

Morphological analysis of the digestive tract of Antillean manatee (*Trichechus manatus manatus*, Linnaeus, 1758) in northeast Brazil

Rebeca Taínes do Nascimento Pinheiro^{1,2}, Radan Elvis Matias de Oliveira^{1,3,4,5}, Augusto Carlos da Bôaviagem Freire^{1,5}, Fernanda Loffler Niemeyer Attademo^{5,6,7}, Fábila de Oliveira Luna⁶, Vitor Luz Carvalho⁸, Flávio José de Lima Silva^{1,4,5,9,10}, and Simone Almeida Gavilan^{1,2,5}

¹Projeto Cetáceos da Costa Branca, Universidade do Estado do Rio Grande do Norte (PCCB/UERN), Mossoró, Brazil

²Universidade Federal do Rio Grande do Norte (UFRN), Centro de Biociências, Laboratório de Morfofisiologia de Vertebrados, Natal, Brazil

³Universidade Federal Rural do Semi-Árido (UFERSA), Programa de Pós-graduação em Ciência Animal (PPGCA), Laboratório de Morfofisiologia Animal Aplicada (LABMORFA), Mossoró, Brazil

⁴Projeto Golfinho Rotador (PGR), Fernando de Noronha, Brazil

⁵Centro de Estudos e Monitoramento Ambiental (CEMAM), Areia Branca, Brazil

⁶Instituto Chico Mendes de Conservação da Biodiversidade/Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (ICMBio/CMA), Santos, Brazil

⁷Universidade Federal de Pernambuco (UFPE), Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal (PPBA), Laboratório de Ecologia Comportamento e Conservação (LECC), Recife, Brazil

⁸Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS), Caucaia, Brazil

⁹Universidade do Estado do Rio Grande do Norte, Departamento de Turismo, Programa de Pós-Graduação em Ciências Naturais-UERN, Natal, Brazil

¹⁰Programa Regional de Pós-Graduação em Desenvolvimento e Meio Ambiente (PRODEMA), Universidade Federal do Rio Grande do Norte (UFRN), Natal, Brazil

*Corresponding author: taines.bio@gmail.com

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Abstract

Morphological studies of the digestive tract are relevant to know the biology and understand the eating habits to conserve species. Our objective was to analyze macroscopically and microscopically the digestive tract of Antillean manatee (*Trichechus manatus manatus*). Samples were collected from 20 animals found dead stranded on the coast of northeastern Brazil or that died during the rehabilitation process in captivity between July 1995 and March 2017. The macroscopic analysis of the gastrointestinal tract was carried out from the *in loco* monitoring of the two necropsies. For microscopic evaluation, tissue fragments were collected during necropsy, fixed with 10% buffered formaldehyde and subjected to routine histological processing, with 5 µm thick slices, and subsequently submitted to the Hematoxylin-Eosin staining method, the Verhoeff method, and the periodic acid-Schiff method. Macroscopically, neonates and adults have the same digestive tract structures, namely esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum. The microscopic analysis demonstrated that the digestive tract is formed by four histological layers (mucosa, submucosa, *muscularis*, and serosa or adventitia). In the esophagus, esophageal glands are absent and there are elastic fibers in the submucosal layer and between the external muscle and adventitial layers. The stomach

has a structure called the cardiac gland, which microscopically presents ontogenetic differences regarding the depth of the lining epithelium and the orderly distribution of cells along the gastric gland. In the duodenal ampulla and in the diverticula, duodenal glands were identified in the submucosa. The luminal portion of the mucous layer of the cecal ampulla, colon, and rectum is lined by keratinized stratified squamous epithelium. The digestive tract of the species Antillean manatee is similar to what has been reported for the order Sirenia.

Introduction

The order Sirenia is represented by the only herbivorous aquatic mammals in the world (Berta et al., 2015) that inhabit rivers, estuaries, coastal environments, and bays (Reynolds et al., 2018). This order is composed of manatees and dugongs, which are part of the Trichechidae and Dugongidae families, respectively. Of the Trichechidae family, two subspecies are still recognized but are under review for *Trichechus manatus*: the Florida manatee (*Trichechus manatus latirostris* (Harlan, 1824)), which occurs in North America, and the Antillean manatee (*Trichechus manatus manatus* Linnaeus, 1758), which inhabits Central and South America (Oliveira et al., 2011). According to the International Union for Conservation of Nature (IUCN), the species *Trichechus manatus* is classified as Vulnerable (IUCN, 2008); however, the species is classified as Endangered in Brazil due to the history of hunting and the various threats it currently faces (Brasil, Ministério do Meio Ambiente, 2022).

Manatees are monogastric animals with morphological adaptations of the digestive system due to the combination of the herbivorous diet and the euryhaline distribution (Reynolds & Rommel, 1996; Reynolds et al., 2018). Although there are studies related to the morphological descriptions of the digestive tract of sirenians (Lemire, 1968; Snipes, 1984; Colares, 1994; Reynolds & Rommel, 1996), the anatomical and histological description of the digestive tract of Antillean manatee from Brazil are still scarce. In addition, studies on the Florida manatee have prevailed among scientists and the information from these studies has been used as a reference for the Antillean manatee (Reynolds & Rommel, 1996; Reynolds et al., 2018).

The anatomical structures of the digestive tract are related to the food nature (Chaves & Vazzoler, 1984) as well as to eating habits (Silva, 2004). In Brazil, the distribution of Antillean manatee in coastal areas, estuaries, and rivers allows for a diet with a variety of food items and food preferences depending on their availability in the environment, which includes marine angiosperms, algae, freshwater macrophytes, and mangrove leaves (Melo et al., 2022). In addition to geographic distribution, this dietary variety is also associated with anatomical adaptations that allow the manatee to search for food in different strata, being able to find food items emerged, floating, submerged, or above the water surface (Melo et al., 2022). While foraging, they may also involuntarily ingest invertebrates that are present among the macrophytes, which may contribute as nutrient supplementation (Best, 1981; Borges et al., 2008).

This study aims to contribute with morphological knowledge to support the understanding of eating habits, disease identification,

and provide actions of conservation strategies for the species. Thus, the objective was to analyze macroscopically and microscopically the digestive tract of the Antillean manatee.

Material and Methods

Study site and Animals

Tissue samples from the digestive system of 20 Antillean manatees of both sexes from July 1995 to March 2017 were used. The samples were collected from five states in northeastern Brazil: Alagoas, Ceará (CE), Paraíba, Pernambuco (PE), and Rio Grande do Norte (RN) and were categorically separated by age group according to the classification by Attademo (2014): neonate (n = 4), calf (n = 4), juvenile (n = 5), adult (n = 3) and undetermined (n = 4), since there was no information about the total length. The samples were obtained from animals that died in a free-ranging environment, during the rehabilitation period, or after reintroduction to the free-ranging environment from: Costa Branca Cetaceans Project - State University of Rio Grande do Norte (PCCB/UERN), National Center for Research and Conservation of Aquatic Mammals (CMA/ICMBio), and the Association for Research and Preservation of Aquatic Ecosystems (AQUASIS), in Natal - RN; Itamaracá Island - PE and Caucaia - CE, respectively. All materials were regulated by SISBIO license No. 13694-3.

Macroscopy

To characterize the anatomical arrangement of the organs, we used photographic records taken during necropsies by the team of veterinarians from the PCCB-UERN. The esophagus was not considered in this macroscopic analysis, due to sample limitations. Therefore, we evaluated from the stomach to the large intestine. The study was based on terms adopted by the International Committee on Veterinary Gross Anatomical Nomenclature (2017) to denominate the identified structures and the results were compared with the literature referring to studies on other species of aquatic mammals.

Microscopy

For microscopic evaluation, the biological tissues collected comprised the esophagus (n = 3), stomach (n = 4), cardiac gland (n = 2), small intestine (n = 9) and large intestine (n = 11).

Biological tissue fragments were removed in minimal autolysis and fixed in 10% formaldehyde aqueous solution. Subsequently, at the Laboratory of Vertebrate Morphophysiology at the Federal University of Rio Grande do Norte, the fragments were subjected to dehydration using baths in gradual and increasing ethanol solutions (ethanol at a concentration of 70% to absolute alcohol), then diaphanized using xylene as an intermediate substance to carry out paraffin impregnation. In all stages, the biological tissues were submerged for 1 h in each bath. After impregnation, the inclusion stage was performed to form the block with the tissue using metal molds and liquid paraffin. Next, the block was sectioned in a microtome (Leica RM 2235) at a 5 µm thickness. The sections were stained with the Hematoxylin-Eosin (HE) staining method, according to Tolosa et al. (2005), and with the Periodic Acid-Schiff (PAS) staining method, according to Behmer et al. (1976).

The Verhoeff staining method was also performed on the esophageal slides to identify elastic fibers, according to Copetti (1996). During the technical procedure, the slides were deparaffinized and hydrated then stained with Verhoeff's solution (ferric hematoxylin) for seven min and discolored with 2% iron chloride. Subsequently, the technique was adapted and Light Green was used for 5 min for background staining.

The analysis of the histological slides was performed under light microscopy and microphotographed under an optical microscope (Olympus BX41) with a digital camera (Nikon DXM1200) attached and with the aid of the *NIS-Elements Advanced Research Software* for image capture.

Results

Macroscopic analysis

The macroscopic analysis allowed categorizing the Antillean manatee as a monogastric animal. Most of the stomach is located to the left of the median plane and between the two hepatic lobes (Fig. 1A). Specifically, the ventral portion of the stomach is in contact with the left lobe of the liver, gallbladder, and small intestine (Fig. 1A). The margins of the stomach are formed by the greater curvature, which is convex and extends to the left, and by the smaller curvature, which is concave (Fig. 1B). The smaller curvature empties into the esophagus and the pylorus, which are nearby (Fig. 1B). The presence of the cardiac gland with a cylindrical shape and with a single opening to the lumen of this region was verified in the dorsal portion of the cardiac region of the stomach (Fig. 1B).

The small intestine of the Antillean manatee is a tube subdivided into duodenum, jejunum, and ileum, which extends past the pyloric sphincter of the stomach to the beginning of the cecal body (Figs 1C, 1D). The loops of the small intestine are located caudally to the stomach. The duodenum is initially formed by the duodenal ampulla, constituted by a pair of duodenal diverticula, which open into the lumen of this portion (Fig. 1C). In the final portion of the duodenum, the post-duodenal ampulla was identified (Fig. 1C). The duodenal ampulla is located distal to the pylorus. The jejunum and ileum segments are located right after the duodenum, which is considered the last portion of the small intestine (Fig. 1D). However, there were no macroscopic features that delimited the jejunum and ileum.

The large intestine is the digestive tract's final portion and segmented into cecum, colon, and rectum (Fig. 1D). The cecum is the initial portion of the large intestine and is formed by the cecal body from which two cecal diverticula arise close to the ileocecal junction (Fig. 1E). The cecal body is connected to the cecal ampulla. In neonates, the cecal body is larger than the cecal ampulla (Fig. 1E). The colon is the largest portion of the large intestine that proceeds to the rectum, forming intestinal loops. Macroscopically, the rectum does not present any characteristic that distinguishes it from the colon (Fig. 1D).

Microscopic analysis

The mucosal layer of the esophagus has keratinized stratified squamous epithelium. The lamina propria is composed of loose connective tissue with the presence of blood vessels, projecting connective papillae into the epithelium with an irregular

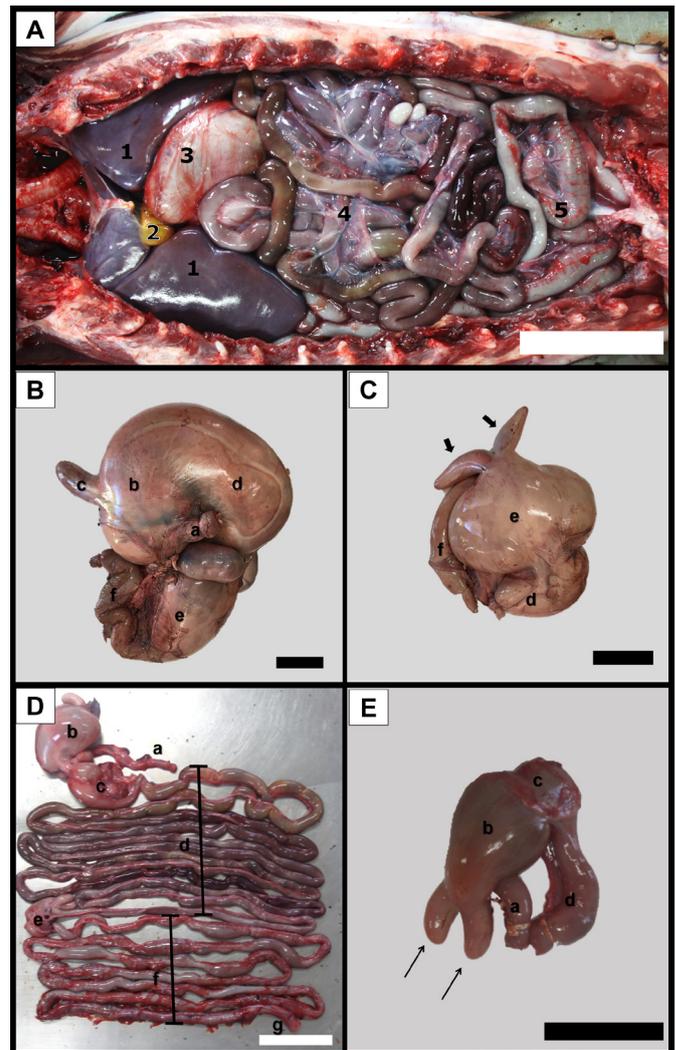


Figure 1. Macroscopic anatomy of the gastrointestinal tract of Antillean manatee. A) Topography of the organs of the digestive tract of a newborn animal, ventral view; hepatic lobes (1), gallbladder (2), stomach (3), small intestine (4), and large intestine (5). Bar: 10 cm; B) and C) Stomach and duodenum of an adult manatee; caudal portion of the esophagus (a), cardiac region (b), cardiac gland (c), pyloric region (d), duodenal ampulla (e), post-duodenal ampulla (f); duodenal diverticula (arrows). Bar: 10 cm; D) Digestive tube of a newborn animal (*ex-situ*), in sequence, the esophagus (a), stomach (b), duodenum (c), small intestine with jejunum and ileum (d); cecum (e); colon (f); and rectum (g). Bar: 10 cm; E) Cecum of a newborn manatee; ileum (a), cecal body (b), cecal ampulla (c), colon (d), and cecal diverticula (arrows). Bar: 5 cm.

arrangement. Underlying the lamina propria, the muscle layer of the mucosa was observed, formed by several layers of smooth muscle fibers, arranged longitudinally with undulations and with the presence of dense connective tissue fibers between the muscle fibers. Due to the undulating arrangement of muscle fibers, using the Verhoeff staining method, the presence of elastic fibers was verified from the *lamina propria* of the mucosal layer to the submucosal layer in neonates and adults. In adults, elastic fibers are abundant in the mucosal and submucosal layers and are present between the *muscularis externa* and the adventitia. The submucosa has connective tissue of varying density, blood vessels, and submucosal nerve plexus. The presence of esophageal glands was not observed in the esophagus. The muscle layer is arranged in two layers, an inner circular layer

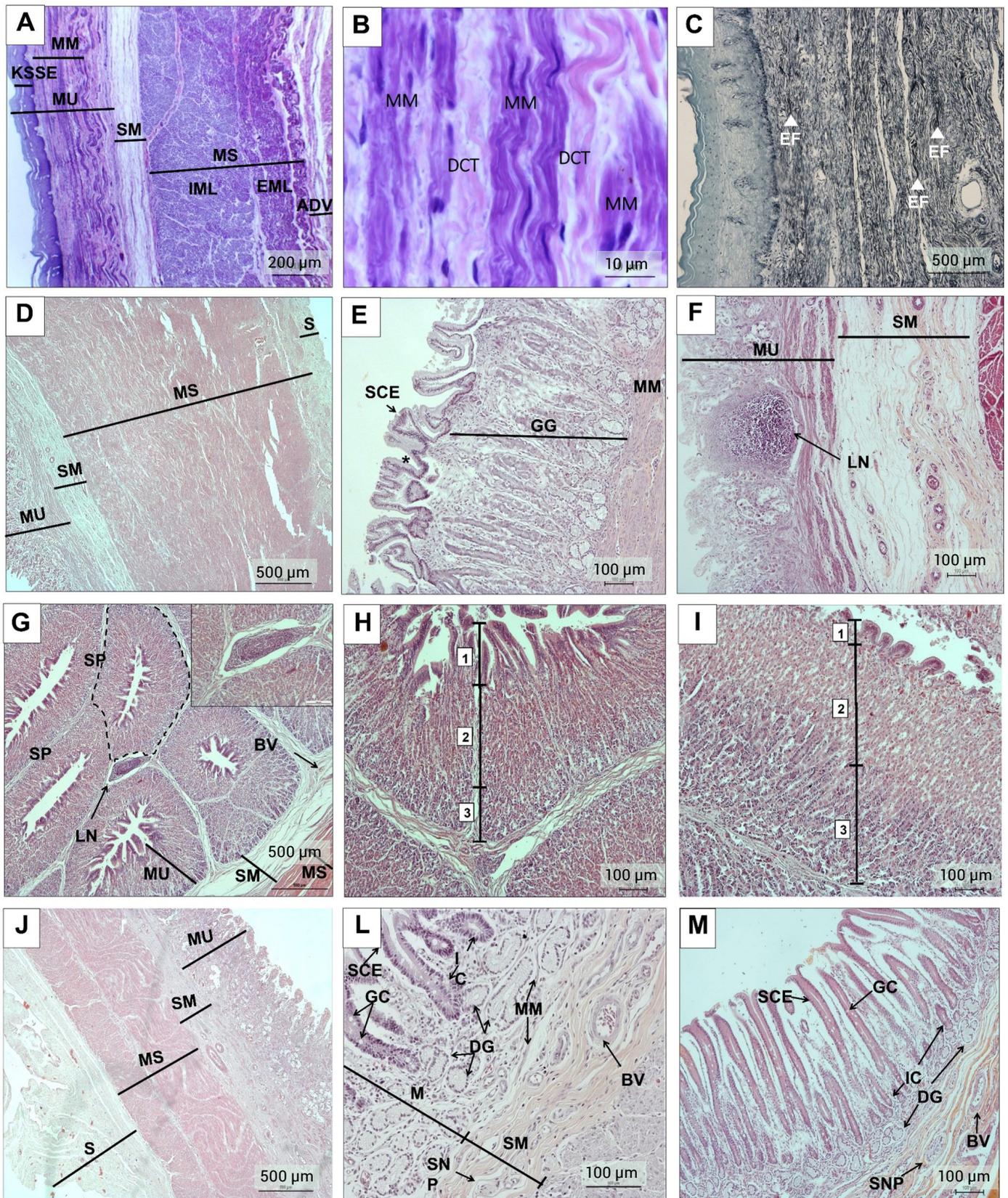


Figure 2. Microscopic characteristics of the gastrointestinal tract of Antillean manatee. A), B), and C) esophagus; D), E), and F) stomach; G), H) and I) cardiac gland; H) and I) comparison of the cardiac gland between two phases with zonations (1, 2 and 3): neonate H) and adult I); J) and L) duodenal ampulla; M) duodenal diverticulum. Abbreviations: adventitia (ADV); blood vessel (BV); dense connective tissue (DCT); duodenal gland (DG); elastic fiber (EF); external muscle layer (EML); ganglia of the submucosal nerve plexus (SNP); gastric gland (GG); gastric pit (*); goblet cell (GC); inner muscle layer (IML); intestinal crypts (IC); keratinized stratified squamous epithelium (KSSE); lymphoid tissue (LT); mucosa (MU); mucosal muscle (MM); muscle (MS); septa (SP); serosa (S); simple muscle (SM).

and an outer longitudinal layer, both made up of skeletal muscle tissue fibers. The presence of ganglia of the myenteric nervous plexus was observed between the muscle layers. In all animals, the adventitial layer presents loose connective tissue with the presence of blood vessels (Figs 2A, 2C).

The gastric mucosa presented an irregular surface covered by simple cylindrical epithelium with muco-secretory cells. This surface invades the *lamina propria* forming the crypts, or gastric pits, which are continuous with the branched tubular gastric glands. In the gastric mucosa, different types of cells were identified in the mucous cells on the surface and in the gastric glands that had a positive reaction to PAS, parietal cells (oxyntic) and chief cells (zymogenic). In gastric glands, mucous and parietal cells are predominant along the length of the glands, while zymogen cells predominate in the basal portion. The gastric glands are located in the *lamina propria*, which is composed of loose connective tissue with blood vessels and lymphocytes. The *muscularis mucosae* is characterized as a thick and diffuse layer, formed by smooth muscle, with blood vessels and nerve plexus ganglia. The presence of the ganglion of the nervous plexus in the *muscularis mucosae* evidences a nervous system in the mucous layer. Lymphoid tissue is present in the mucous layer, specifically in the *lamina propria*. No glands were seen in the submucosa, but the presence of blood vessels was identified. The muscle layer is formed by smooth muscle and subdivided into an inner circular layer and an outer longitudinal layer. The myenteric nervous plexus was located between the circular and longitudinal layers. The serosa layer is formed by loose connective tissue lined by mesothelium (Figs 2D - 2F).

The cardiac gland structure was identified in the cranial portion of the cardiac region of the stomach, which presents the four histological layers of the digestive tract: mucosa, submucosa, *muscularis*, and serosa. The mucous layer invaginates and forms lobules divided into zones. Zone 1 is covered by simple cylindrical mucus-secreting epithelium forming the gastric crypts with secretions from the gastric glands of the other zones. In zone 2, mucus-producing cells predominate, located mainly in the upper region of the glands and parietal cells. In zone 3, there is the presence of parietal cells and mainly principal cells. The *muscularis mucosae* was not evident. The submucosal layer is composed of loose connective tissue that forms the septa between the lobules in which the presence of blood vessels and a lymph node was verified. The muscle layer is formed by smooth muscle fibers and the identification of fiber orientations was not clear along the muscle layer, but the myenteric nerve plexus and blood vessels were identified. The serosa layer is formed by loose connective tissue with mesothelium (Figs 2G - 2I).

The analyses showed differences in the zones that compose the lobes of the cardiac gland between newborn and adult animals. In neonates, the gastric crypts (Zone 1) are deeper compared to adult animals. In the transition from Zone 1 to Zone 2, in newborns, the gastric crypts are present in oblique and transversal cuts, possibly indicating sinuous paths. In adults, the gastric glands (Zones 2 and 3) are longer and easier to identify when distinguishing the zones. In Zone 2, mucus-secreting cells and parietal cells were observed in animals of both age groups. In turn, in Zone 3, principal and parietal cells are present in neonates and adults, but with predominance of principal cells in adults (Figs 2H, 2I).

The duodenal ampulla and duodenal diverticula of the Antillean manatee have similar histological characteristics. In the mucous layer, the villi are directed toward the lumen and lined by absorptive columnar epithelial cells or enterocytes, interspersed with goblet cells. The absorptive cells, tall columnar cells with oval nuclei at the basal portion, and the goblet cells are distributed along the length of the villi. This epithelial lining is continued into the intestinal crypts in the *lamina propria*, which are glands characterized as simple tubular. The PAS reaction was positive in goblet cells, duodenal glands, and intestinal crypts as a result of the numerous goblet cells. The smooth muscle fibers of the *muscularis mucosae* are dissociated bypassing the duodenal glands and lymphoid tissue in the mucosal layer. The duodenal glands that secrete mucus into the intestinal crypts are between the *muscularis mucosae*, extending into the *lamina propria*. These glands are characterized as composite acinar formed by columnar mucous cells with a nucleus in the basal portion. In the submucosa, blood vessels and submucosal nerve plexus ganglia were found in the dense connective tissue. The muscle layer is divided into an inner circular layer and an outer longitudinal layer and is composed of smooth muscle fibers with the presence of myenteric nerve plexus ganglia and blood vessels between the layers. The serosa layer is formed by loose connective tissue with few blood vessels (Figs 2J - 2M).

The histological characteristics are similar in the jejunum and ileum. The intestinal villi and crypts of the mucous layer were not characterized, as they were damaged, possibly due to the decomposition stage of the carcasses. The lymphoid tissue is in the mucous layer in a newborn individual. In the Antillean manatee, Peyer's patches were found ungrouped. The *muscularis mucosae* has an undulating orientation in the jejunum. The submucosal layer is formed by predominantly loose connective tissue with the presence of submucosal nerve plexus ganglia and blood vessels. In the muscle layer, the inner circular layer and the outer longitudinal layer have an abundant presence of myenteric nerve plexus ganglia between the layers. In the serosa layer, loose connective tissue with mesothelium was observed.

In the mucous layer of the cecal ampulla, the epithelium in the apical portion is keratinized cubic stratified and the intestinal crypts have simple columnar epithelium and the presence of goblet cells in the basal portion. The intestinal crypts showed a positive reaction to the PAS technique due to the abundant presence of goblet cells. The *muscularis mucosae* appears as a thick layer with smooth muscle fibers in the longitudinal direction. In the mucous layer, lymphoid tissue and mucous nerve plexus ganglion are present. The submucosal layer has blood vessels in the loose connective tissue. In the muscular layer, three layers were observed in different orientations, subdivided into internal circular, medium longitudinal, and external circular. The myenteric nerve plexus is abundant in the inner circular layer. In newborns, two layers with an internal circular and an external longitudinal orientation are identified, with the ganglion of the myenteric nervous plexus present in both layers. The serosa layer is formed by loose connective tissue. No adventitial layer was seen in the cecal ampulla.

In the mucous layer of the colon, above the glandular epithelium, the epithelium is keratinized stratified squamous. From the luminal portion to the basal portion of the mucosa, the glandular

epithelium is characterized as simple cubic, epithelium composed of cells called enterocytes, with the presence of goblet cells predominating in the lower portion. The goblet cells showed a positive reaction to the PAS technique in the villi and intestinal crypts. A small number of columnar cells were identified in the medial and basal portions of the glands. In the mucous layer, lymphoid tissue was found regularly distributed, with the presence of *muscularis mucosae* filaments among them. The *muscularis mucosae* has smooth muscle fibers in a circular arrangement. The submucosal layer contains loose connective tissue, with blood vessels and ganglia of the submucosal nervous plexus. The muscle layer is subdivided into an inner circular layer and an outer longitudinal layer with the presence of myenteric nerve plexus ganglia between them. The serous layer of variable thickness is formed by loose connective tissue with the abundant presence of blood vessels.

The epithelium is characterized as keratinized stratified squamous on the luminal surface of the mucous layer of the rectum. However, columnar cells called enterocytes and goblet cells were identified below the luminal surface, and a simple epithelium with cubic cells was observed in the basal region of the mucous layer. Goblet cells showed a positive reaction to PAS staining. This composition of the epithelium extends to the cells that form the intestinal crypts. The *muscularis mucosae* has a circular orientation and the presence of lymphoid tissue was verified between the muscle fibers. The submucosal layer has blood vessels. The muscle layer is constituted by the internal circular layer and the external longitudinal layer, among which the presence of the myenteric nervous plexus can be identified. The serous layer is composed of loose connective tissue with the presence of blood vessels and delimiting the layer by the mesothelium.

Discussion

Sirenians present digestive tract development with adaptations for being herbivores and carrying out post-gastric fermentation, which are characteristics similar to elephants, hyraxes, and horses (Horstmann, 2018; Reynolds et al., 2018; Thitaram et al., 2018; Clauss et al., 2020). The anatomical adaptations of the results obtained in this study of Antillean manatee include accessory structure in the stomach (cardiac gland), a pair of diverticula in the duodenum, and cecum, similar to anatomical adaptations of the digestive tract among other sirenians (Reynolds & Rommel, 1996).

Research points to similarities in the gastrointestinal tract between sirenians and green turtles (*Chelonia mydas*), due to similar eating habits and sharing the same habitat (Burn & Odell, 1987; Reynolds & Rommel, 1996). Through foraging, sirenians and green turtles in the adult phase are marine macroherbivores that play an important ecological role in seagrass meadows and their associated fauna (Thayer et al., 1984; Valentine & Heck, 1999; Farias et al., 2023). Sirenians are efficient in cellulose digestion (Bjorndal, 1979; Burn, 1986) through post-gastric microbial fermentation, synthesizing volatile fatty acids (VFA). The same process occurs with sea turtles (Yuan et al., 2015; Campos et al., 2018; Farias et al., 2023). Along the gastrointestinal tract, sirenians and adult green turtles show patterns of similarity

in VFA concentrations with higher levels in the cecum and colon segments (Bjorndal, 1979; Burn & Odell, 1987; Reynolds & Rommel, 1996).

The histological features of the esophageal mucosal layer of Antillean manatee presented keratinized stratified squamous epithelium, which has also been described in Amazonian manatees (Colares, 1994) and is commonly found in birds and other herbivorous animals, such as horses, rabbits (Kisia, 2016; Thitaram et al., 2018), and green turtles (Melo et al., 2022). In a marine environment, the keratinized stratified squamous epithelium is also responsible for waterproofing and preventing dehydration of the animal by ingestion of salt water in addition to protecting against mechanical aggression from the food, similar to what was identified in herbivorous sea turtles (*Chelonia mydas*) (Reynolds & Rommel, 1996; Magalhães et al., 2012; Chen et al., 2015; Farias et al., 2022; Melo et al., 2022;).

The absence of esophageal glands in the submucosal layer of Antillean manatee resembles those of the Amazonian manatee (Colares, 1994) and capybaras (Velásquez et al., 2016), contrasting with what is seen in Asian elephants with abundant mucus-secreting glands to protect the esophageal mucous layer, but they have non-keratinized stratified squamous epithelium (Thitaram et al., 2018).

The aglandular submucosa layer of the esophagus observed in Antillean manatee may indicate the presence of the keratin layer as exclusive protection of the esophageal mucosa (Velásquez et al., 2016) and that the esophagus is an organ that is only related to food conduction to the stomach (Magalhães et al., 2012).

The presence of elastic fibers was verified by comparing the microscopic structure of the esophagus in Antillean manatee from different stages of development (neonate and adult). In addition to the presence of elastic fibers in the submucosal layer in both developmental stages, adult individuals presented a network of elastic fibers between the outer muscle and adventitia layers, providing the esophagus with great elasticity, a function similar to the fibers found in the esophagus of horses (Stick, 2012).

The existence of the elasticity property in the esophagus of the adult manatee may be related to the foraging habit. According to Reynolds (1981), the manatee forages 6 - 8 hours a day and can feed up to 10% of its body weight. In the initial period of life, the calf can be stimulated by the mother's behavior in having an interest in plant consumption, which provides learning of the foraging technique (Hartman, 1979; Hayssen, 1993).

Although similar, the stomach of the Amazonian manatee has characteristics that differ from those of the Florida manatee and the Antillean manatee studied here. In the Florida and the Antillean manatees, the parietal cells and the main ones are distributed in the gastric mucosa, while in the Amazonian manatee, these cells are present only in the cardiac gland. Regarding the stomach topography, it is located on the right of the mid-sagittal plane in the Amazonian manatee and on the left in the Florida and Antillean manatees (Colares, 1994; Reynolds & Rommel, 1996). These divergent morphological characteristics may be related to adaptations to the aquatic environment, as Amazonian manatees inhabit a freshwater environment with variations in seasonal floods with changes in food availability between the wet and dry seasons, while Florida and Antillean manatees inhabit coastal and estuary environments, which exhibit salinity variations (O'Shea & Powell, 2001).

The stomach glandular mucosa of Antillean manatee is covered by surface mucous cells that produce mucus to protect the gastric epithelium. In horses, one of the defense mechanisms is the production of mucin with bicarbonate to avoid lesions in the gastric mucosa (Aranzales & Alves, 2013), forming an alkaline barrier that protects the mucosal layer against the action of enzymes, acidity in the stomach lumen (Nasciutti et al., 2016), and polysaccharide mucus that can protect against friction caused by grains of sand ingested with food, developing a function similar to that of keratin (Silva et al., 2005).

In the greater curvature region of the Florida manatee stomach, mucous glands were identified in the mucosal and submucosal layers that allow for abundant mucus production (Reynolds & Rommel, 1996). However, in the analyzed specimens of Antillean manatee, gastric glands with mucus-producing cells were located only in the mucous layer of the stomach.

One of the main atypical characteristics found in the stomach of sirenians is the presence of the cardiac gland, also verified in this study on Antillean manatee. The presence of the cardiac gland differentiates sirenians from other taxa in terms of stomach morphology (Langer, 2001). This structure was observed among the animals that formerly composed the ancient group of ungulates (Langer, 2001). Structures similar to the cardiac gland have only been found in some herbivorous mammals (Reynolds & Rommel, 1996).

The types of cells are similar in the comparative analysis of the mucous layer of the cardiac gland of newborn and adult Antillean manatee. The differences found refer to the depth of the lining epithelium and the orderly distribution of cells along the gastric gland, which may be related to the feeding habits during the development phases, since the primary function of the cardiac gland is the separation of acidic and digestive enzymes from abrasive plant material that are passing through the stomach (O'Shea & Powell, 2001; Horstmann, 2018) and the production of mucus, contributing to the digestion process (Reynolds & Rommel, 1996; Harshaw, 2012; Horstmann, 2018). Lymphatic nodules were also found in the cardiac gland in Antillean manatee newborns, but not in the adult individual. Reynolds and Rommel (1996) also did not identify the presence of lymphoid tissue in the cardiac gland of the Florida manatee adults.

Anatomically, there is a similarity between the duodenum and duodenal diverticula of sirenians with other herbivorous mammals, differing in size (Reynolds & Krause, 1982; Reynolds & Rommel, 1996). Similar to sirenians, cetaceans also have a duodenal ampulla (Rommel & Lowenstine, 2001; Horstmann, 2018). The absence of Paneth cells in the duodenum of the individuals evaluated in this study corroborates histological reports in the duodenum of manatees, already described in the literature, but these cells are present in most large terrestrial herbivores (Dellmann, 1971; Colares, 1994; Mead, 2009). In caracara bird (*Caracara plancus*), the presence of lymph nodes along the small intestine may be related to the absence of Paneth cells, as a contribution of lymph nodes to defense against foreign bodies in the intestinal mucosa (Almeida et al., 2016). In the present study, the Antillean manatee also had lymphoid tissue in all segments of the small intestine, besides the presence of duodenal glands in the duodenum and diverticula, which were also recorded in dugongs (*Dugong dugon*) and Florida manatees (Reynolds & Rommel, 1996).

In this study, the oval shape of the cecum was verified in the different ontogenetic phases of the Antillean manatee. According to Mead (2009), the cecum of sirenians presents different models depending on the phylogeny. In manatees, the cecal body has an oval shape, while the cecum of dugongs has a conical shape. Regarding the size of the cecal ampulla in Antillean manatee neonates in this study, Snipes (1984) reports an increase in the volume of the cecal ampulla during ontogenetic development due to the need for a food reservoir in adults.

The large intestine of sirenians is the main region where digestion of organic matter, nitrogen, and crude fat occurs, as well as water reabsorption. The cecum and proximal colon are the sites where cellulose digestion mainly occurs, leading to the classification of sirenians as post-gastric fermenters (Burn, 1986; Reynolds & Rommel, 1996; Horstmann, 2018; Reynolds et al., 2018). Manatees have a high digestibility efficiency for cellulose compared to other herbivorous mammals, such as the horse, because of the slow passage rate due to the large size of both their body and large intestine (Burn, 1986; Reynolds & Rommel, 1996), allowing thus a better nutritional use (Amaral et al., 2023).

Macroscopically, in the cecum, a paired cecal *muscularis diverticula* was seen in the Antillean manatee. The paired cecal diverticula has been observed in other manatees, anteaters, armadillos, hyraxes, kangaroos, wombats, beavers, capybaras, sloths, pika, and antelopes (Snipes, 1984). In dugongs, only one diverticulum is present in the cecum (Horstmann, 2018). According to Smith et al. (2017), the presence of the appendix or cecal diverticula in mammals is correlated with immune function due to the concentration of lymphoid tissue.

In each macroscopic segment of the cecum of the Antillean manatee, histological structures were similar to those in the Florida manatee (Snipes, 1984). In this study, the cecum of Antillean manatee presented lining epithelium classified as keratinized stratified squamous. In the Florida manatee, Snipes (1984) reports the same classification of the epithelium, contrasting with Reynolds and Rommel (1996) who state that the epithelium is non-keratinized. The functionality of this characteristic is related to the protection mechanism, in addition to the production of mucus by the goblet cells, against abrasions due to digesta with a hard consistency of plant elements that have not yet been digested (Snipes, 1984; Horstmann, 2018).

The unusual epithelial lining of the keratinized stratified squamous type identified in the cecum of Antillean manatee is similar to that in the colon and rectum segments. In dugongs and Florida manatees, the epithelium differed from that in Antillean manatee by the absence of keratin (Reynolds & Rommel, 1996).

The colon and rectum of Antillean manatee present the absorptive cells or enterocytes, which are responsible for nutrient absorption. The absorption of water and ions in the large intestine is relevant to keeping water balance in manatees, considering that they are euryhaline animals (Snipes, 1984; Reynolds & Rommel, 1996). Horstmann (2018) reports the absence of enterocytes in the small intestine of sirenians but describes the presence of these cells in the large intestine.

Thus, the digestive tract of Antillean manatee has the four histological layers (mucosa, submucosa, muscle, and adventitia/serosa) from the esophageal portion to the rectum, considered as standard for vertebrates (Farias et al., 2022). However, adaptations

in the epithelium of the mucous layer along the digestive tract were highlighted to protect against abrasion and dehydration due to the food type and accidental ingestion of salt water, respectively.

Furthermore, morphological ontogenetic changes reported in this study (location of elastic fibers in the esophageal layers and changes in the mucous layer of the cardiac gland) coincide with the developmental stages related to the feeding habits of the species.

The present work presented morphological descriptions of the digestive tract of Antillean manatee in northeastern Brazil that were not previously reported in the literature but already described in the dugong, and Amazonian, African, and Florida manatee.

Our results confirm that Antillean manatees in northeastern Brazil are monogastric animals and present digestion in the large intestine, classified as non-ruminants and post-gastric fermenters.

The comparative analysis with the subspecies of the Florida manatee showed unusual morphological characteristics in the subspecies of the northeastern Brazil Antillean manatee, namely the location of the mucous glands in the stomach, presence of ganglion of the nervous plexus in the mucous layer, and presence of keratinization in the epithelium of the large intestine.

Therefore, the morphological description of the digestive tract of the Antillean manatee becomes relevant for future research in several areas of biology, such as phylogenetics, in terms of identification of digestive dysfunctions, avoiding compromising the health of the animals as well as the knowledge about eating habits to develop successful strategic actions for conservation of this endangered species in Brazil.

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