



Anatomical characterization of the digestive system of the pufferfish (*Chilomycterus spinosus spinosus*)

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ABSTRACT. Descriptive studies of the fish digestive system are fundamental because they provide information on the biology of the species. Thus, the objective of this study was to morphologically describe the digestive system of the pufferfish, *Chilomycterus spinosus spinosus*. For this, adult specimens of pufferfish (n = 10) of both sexes were used. The animals were fixed with 10% aqueous formaldehyde solution, dissected, analyzed descriptively and photographed. The results demonstrate that the pufferfish has a morphologically modified digestive system, which is adapted to the defense behavior. This species presents a pouch-shaped diverticulum, that is called abdominal pouch, which allows the expansion of the celomatic cavity and the temporary storage of food. Although it is used to store food, macroscopically the abdominal pouch does not show gastric folds. However, this absence is compensated by a small intestine containing innumerable villi.

Keywords: comparative anatomy; digestive tract; marine teleost; tetraodontiformes; morphology.

Received on September 18, 2018.

Accepted on April 12, 2019.

Introduction

Descriptive anatomical studies of marine animals are fundamental, since they provide information about the biology of the species and can be used as conservation strategies, such as to suggest the establishment of new protection areas, disease control and water quality. In addition, these studies promote subsidies for the elaboration of more adequate techniques of sustainable management of animals (Arkema, Abramson, & Dewsbury, 2006; Curtin & Prellezo, 2010), such as protection and recovery of endangered species.

Pufferfish is a marine teleost belonging to the Tetraodontiformes family, and it occurs in tropical and subtropical regions of the Atlantic, Indian and Pacific Oceans (Díaz & Gómez-López, 2003; Acero & Polanco, 2006). This fish presents great importance in the trophic balance of the marine aquatic ecosystems, being a secondary consumer and having as a food habit the intake of sessile invertebrates (Figueiredo & Menezes, 2000). The Pufferfish possess a highly modified and specialized digestive system that allows its body to inflate by ingesting water or air (Wainwright & Turingan, 1997; Fagundes, Rotundo, & Mari, 2016). This morphological adaptation takes part in a defense mechanism used by the species against predators (Brainerd, 1994; Wainwright & Turingan, 1997). When threatened, the animal swallows large amounts of water, directing it to a pouch located in the distal portion of the anterior intestine (Fagundes et al., 2016; McGee & Clark, 2018). This adaptation allows the expansion of the coelomic cavity, transforming the animal into a sphere with small spines on the body surface (Mittal & Banerjee, 1976). Although there are reports of this anatomical adaptation of the digestive system in the pufferfish (Brainerd, 1994; Fagundes et al., 2016), detailed studies that describe the morphology of the gastrointestinal tract of this species are scarce in the literature.

Given the importance of the digestive system morphological description and the scarcity of detailed information regarding the anatomical aspects of these organs, the aim of the present study was to anatomically describe the digestive system of the pufferfish, *Chilomycterus spinosus spinosus*.

Material and methods

This study was conducted in accordance with the ethical principles of the Brazilian College of Animal Experimentation (CONCEA). In addition, we submitted this work for the approval of the Ethical Committee on the Use of Animals (Ceua) of the *Centro Universitário de Rio Preto* (UNIRP), under protocol number 01/18. Adult specimens of pufferfish ($n = 10$) of both sexes were collected from the northern coast of São Paulo, in the city of Ubatuba. They presented average total body length of 24.53 ± 1.69 cm and weight of 136.5 ± 31.62 g. The animals used in the study were stored in the collection of the Laboratory of Zoology of the *Centro Universitário de Rio Preto* (UNIRP). The specimens were obtained from artisanal fisheries and stored in containers with a capacity of 10 liters. Subsequently, the animals were anesthetized with benzocaine (9 mg L^{-1}) and fixed with 10% aqueous formalin solution (subcutaneous, intramuscular and intracavitary injections), remaining in this condition until the study period. For the macroscopic evaluation of the digestive system, the specimens were opened by means of a longitudinal incision in the coronal plane of the abdomen ventral wall. Then, the abdominal cavity lateral wall was folded to perform the anatomical evaluation and the photographic images *in situ*. The segments of the digestive system were illustrated macroscopically through a photographic camera in different views according to the anatomical planes of delimitation. The anatomical boards of the species under study were made using a microcomputer, by means of photo digitalization in Adobe Photoshop 4.0.® The diagramming was carried out using Microsoft Power Point®.

Results

The digestive system of the studied species was divided into: oral cavity, anterior intestine (esophagus and abdominal pouch), middle intestine (intestine proper) and posterior intestine (rectum and anus). Macroscopically, the digestive tube showed to be elongated, covered by a clear peritoneum and with little pigmentation. The organs were disposed along the peritoneal cavity, occupying mainly the two caudal thirds, and they were united through the peritoneum. The mean total length of the digestive system was 16.26 ± 2.67 cm, and the mean total weight of 6.94 ± 2.21 g (Figure 1).

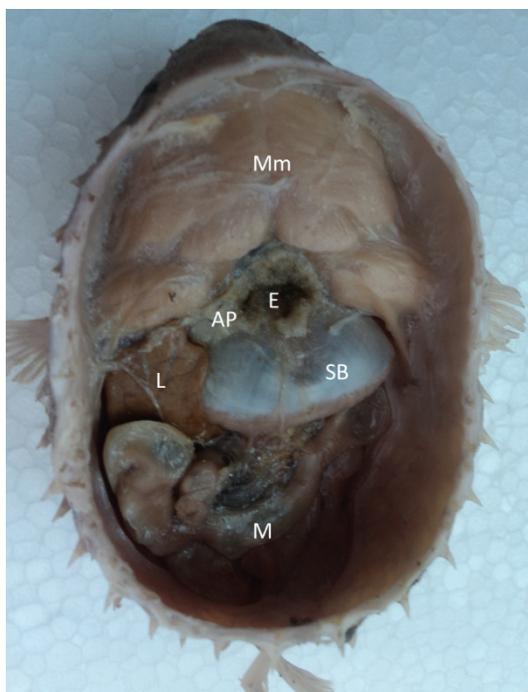


Figure 1. Ventral view evidenced the digestive system of the pufferfish, *Chilomycterus spinosus spinosus*. Longitudinal incision in the coronal plane near the ventral margin of the pectoral fin demonstrated the musculature (Mm); esophagus (E); part of the abdominal pouch (AP), liver (L), swim bladder (SB) and midgut (M). Bar scale = 1 cm.

Oral cavity

The mouth of the pufferfish is relatively small and terminal. The oral vestibule of this species shows fleshy lips and it is composed by several papillae. The maxilla of this species presents itself in the form of a

beak, containing teeth shaped as plaques that are the result of the fusion of several dentigerous units (Figure 2A).

Anterior intestine (esophagus and abdominal pouch)

The esophagus of the pufferfish is short, difficult to identify and arranged along the median sagittal plane. This organ contains thick walls, which are distensible, and it begins in the cephalic region, soon after the oral cavity, being continuous with the orobranchial cavity and ending in the abdominal pouch (Figure 2B). The distal portion of the anterior intestine of this species shows a sac-shaped diverticulum, called as abdominal pouch. When inflated with water, this anatomical structure expands, allowing the increase in volume of the coelomic cavity (Figure 2B, C, D, E). In addition, the abdominal pouch also displays the temporarily function of storing food before directing it to the midgut. In some dissected specimens, food items were found in this region. Macroscopically, no gastric folds were observed in the mucosa of the abdominal pouch, suggesting that this structure does not perform functions of chemical digestion of food. In addition, a well-developed pyloric sphincter was observed when the abdominal sac was separated from the midgut (Figure 2E).

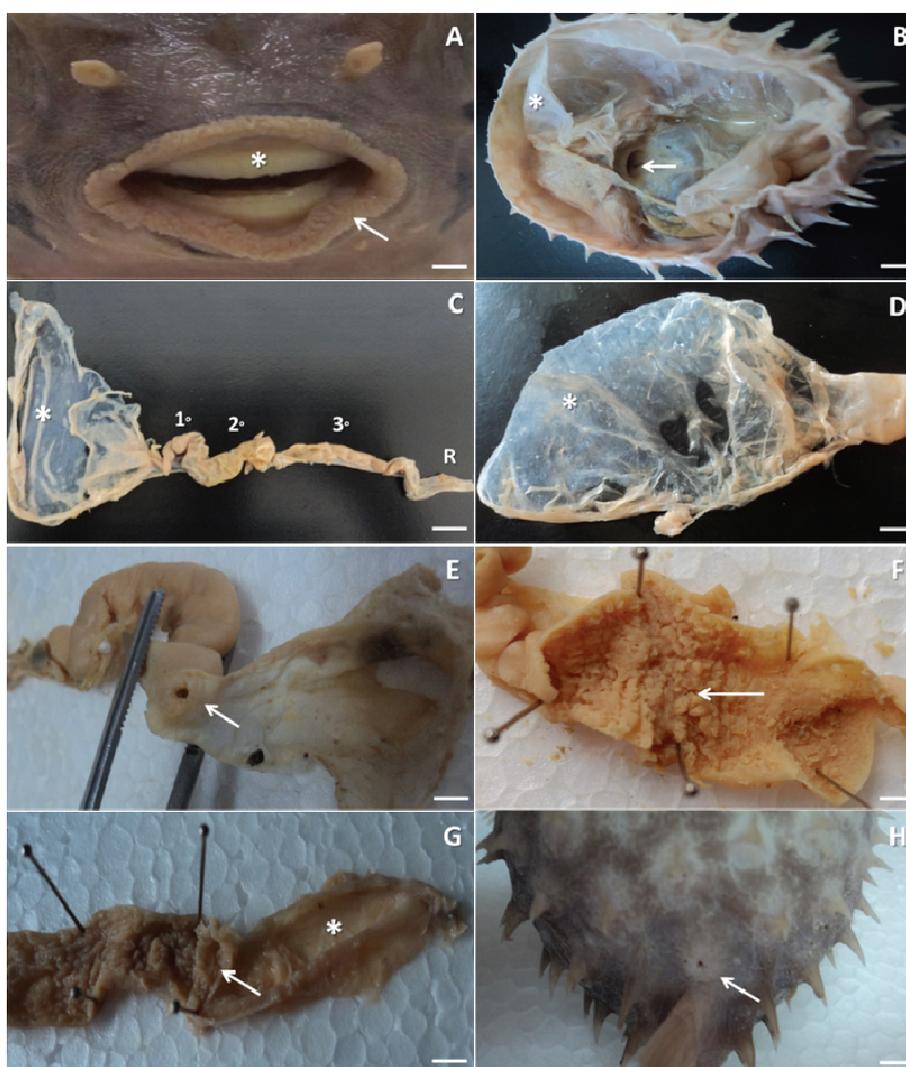


Figure 2. Photographs of the anatomical structures present in the digestive system of the pufferfish, *Chilomycterus spinosus spinosus*. (A) oral vestibule with the presence of papillae on the lips (arrow) and teeth in the form of plaques (asterisk). (B) ventral view of the digestive system, which evidenced the arrangement of the abdominal pouch in the peritoneal cavity (asterisk) and the distal portion of the esophagus (arrow). (C) segments of the digestive system, showing the abdominal pouch (asterisks), intestinal loops (1°, 2° and 3°) and rectum (r). (D) abdominal pouch showing the absence of gastric folds in the mucosa (asterisk). (E) details of the pyloric sphincter (arrow), responsible for separating the abdominal pouch from the midgut. (F) details of the first midgut loop demonstrating the large amount of villi (arrow). (G) ileorectal valve (arrow) that separates the last portion of the midgut from the rectum (asterisk). (H) ventral view showing the anal orifice (arrow). Scale bar = 1 cm.

Midgut

The midgut was present in the form of tubular organ, long and with convolutions. It was located in the craniocaudal region and displayed three segments, called intestinal loops (Figure 1). The first intestinal loop was situated in the right antimere, inferiorly to the right hepatic lobe and after the esophageal sphincter. A macroscopic analysis of the mucosa of this first intestinal segmentation demonstrated a large number of villi or intestinal folds (Figure 2F). No evagination of the intestinal walls, called pyloric caecum, was seen in the first intestinal loop. The second intestinal loop started after the first flexure, in the right antimere, and extended over the underside of the gas vesicle toward the left antimere. The third and last intestinal loop began after the second flexure, extending in the caudal direction, above the lateral face of the left antimere. This intestinal loop was rectilinear and short.

Posterior intestine (rectum and anus)

The rectum corresponded to the last intestinal portion extending from the ileorectal valve to the anal orifice. This intestinal portion was linear, short and devoid of intestinal folds in the mucosa (Figure 2G). Furthermore, there was a change in the diameter and pattern of convolutions in the rectum. The opening of the anus was located caudoventrally between the pelvic fins and the genital ostium (Figure 2H).

Accessory digestive organ (liver and gallbladder)

Macroscopically, the liver presented a homogeneous structure, with a reddish-brown coloration, due to its rich vascularization. The organ was situated in the right sagittal plane, in the latero-ventral region, with the presence of the gallbladder, and with the bile duct opening into the first intestinal loop. During the dissection, no pancreas was found in the animals (Figure 1).

Discussion

The anatomical and functional variations of the digestive system of teleosts are often related to diet (Pavlov & Kasumyan, 2002; Wilson & Castro, 2011). However, in some species, these alterations may also be related to the defensive behavior, as in the case of the pufferfish (Brainerd, 1994; Wainwright, Turingan, & Brainerd, 1995; McGee & Clark, 2018). Morphological variations usually occur along phylogenetic evolution, providing certain adaptations to the animals (Rabosky, Santini, Eastman, Smith, Sidlauskas, Chang, & Alfaro, 2013). In this study, we observed that the pufferfish has a morphologically modified digestive system, which is adapted for defensive behaviors. This species has a pouch-shaped diverticulum, located in the anterior intestine, that allows the expansion of the coelomic cavity and the temporary storage of food.

The mouth of the species studied is small and tubular, similar to that observed in herbivorous and bentophagous teleosts (Pavlov & Kasumyan, 2002; Salvador-Jr, Salvador, & Santos, 2009). The lips are fleshy and composed of several papillae, which are possibly destined to the foraging of benthic invertebrates (Rodrigues & Menin, 2005). The maxilla is shaped like a beak, and contains several dentigerous units fused in the form of plates, allowing the animal to consume prey with shells (gastropods and bivalves) and carapace (crustaceans) (Chen & Yang, 2005; Chi-Espínola & Vega-Cendejas, 2013).

The esophagus is morphologically designed for the passage of food, since it presented short, linear and thick walls. These anatomical characteristics corroborate with the ones found in *Sphoeroides testudineus* (Fagundes et al., 2016), which is a species from the same family of the *Chilomycterus spinosus spinosus*. The anterior intestine presented a pouch-shaped diverticulum that allows the animal to inflate through the expansion of the coelomic cavity. This morphological adaptation of the digestive system is a defense mechanism used by the species against predators (Brainerd, 1994; Wainwright & Turingan, 1997). According to Myer (1989), pufferfish are slow swimming fish. Thus, to protect itself, the animal promotes oral cavity depression (Wainwright et al., 1995), directing large amounts of water towards the abdominal pouch (Fagundes et al., 2016; McGee & Clark, 2018). According to Wilson & Castro (2011), the water retention in this compartment and, later, the expansion of the celomatic cavity, is possible due to the presence of a well-developed esophageal sphincter in the pufferfish species. The abdominal pouch is also a storage place, because in some animals, one can find food in this compartment. According to Zhao, Song and Wang (2010), in *Takifugu obscurus*, the abdominal pouch, besides causing expansion, also has the function of accommodating the food. The morphological adaptations observed in this structure of the

pufferfish were also found in other species, such as *Takifugu obscurus* (Zhao et al., 2010) and *Sphoeroides testudineus* (Fagundes et al., 2016).

The spatial arrangement and length of intestinal loops of teleosts are usually compatible with eating habits, body morphology, among other aspects (Baldisserotto, 2002; Wilson & Castro, 2011). However, the villi present in the intestinal mucosa are related to the physiological mechanisms of digestion and nutrients absorption (Wilson & Castro, 2011). Both relationships above can be evidenced in the species under study. As the pufferfish does not have a stomach with digestive functions, the midgut becomes responsible for the physiological mechanisms of digestion and nutrients absorption (Wilson & Castro, 2011). Thus, the intestinal loops are directly linked to the adaptations of the digestive system (García-Gasca, Galaviz, Gutiérrez, & García-Ortega, 2006) and the villi for nutrient uptake (Fagundes et al., 2016). The rectum of the pufferfish corresponded to the last intestinal portion, which extends from the ileorectal valve to the anal orifice. This portion was linear, short and devoid of intestinal folds. According to Wilson & Castro (2011), the rectum of teleosts can be differentiated from the midgut by the decreasing vascularity and villi. In addition, the midgut is separated from the rectum in some species of fish by a small constriction, in which the ileorectal valve is located.

Conclusion

In conclusion, the results obtained in this study demonstrate that the pufferfish displays a morphologically modified digestive system, which is adapted to the defense behavior. This species presents a bag-shaped diverticulum, known as abdominal pouch, which allows the expansion of the coelomic cavity. In addition, this compartment has the function of temporarily storing food before directing it to the midgut. Although it is used to provisionally store food remains, the abdominal pouch does not display gastric folds. However, this absence is compensated by a midgut containing innumerable villi.

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