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Morphology of the Digestive Apparatus in *Oligoryzomys nigripes* (Rodentia, Sigmodontinae)

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Abstract

Although the Genus *Oryzomys* is widely distributed in the Americas, there are very few published data on the morphology of this rodent group. Notably, in relation to the digestive apparatus, the data are sparse and fragmented. In addition, because rodents are important animal models, this information may be useful in recent and promising areas for applied studies, such as clinical, therapeutic, and surgical models. Here, we describe the morphological characteristics of the digestive system in *Oligoryzomys nigripes* using gross morphology and light and scanning electron microscopy. In total, five specimens were used. After an incision from the oral to pelvic cavities, all organs of the digestive system were exposed and dissected. Next, all structures from the oral cavity, pharynx, esophagus, stomach, intestines, liver, and pancreas were processed and evaluated by microscopy. The results revealed that the digestive system of *O. nigripes* consisted of an oral cavity, in which the tongue, minor and major salivary glands, and dental arch were predominant. Behind the oral cavity, the pharynx, esophagus, stomach, intestines, liver, and pancreas were identified and presented typical morphological characteristics of rodents. In conclusion, our results suggest that the morphology of the *O. nigripes* digestive system differs from the previously described data for other rodent species, mainly due to the absence of the molar teeth and gallbladder in this species.

Keywords

Cricetidae, Microscopy, Macroscopic, Rodents

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1. Introduction

Rodents represent the most diversified group in Mammalia and the families Muridae and Cricetidae are the most diverse clades [1]. Within the subfamily Sigmodontinae, the tribe Oryzomyini has the greatest species richness and currently consists of 28 recognized genera [1] [2]. The genus Oryzomys is widely distributed from the northern Central America to the southern region of South America; consequently, it has adapted to various habitats [3]-[5].

According to Emmons (1997) [3], rodents have two large pairs of chisel-like incisors and three to five molars (fewer molars have been found in a few rare cases), which are separated from the incisors by a large space known as the diastema. The particular characteristics of the rodent's dentition have had a significant impact on their evolution, resulting in great diversity, especially in relation to the habits and niches that these species inhabit. In addition, taxonomically, rodents are divided into three groups according to the arrangement of the jaw muscles and associated skull structures. The Sciuromorpha (squirrel-like rodents) have a simple jaw muscle that extends onto the snout in front of the eye. The Myomorpha (mouse-like or rat-like rodents) have jaw muscles that anchor to the side of the nose—these are the most efficient among the rodents. The Caviomorpha (cavy-like rodents) have large cheekbones and muscles anchored to the side of the face [6].

Although these animals are widely distributed throughout the Americas, few studies of this group have been conducted. In contrast with murine species, for which extensive literature is available [7]-[10], for cricetid rodents, the data are sparse. Notably, ecological studies [3]-[5] [11] [12], epidemiological data [13]-[15], and a few developmental studies [16]-[18] have been developed for these taxa.

In the scientific field, rodents have been used as experimental models, including for homology to humans in studies on pathologies that affect the digestive system, including malnutrition and re-nutrition [19]-[21]. In relation to the digestive system, Cooper and Schiller (1975) [22] analyzed the guinea pig (*Cavia porcellus*, Caviidae) components of this system and concluded that it consisted of the digestive tract (gut) and accessory glands. The alimentary canal extends from the mouth to the anus and consists of the following segments: oral cavity, pharynx, esophagus, stomach, small intestine (which is divided into the duodenum, jejunum, and ileum), and large intestine (which is divided into the cecum, colon, rectum, and anus). The accessory glands identified include the salivary glands, liver, and pancreas.

The study of the digestive tract may reveal general dietary habits, and its structure is occasionally used in systematic classification [23]. Although this system presents a simple morphological and structural pattern in mammals, it is extremely important for the knowledge of the gross and microscopic particularities of the digestive tract in other species, especially in those with potential to be used as animal models. In addition, many organs that compose the digestive system have recently been used as sources of stem cells, and there are promising results for the use of these cells in regenerative medicine [24].

Due to the absence of data regarding the morphology of the digestive apparatus in cricetid rodents, the aim of the present study was to describe the organs that compose the digestive system in *Oligoryzomys nigripes* using gross morphology and light and scanning electron microscopy. The results presented here may facilitate not only applied studies on the morphology of the organs that compose this system in rodents but also comparative analyses.

2. Materials and Methods

2.1. Sample Collection

The five *O. nigripes* specimens used in this study were donated to the Anatomy Museum of the School of Veterinary Medicine and Animal Science, University of Sao Paulo during a fauna rescue effort in an area used to construct a hydroelectric dam in São Joaquim da Barra, Sao Paulo, Brazil. The specimens were used in other projects in the same institution and were registered with the bioethics committee under number 1148/2007.

All experimental animals were adults and were fixed in 2.5% glutaraldehyde. The animals were initially used for macroscopic description, and samples of the digestive organs were processed for light and scanning electron microscopy

2.2. Macroscopic Description

First, an incision along the midsagittal plane was performed to better visualize the organs of the digestive system

in the cervical and thoracic regions, as well as in the abdominal and pelvic cavities (pharynx, esophagus, stomach, intestines, liver, and pancreas). To analyze the oral cavity, the temporomandibular joint was dismantled at both antimeres so that all structures in the mouth and oral cavity could be observed, dissected, and photographed. A stereo microscope (Zeiss Stemi SV6, Germany) was used to better visualize the samples. All results were photo-documented (Sony MVC-CD500).

2.3. Light Microscopy

For histological analysis, the samples fixed in 2.5% glutaraldehyde were washed in phosphate buffer or distilled water to remove the fixative, dehydrated in a graded alcohol series (70% to 100%), cleared in xylene, and embedded in paraffin (Histosec-MERCK, lot K91225309).

The paraffin blocks were sectioned into 5 µm portions using an automated microtome (Leica RM2165), and the slides were deparaffinized and stained using hematoxylin and eosin [25]. Finally, the samples were analyzed, and the morphological features were photo-documented (Nikon Eclipse 80i microscope).

2.4. Scanning Electron Microscopy

Samples of the tongue were washed in 0.1 M phosphate buffer, pH 7.4 and then, postfixed in 1% osmium tetroxide, followed by critical point drying (Balzers CPD 020). The samples were then placed on a metal support for gold plating ("sputtering" Emitech K550). The results were analyzed in a scanning electron microscope (ME Leo 435 VP).

The nomenclature was based on the International Committee on Veterinary Gross Anatomical Nomenclature and International Committee on Veterinary Histological and Embryological Nomenclature, 2005.

3. Results

3.1. Gross Morphology

The oral cavity was divided into a vestibule and oral cavity proper. Externally, the vestibule consisted of the region between the cheeks and lips. Internally, it consisted of the region between the teeth and gums. The dental arch consisted of two incisors, two prominent canines, and four premolars on each side, for a total of 22 teeth. The oral cavity proper was located in the region between the dental arches. It was defined dorsally by the hard palate and the palatine process of the maxilla and was defined caudal-dorsally by the soft palate, which was identified by palatine folds that were oriented caudally on its surface and ventrally toward the tongue. The oral cavity could be generally divided into three distinct parts, the apex at the narrowest region, the body, and the root at the widest region (**Figure 1[A]**). The lingual surface was identified by the presence of unevenly distributed papillae. The following types of papillae were observed: vallate, filiform, and fungiform. Minor salivary glands were observed on the cheek, palate, and the floor of the mouth and tongue. The major, sublingual, submandibular, and parotid salivary glands were found in the parotid-auricular region. The pharynx was a continuous membranous tube of muscle that connected the oral cavity to the esophagus and was composed of three segments, the oropharynx, nasopharynx, and laryngopharynx. The esophagus extended from the pharynx to the stomach and was divided into the cervical, thoracic, and abdominal esophagus (segment connected to the stomach in the abdominal cavity), and longitudinal muscle lines were easily observed at the macroscopic level (**Figure 1[A]**). After the esophageal hiatus, the esophagus was connected to the stomach by the gastric cardia (**Figure 1[B]**).

The stomach had a J shape because of the presence of two curvatures, the greater and lesser curvatures, where the greater omentum and lesser omentum were attached. Anatomically, the stomach was divided into the following three regions: the cardia, which was located to the right of the abdominal midline, was connected to the esophagus and was a gastric sphincter; the body, which was the larger middle region of the stomach; and the pylorus, which was located toward the left near the duodenum.

The intestine was a muscular tube that extended from the pyloric portion of the stomach to the anus. The intestinal diameter was not uniform throughout its entire length and could be anatomically divided into the small and large intestines. The small intestine consisted of three regions: duodenum, jejunum, and ileum. The duodenum had two curvatures, an ascending curvature, where the pancreas was located, and a descending curvature. The jejunum had a greater region that could be identified by the presence of mesentery, and the ileum possessed

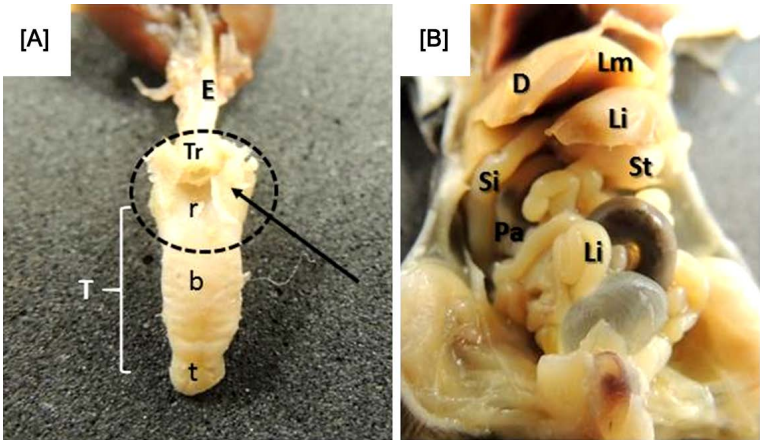

Figure 1. Gross morphology of the *Oligoryzomys nigripes* digestive apparatus. [A] Detail of the tongue (T) and laryngopharynx (circle) (*ex situ*). Observe the tongue regions: the tip (t), body (b), and root (r), as well as the pyriform sinus. Trachea (Tr) and esophagus (E); [B] A ventral view (*in situ*) showing the abdominal organs: pancreas (Pa), liver (Lm—left medial and Li—left lateral lobes), stomach (St), small intestine (Si), and large intestine (Li). Note the topography of the digestive organs in relation to the diaphragm (D).

a minor region, which was identified by the presence of the ileocecal fold that marked the anatomical division between the jejunum and ileum. The large intestine also consisted of three regions. The first region was the cecum, a small tube-shaped structure with a blind end. The second region was the colon, which could be subdivided into ascending, descending, and transverse regions and was located in the caudal region of the abdominal cavity. The third region was the rectum, which was located toward the pelvic cavity and formed the final segment of the large intestine.

The liver, located in the cranial region of the abdomen, was covered by the peritoneum. The liver had four lobes, a left lateral lobe, a left medial lobe, a quadrate lobe, and a right lateral lobe (**Figure 1[B]**). The presence of the hepatic artery and portal vein were observed. There was no evidence of a gallbladder. The pancreas was elongated, connected to the duodenum, and oriented toward the greater curvature of the stomach (**Figure 1[B]**).

3.2. Histology

At the microscopic level, the oral cavity was lined by stratified squamous epithelium that was keratinized on the gingiva and non-keratinized on the palate, lips, cheek, and floor of the mouth.

The crowns of the teeth were coated with enamel, and the roots were coated with cementum. Root portions were not observed on the incisors. Dentin was observed below the cementum and enamel. Surrounding the dentin was a pulp cavity that was filled with a loose connective tissue known as dental pulp (**Figure 2[A]**). The hard palate consisted of non-keratinized stratified squamous epithelium, and the connective tissue was dense and unmodeled. We observed a muscle layer, palatine glands, and periosteum (**Figure 2[B]**). The soft palate also consisted of a non-keratinized stratified squamous epithelium, and striated skeletal muscle. Mucous glands were also present. The salivary glands contained mucosal lobes that contained serous and mucous cells. The secretory endings formed a duct system, and the intercalated ducts were composed of cuboidal epithelial cells. Several of these ducts formed striated ducts that converged into larger ducts to form excretory ducts, which consisted of an initial region composed of stratified cuboidal epithelium and a more distal region composed of stratified columnar epithelium (**Figure 2[C]**).

The tongue consisted of a stratified squamous epithelium over muscle layers. Filiform papillae covered the entire surface of the tongue, with fungiform papillae distributed between the filiform papillae (**Figure 2[D]**, **Figure 2[E]**, and **Figure 2[F]**). The esophagus was lined with a non-keratinized stratified squamous epithelium, and a lamina propria filled with esophageal glands was observed below the muscle layer (**Figure 2[G]**). The stomach was lined with a simple columnar epithelium that invaginated toward the lamina propria to form gastric pits in which branched tubular glands were observed. These glands were named according to the region of the

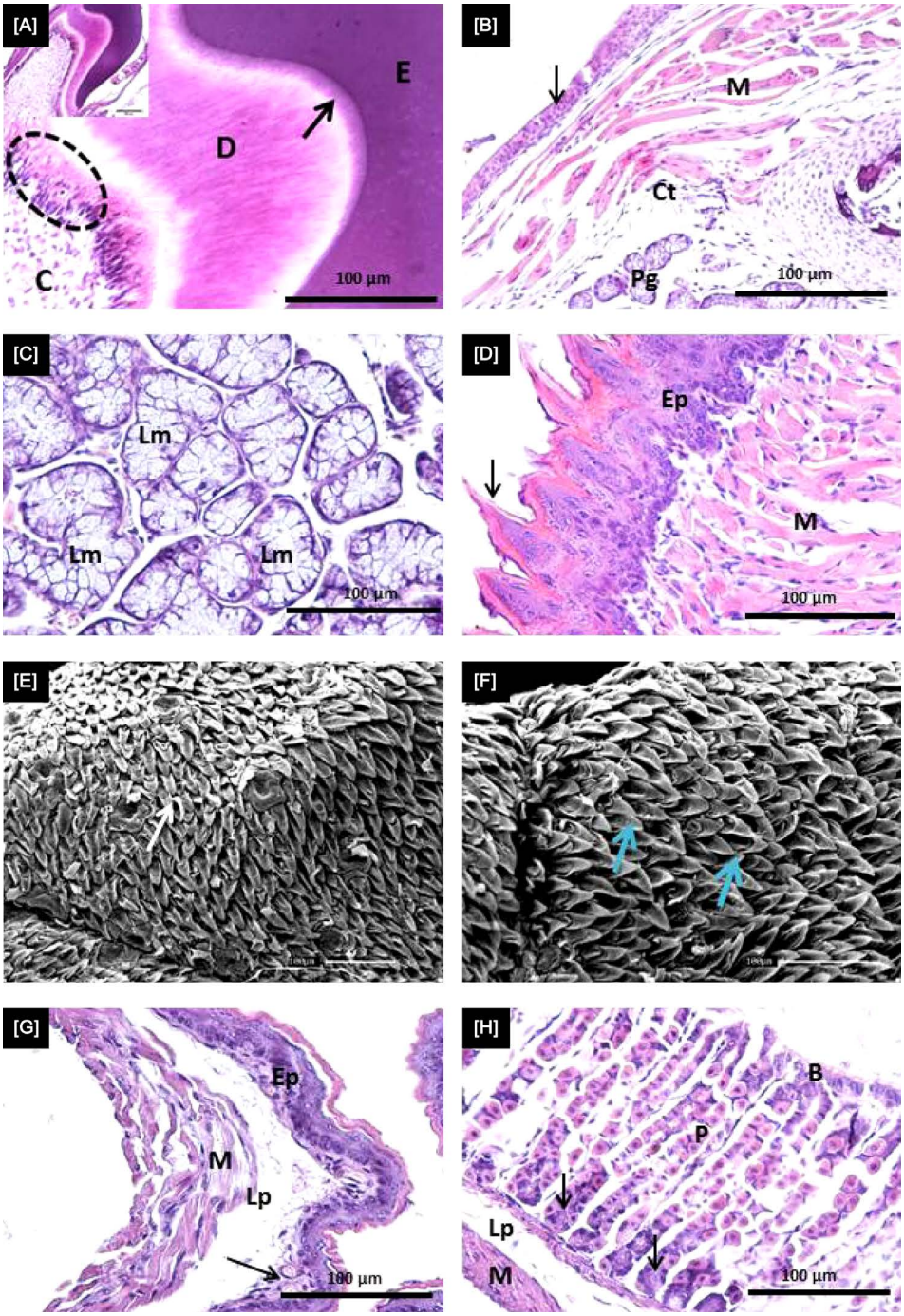

Figure 2. Histology and scanning electron microscopy of the digestive apparatus in *Oligoryzomys nigripes*. [A] Note the tooth displaying the enamel (E), dental crown (arrow), dentin (D), odontoblasts (circle), and cementum (C); [B] Hard palate showing the non-keratinized stratified squamous epithelium (arrow), muscle layer (M), dense unmodeled connective tissue (Ct), palatine glands (Pg), and periosteum (Pe); [C] Mucosal region of the salivary gland with mucous lobes (Lm); [D] Tip of the tongue displaying the filiform papillae (arrow), stratified squamous epithelium (Ep), and muscle layer (M); [E] and [F] Scanning electron micrograph of the tongue showing the papillae (arrows); [G] Esophagus showing the stratified squamous epithelium (Ep), lamina propria (Lp), esophageal glands (arrow), and muscle layer (M); [H] Stomach showing the basal (B), parietal (P), and mucous (arrows) cells. Observe also the lamina propria (Lp) and muscle (M).

stomach in which they are located (cardia, fundic, and pyloric). The lamina propria was composed of loose connective tissue. A layer of smooth muscle known as the muscularis mucosae separated the mucosa from the submucosa. Mucosal, parietal, zymogenic, and enteroendocrine cells were observed (**Figure 2[F]**).

Villi were observed in the small intestine (**Figure 3[A]** and **Figure 3[B]**). The epithelium of the villi was composed of absorptive cells and goblet cells (**Figure 3[A]**). The lamina propria was composed of loose connective tissue with a deeper muscularis mucosa, a submucosa that contained submucosal glands (**Figure 3[A]**), and a muscle layer. Crypts containing many goblet cells were observed in the large intestine. The lamina propria was also observed to be followed by a muscular layer composed of smooth muscle and skeletal muscle (**Figure 3[C]** and **Figure 3[D]**).

Hepatic lobules and centrilobular veins were observed in the liver. The hepatic lobes consisted of hepatocytes with sinusoid strands between them (**Figure 3[E]**). Islets (Langerhans islets), centroacinar cells, secretory cells, and acini surrounded by a basal lamina were observed in the pancreas (**Figure 3[E]**).

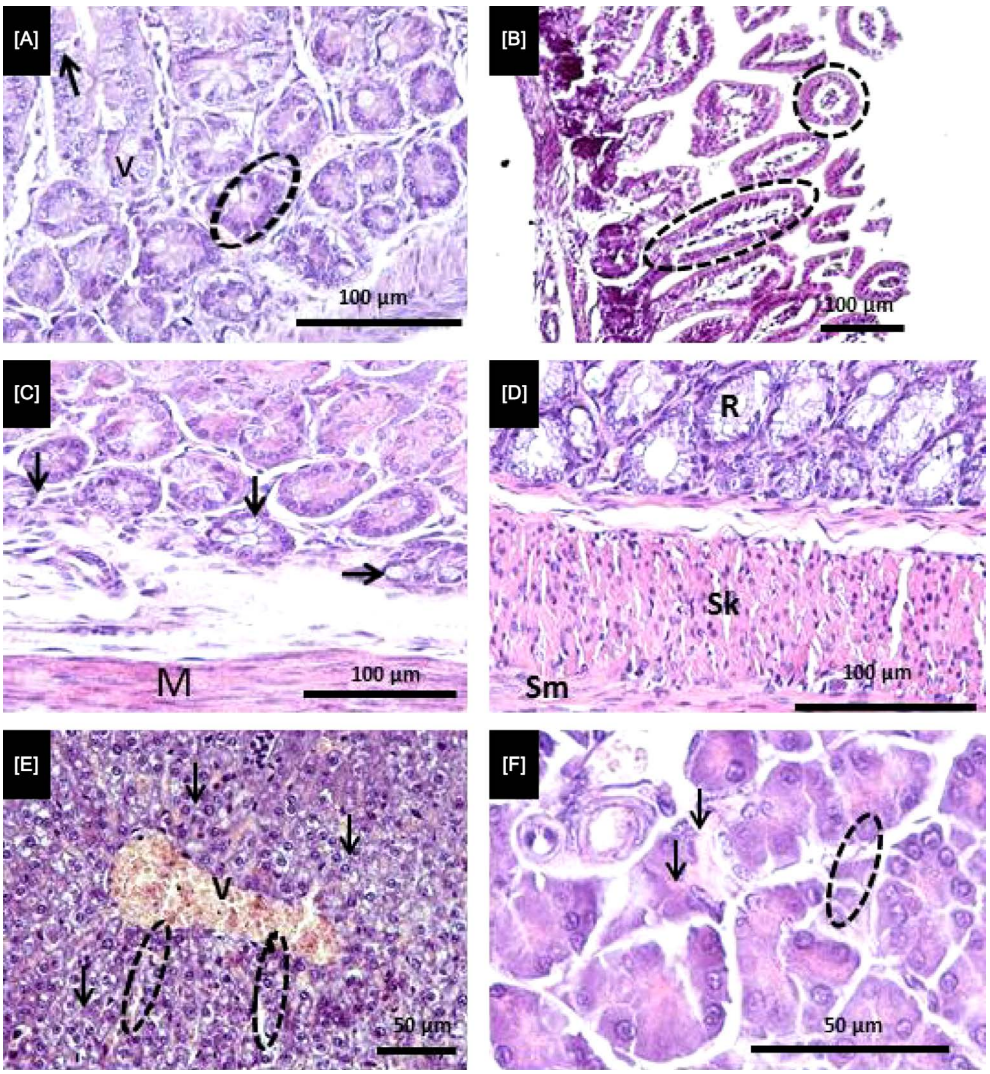

Figure 3. Histology of the organs of the digestive apparatus in *Oligoryzomys nigripes*. [A] Duodenum showing goblet cell (arrow), submucosal glands (circle), and villi (V); [B] Detail of the jejunum. Note the smaller villi with variable height (circles); [C] Cecum showing the muscular layer (M) and unbranched simple tubular glands; [D] Rectum showing a layer of smooth muscle (Sm), skeletal muscle (Sk), and a glandular connection to the rectum (R); [E] Liver showing a centrilobular vein (V), sinusoid strands (circles), and hepatocytes (arrows); [F] Detail of the acini and centroacinar cells (arrows) in the pancreas, as well as the secretory cells (circle).

4. Discussion

The digestive system is responsible for several important functions mainly related to digestion, yielding energy for the body and hormone production [26]. The oral cavity receives, chews, and grinds the food; thus, it initiates the process of digestion. The following oral cavity structures were observed in *O. nigripes*: a vestibule, which is the space between the cheeks and lips, teeth, and tongue. These same structures were observed for the guinea pig (*Cavia porcellus*) by Cooper and Schiller (1975) [22]. It is known that the *O. nigripes* are heterodont, having cutting teeth (two incisors, two canines, and four premolars on each side, distributed into pairs of incisors bones and the body of the mandible) that work to collect food and crush or grind. Particularly in rodents, the teeth are calcified structures adapted for feeding and mechanically reducing the size of the food [27].

These findings related to the type and numbers of teeth are different than those observed for the agouti (*Dasyprocta leporina*), which lacks canines and has molars [28]. Castro *et al.* (2007) [29] reported that in mice, the absence of premolars and a total of 16 teeth and incisors divided between the maxillary and mandibular molars were observed. Rabbits exhibited four maxillary and two mandibular incisors and five molariforms, diverging from the data collected in our research.

Similar to what is known for rabbits [30] the *O. nigripes* tongue is divided into a root, body, and tip. The tongue assists in the process of chewing and swallowing food [31]. Although filiform, fungiform, and vallate papillae were found on the tongue surface, no litter papillae were observed. By contrast, all four types of papillae have been reported for rabbits [30] and rats [32].

To complete digestion, salivary glands play an important role in the breakdown of molecules (chemical digestion) by producing and releasing mucus and enzymes. Here, we observed minor and major salivary glands (sublingual, submandibular, and parotid). Similar findings were reported for other rodent species [33], as well as for European hamsters [34] These glands are critically important for digestion because they produce substances that lubricate the food, help to digest starch, and assist with swallowing [31] [35].

The *O. nigripes* esophagus is a hollow, muscular tube that transports food from the oral cavity to the stomach [22]. It consists of the same tissue layers that can be found throughout the entire digestive tract, namely, submucosal, muscular, and adventitia layers [22] [35].

The stomach is a hollow cavity that stores the food until it can be digested by the digestive enzymes [26]. Anatomically, the stomach was divided into three regions: cardia, body, and fundus. The stomach displayed two curvatures, the greater and lesser curvatures, which have been previously described by Yapi *et al.* (2012) [36] and Scopin *et al.* (2011) [37] in rats. Cooper and Schiller (1975) [22] described the guinea pig stomach as having two curvatures, the greater and lesser curvatures, and four regions, a cardia, a fundus, a body, and a pylorus. In the cardia region, we found glands that produce mucus to protect the stomach wall and lysozyme, a substance responsible for destroying invading microorganisms. Glands found in the body and fundus regions produce mucus and acids that act on the food [8] [35].

The intestine is a hollow muscular tube with an irregular shape and is divided into two regions, a small and large intestine [31]. Here, we observed that the small intestine was subdivided into three regions (duodenum, jejunum, and ileum), which is consistent with the observations reported by Pérez *et al.* (2011) [38] in the chinchilla and by Bredo and Odo (2010) [8] in the Wistar rat. Similarly, the large intestine was subdivided into a cecum, colon, and rectum, which is also consistent with the observations reported by Pérez *et al.* (2011) [38] in the chinchilla and by Cooper and Schiller (1975) [22] in the guinea pig. The small intestine is responsible for completing the digestive process and absorbing nutrients. Intestinal villi were observed in the small intestine, and the villi epithelium consisted of absorptive and goblet cells, which is consistent with the observations reported by Konig (2007) [26], Junqueira, and Martins (1947) [32] and by Dyce *et al.* (2010) [31]. The large intestine did not have villi but crypts for absorbing water. The large intestine also contained several goblet cells that produced mucus. These large intestine features aid in feces production and fecal movement during defecation [26] [31]. The intestine terminates at the anus, a set of sphincter muscles that form the final portion of the digestive system and are required for defecation [26].

The two accessory glands are the liver and pancreas. The liver of *Oligoryzomys* consists of four lobes: a left lateral lobe, a left medial lobe, a quadrate lobe, and a right lateral lobe. By contrast, Oliveira *et al.* (2006) [39] studying the rock cavy (*Kerodon rupestris*) and Barbino *et al.* (2011) [40] studying two guinea pig species from suborder Hystricomorpha described an additional caudate lobe in the liver and the presence of a gallbladder. These two structures that were not observed in *Oryzomys* are a distinct characteristic and may be an adaptation

that differentiates these two rodent taxa. The pancreas is a gland involved in the production of hormones that aid intestinal digestion [26]. It is divided into small and irregular lobes, composed of acinar glands and Langerhans islets [32] [35].

5. Conclusion

In conclusion, the *O. nigripes* digestive system differs morphologically from the previously data described for other species, especially those species belonging to the order Rodentia because of a lack of molars and a gallbladder in *O. nigripes*. The findings presented in this study may benefit future studies focused on establishing specific and standardized diets for this species and its relatives in captivity. Furthermore, these findings may also benefit future studies focused on malnutrition and re-nutrition, which already use rodents as experimental models. The standardization of diets that meet the nutritional needs of a species helps to prevent developmental disorders that are caused by the absence or overabundance of specific nutritional elements. A more detailed study investigating the functional mechanisms of the liver in the absence of a gallbladder may provide important insights into the bile elimination pathways into the intestine. In addition, recently, many organs that compose the digestive system are used as sources of stem cells, and there are promising results for the use of these cells in regenerative medicine. Our anatomical and cellular findings regarding the different organs of this digestive system may contribute to the identification of new stem cell sources, including specificity and functional impairments that may be applicable for cellular therapy.

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