmoregulation by aiding renal function, where excess salt is transported into the cells via osmosis, which in turn releases via exocytosis, into the canalicular lumen.

Keywords: osmoregulation, turtles, morphology, scanning electron microscopy, glandular tissue.

RESUMO
No grupo dos répteis há muitas espécies marinhas cujos alimentos têm grande concentração de sais ficando com o plasma sanguíneo muito concentrado. Tartarugas, lagartos e aves marinhas têm glândulas de sal que, por meio de transporte ativo, excretam o excesso de sal ingerido. O objetivo deste estudo foi descrever a morfologia das glândulas de sal da tartaruga - verde (*Cheloniamydas*). A glândula de sal foi retirada e analisada macroscopicamente e microscopicamente através da microscopia de luz e eletrônica de varredura.Macroscopicamente as glândulas de sal são pareadas na região póstero-lateral dos olhos, de coloração marrom-avermelhada e aspecto lobulado. Na região mais apical da glândula seu formato é côncavo aderido ao olho e na região basal é triangular. Microscopicamente observou-se que os lóbulos são separados por vasos sanguíneos e tecido conjuntivo. As glândulas possuem tecido glandular tubular simples que emergem para um ducto central. Os achados morfológicos sugerem que esta glândula ajuda na osmorregulação sanguínea

auxiliando a função renal, onde o excesso de sal é transportado para o interior das células, via osmose, que, por sua vez lança via exocitose, para o interior do lúmen canalicular.

Palavras-chave: osmorregulação, tartarugas, morfologia, microscopia eletrônica de varredura, tecido glandular.

1 INTRODUCTION

The green turtle, *Cheloniamydas*, is widely distributed across the seas from the tropics to the temperate zones, being the species of sea turtle that presents more coastal habits, with reports of occurrences in estuaries of rivers and lakes. It belongs to the *Cheloniidae* family, inhabiting the tropical and subtropical oceans - Atlantic, Pacific and Indian Oceans (Ernest & Barbour, 1989). The name green turtle comes from the greenish coloration of its fat and is not related to the external appearance, which reveals a circumstance of knowledge of the species through hunting for human consumption (Hirth, 1997; Pritchard &Trebbau, 1984). These turtles can exceed 360 kg in weight (Hickman, Roberts & Keen, 2013). Despite the declining populations, the species maintains many traces of its historical distribution which includes feeding areas, migration corridors and nesting beaches that are interspersed along the oceans (Hirth, 1997).

Generally the kidneys are the organs responsible for osmoregulation in vertebrates, that is, they have the function of promoting the control of the concentrations of salts in the organism, in order to maintain the adequate conditions to the metabolic activities, but in the marine vertebrates, they are not able to alone with the high concentration of dissolved salts in the organism due to the highly saline environment. Thus, accessory structures are used to assist them in such function, with the exception of marine mammals (Nicolson & Lutz, 1989). The function of osmoregulation in the elasmobranchs is given by the rectal gland, the sea serpents by the sublingual gland, the sea birds and lizards is given by the nasal gland and sea turtles by the salt gland (Schmidt-Nielsen &Fänge, 1958). Without the help of these structures the animal collapses into osmotic. Salt glands are exocrine structures with the osmoregulation function, thus maintaining homeostasis. The appearance of these glands was prior to the adaptation of the limbs to the swim, becoming efficient fins (Hirth, 1997; Pritchard &Trebbau, 1984; Wyneken, 2001).

The first to describe the salt glands were Schmidt-Nielsen &Fänge in 1958, who found high amounts of sodium chloride in ocular secretions excreted through post-orbital ducts (Linzell&Peaker, 1997). When the turtles are on the ground for spawning, this secretion is visible through the tear ducts, and human interpretation correlates with crying with the pain caused by spawning, which has always been a speculation rejected by the naturalists, who until the end of the the 1950s speculated that they served to keep the sand away from the eyes (Schmidt-Nielsen

&Fänge, 1958). Due to the scarcity of image on the subject of salt glands, this study seeks to provide current material through morphological description using light microscopy (ML) and scanning electron (SEM) techniques.

2 MATERIAL AND METHOD

Four green turtle specimens (*Cheloniamydas*), found dead, stranded on Guaraú beach, Peruíbe - SP, were obtained with authorization and licenses approved by ICMBio / SISBio: 50132-1, CEUA-IBIMM: 005/18 and the ethics committee of the university, registered under CEUA nº 4856210818.

For histological analysis, samples of the salt gland were collected, cut and fixed in 10% formaldehyde solution for 48 hours. After the samples were dehydrated in increasing series of ethanols (70 to 100%) and diaphanized in xylol, with subsequent inclusion in paraffin. 5 μm thick sections were made on the microtome (Leika, German) and stained with Hematoxylin-Eosin, and Masson's Trichrome and Periodic Acid Schiff (PAS). The images were obtained through the Nikon Eclipse-800 light microscope (ML).

Scanning electron microscopy (SEM), after fixation with 10% formaldehyde solution, were dehydrated in increasing series of alcohols in concentrations of 70%, 80%, 90% and 100%, dried in a critical point apparatus LEICA EM CPD 300, glued with carbon glue on metallic (stub) and metalized silver sputting bases on the EMITECH K550 metallizer, and photographed on LEO 435VP scanning electron microscope (SEM). All images were obtained at the Advanced Center in Diagnostic Imaging - CADI-FMVZ-USP.

3 RESULTS

Macroscopically, the salt gland is reddish-brown in color, and is caudally positioned on each side of the eye socket (Figure 1A). It presents a rough appearance, and its shape in the basal portion is triangular, while in the apical portion it is concave (Figure 1B). The basal portion of the gland is closely related to the groove of the lower eyelid rhyme (Figure 1C), and it is attached to the orbit of the eye (Figure 1D).

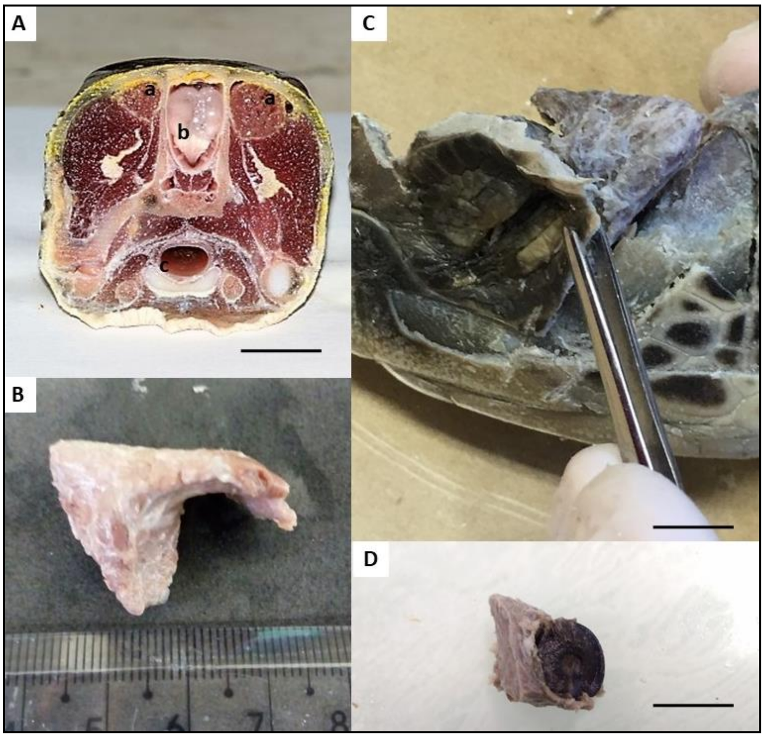

Figure 1: Head of turtle - green, *Cheloniamydas.* A- Cross section of the head, post-orbital region, (a) salt gland, (b) brain (c) trachea. B- salt gland withdrawn from the post-orbital region. C- salt gland related to the groove of the eyelid rhyme. D-gland attached to the orbit. Bar 1 cm.

It has been observed in ML and SEM that the salt glands are arranged in lobes, and these are surrounded by blood vessels (arterioles and venules), which are located in the interlobular space. The intralobular and perilobular connective tissue, together with the collagen fibers, surround the periphery of the lobe and interpose between the adjacent tubular structures that converge to the central duct region (Figure 2 ABC). The salt glands have simple cylindrical epithelial tissue, forming simple tubular structures that emerge into a central duct (Figure 2DEF). The simple cylindrical epithelium presents its nuclei in an eccentric and centrifugal position to the intraluminal canaliculus (Figure 2E). The salivary gland when analyzed by the histochemical PAS technique was strongly marked in the region of the tubules and central duct region, evidencing the presence of mucopolysaccharide (Figure 2EG).

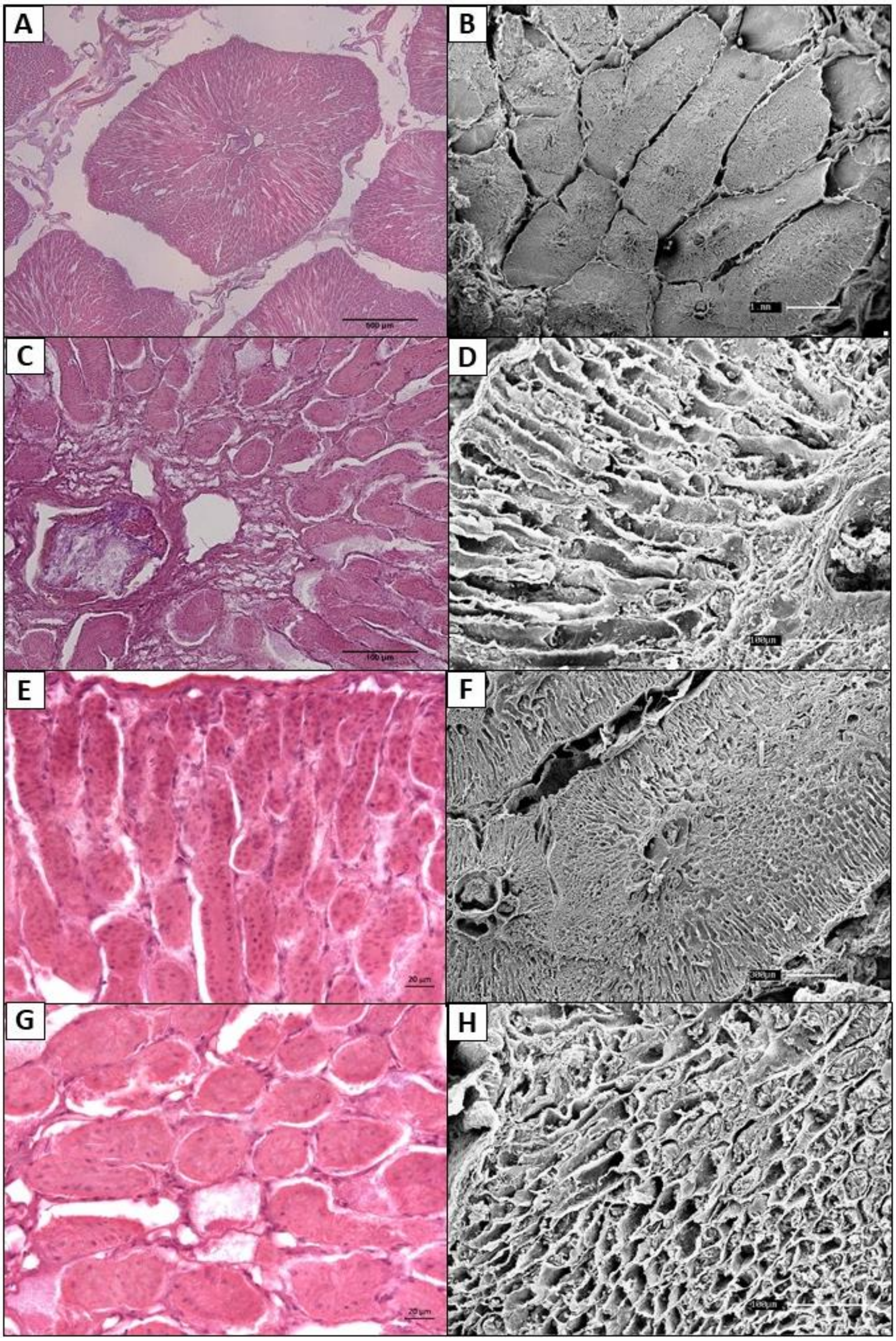

Figure 2: Photomicrograph of the salt gland of *Cheloniamydas*. Representing in ML with HE staining. C ML with Masson staining, and E and G with PAS staining. In images B, D, F and H - SEM of the salt gland lobes.

We note that the vascularization of the salt gland occurs through the emergence of arteries and veins, which are subdivided into arterioles and venules, in order to reach the capillary function for osmoregulatory exchange. Such structures are distributed at the periphery of the salt gland, and interspersed with the lobes reaching the tubules.

4 DISCUSSION

Sea turtles represent a primitive and unique component of biological diversity, being an important part of marine ecosystems (Santos *et al*., 2011). Its origin is terrestrial, but they have evolved and adapted to the marine environment, differing from other reptiles. Its locomotive limbs evolved into fins with small claws and its carapace became flatteneddorsoventrally, becoming lighter and hydrodynamic. Another important development concerns the appearance of the salt gland, located behind the eyeballs, which have the function of filtering excess sodium from the animal's organism (Wyneken, 2001).

Salt glands are exocrine glands made up of a set of lobules, which are joined by connective tissue, whose function is to control the equilibrium of the concentration of salts in the body of the organism, allowing them to drink salt water and to feed on plants or animals, such as marine invertebrates with a high ionic concentration (Parrilla-Bel*et al*., 2016).

Its anatomical location varies according to the animal species, be it reptile or bird. Salt glands in marine reptiles may be nasal (lizards), lacrimal (turtles), sublingual or premaxillary (serpents) or lingual (crocodiles) (Minnich, 1982; Linzell&Peaker, 1997; Fernández&Gasparini, 2000). In our study, its anatomical position is hind laterally aimed at the eyeball on both sides of the head, a reddish-brown color.

According to Hirayama (1998), the gland presents as a short and thick duct, whose outlet occurs in the lateral corner of the orbit, this fact was not observed in our study. As to its shape, the gland has a triangular aspect, confirming the work of Holmes and Mcbean (1964), while Wyneken (2001) points it as a round shape.

The salt gland is composed of fractured lobes by connective tissue and presence of blood vessels (Schmidt-Nielsen, &Fänge, 1958), corroborating our study and reiterating the existing morphophysiological knowledge of a peripheral arterial vascularization in blood vessels. Salt molecules through the arterial capillaries, between the glandular structures to near the central duct. During the course, the excess transport into the cell through the osmosis, which in turn launches, via exocytosis, into the canalicular lumen and launched towards the central duct. Blood, with less salt, is then used by venules via venous capillaries (Parrilla-Bel*et al*., 2016).

These findings show that the morphological structure of the salt gland of the *Cheloniamydas* turtle presents histological similarities with those of seabirds, differing structurally from this turtle because it presents more rounded lobes and with clover-like contours, and the epithelium presents a stratified aspect cuboidal, rather than the simple cylindrical aspect of *Cheloniamydas.*These findings suggest homology between these structures, suggesting a common evolutionary origin, as opposed to the sharks' rectal gland, which presents histological structure and differentiated location, inferring to be analogous structures, therefore, diverse embryological evolution.

5 CONCLUSION

Morphological findings confirm that the architecture of the salt gland has the function of blood osmoregulation, helping the renal function of the green turtle, which through active transport excrete excess salts, promoting the desalination of ingested water.

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RESEARCH HIGHLIGHTS

The architecture of the salt gland is adapted to blood osmoregulation function, aiding renal function by excreting salt while the kidneys excrete uric acid.

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