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Oropharyngeal morphological aspects of *Arapaima gigas* (Schinz, 1822)

Luana Félix de Melo[1]
Julia Bastos de Aquino[1]
Keila Silva Pinto[2]
Jerônimo Vieira Dantas Filho[2]
Maria Angelica Miglino[1]
Sandro de Vargas Schons[2]*
Rose Eli Grassi Rici[1]

[1]Universidade de São Paulo, Programa de Pós-Graduação em Anatomia de Animais Domésticos e Silvestres, Escola de Medicina Veterinária e Zootecnia – São Paulo (SP), Brazil.

[2]Universidade Federal de Rondônia, Programa de Pós-Graduação em Ciências Ambientais –Rolim de Moura (RO), Brazil.

*Corresponding author: Sandro de Vargas Schons, Universidade Federal de Rondônia, Programa de Pós-Graduação em Ciências Ambientais, Av. Norte Sul, 7300, Nova Morada, CEP: 76940-000 – Rolim de Moura (RO), Brazil.
E-mail: sandroschons@unir.br

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ABSTRACT

The study of the functional anatomy of the digestive system of fish, in particular the oropharyngeal cavity, is of great importance because it allows inferences about the feeding habit, mechanisms of capture, selection, and processing of food carried out by different species. Thus, the aim of this study was to describe the anatomical adaptations of the oropharyngeal cavity of the pirarucu (*Arapaima gigas* Schinz, 1822) using scanning electron microscopy (SEM) techniques. The oropharyngeal cavity of six specimens of pirarucu was collected in juvenile phase, from Aquaculture Research Center at the Universidade Federal de Rondônia (UNIR), created for commercial purposes. The anatomical pieces were fixed in 10% buffered formalin and processed for SEM analysis. Anatomically, the oropharyngeal cavity of the pirarucu is composed of five pairs of branchial arches, apical portion of the tongue, floor of the tongue, lower pharyngeal area, and upper pharyngeal plate. In SEM, we observed that the mucosa of the apex of the tongue and the upper pharyngeal roof have a smooth texture and are covered by squamous cells with numerous small openings scattered over the surface. The portions of the floor of the tongue and the lower pharyngeal area, on the other hand, have adaptations in the form of a projectile and numerous sensory papillae, giving a rough texture to the region. Thus, the oropharyngeal cavity of pirarucu is adapted for the capture, apprehension, and swallowing of its prey, with signs of carnivory.

Keywords: fish native to the Amazon; pharynx; pirarucu; scanning electron microscopy; tongue.

Aspectos morfológicos orofaríngicos de *Arapaima gigas* (Schinz, 1822)

RESUMO

O estudo da anatomia funcional do aparelho digestivo dos peixes, em particular da cavidade orofaríngea, é de grande importância, pois permite inferências sobre o hábito alimentar, mecanismos de captura, seleção e processamento de alimentos realizados por diferentes espécies. Assim, o objetivo do estudo foi descrever as adaptações anatômicas da cavidade orofaríngea do pirarucu (*Arapaima gigas* Schinz, 1822) por meio de técnicas de microscopia de varredura eletrônica (MEV). A cavidade orofaríngea de seis espécimes de pirarucu foi coletada na fase juvenil, no Centro de Pesquisa em Aquicultura da Universidade Federal de Rondônia (UNIR), criado para fins comerciais. As peças anatômicas foram fixadas em formalina tamponada a 10% e processadas para análise em MEV. Anatomicamente, a cavidade orofaríngea do pirarucu é composta por cinco pares de arcos branquiais, porção apical da língua, assoalho da língua, região faríngea inferior e placa faríngea superior. Na MEV, a pesquisa observou que a mucosa do ápice da língua e o teto faríngeo superior possuem textura lisa e são recobertos por células escamosas com numerosas pequenas aberturas espalhadas pela superfície. As porções do assoalho da língua e da região faríngea inferior, por outro lado, apresentam adaptações em forma de projétil e numerosas papilas sensoriais, conferindo textura áspera à região. Assim, a cavidade orofaríngea do pirarucu é adaptada para a captura, a apreensão e a deglutição de sua presa, com sinais de carnivoria.

Palavras-chave: faringe; língua; microscopia eletrônica de varredura; peixes nativos da Amazônia; pirarucu.

INTRODUCTION

Pirarucu (*Arapaima gigas* Schinz, 1822) is a bony fish, with a relatively elongated body and a dorsoventrally flattened head, belonging to the Arapaimidae family, inserted in order of Osteoglossiformes and class of Actinopterygii (ray-finned fish). This species is described by Schinz in 1822, whose lineage has its origin before the drift of Africa and South America, which occurred in the Jurassic period (Silva and Duncan, 2016). It is among the largest species of primary freshwater fish, being endemic to the Amazon region (Lundberg and Chernoff, 1992; Chávez, 2002). Due to this characteristic, this fish aroused great interest for fishing exploitation, becoming the

first fishing economic base of considerable scale in the Amazon region, in part, due to belief in the overabundance of its stocks (Goulding, 1983; Baldisserotto et al., 2020).

Pirarucu is a fish with a subcylindrical body, slightly long, dorsoventrally depressed and flattened head (Santos et al., 2007), and has a large and oblique terminal mouth, with a characteristic of carnivorous fish, thus facilitating the capture of prey (Sinha and Moitra, 1975; Bone and Marshall, 1982; Baldisserotto et al., 2020). The digestive tract morphology has been described in several species of fish (Tibbetts, 1997; Fishelson et al., 2011; Faccioli et al., 2014). However, fish from the Arapaimidae family eat in a variety of ways, which lead to very specialized eating behavior, indicating wide variations in both morphology and functions, which portrays the high diversity of teleosts and their different positions in the trophic chain (Bone and Marshall, 1982; Baldisserotto et al., 2020).

Pirarucu (*A. gigas*) is a species native to the Amazon region of interest to fish farming in Rondônia state, Brazil. In a natural environment, it can reach up to 200 kg of total weight, and its high economic importance has determined the growing interest in its commercial exploitation by fish farmers (Oliveira et al., 2014). Rondônia state is the largest producer of native fish in Brazil, corresponding to a total of 68,800 tons of fish produced in 2019 (Peixe BR, 2020), and has pirarucu as one of the most cultivated fish and, together with tambaqui (*Colossoma macropomum*), represent about 85% of the fish grown in Rondônia state (Meante and Dória, 2017).

Pirarucu is an important fish for fisheries and aquaculture in Western Amazon, but there is currently an embargo on its production, some environmental authorities have considered the pirarucu an invasive species in microenvironments and others consider it a sensitive species to extinction (Pinto et al., 2022). In addition, the Amazonian pirarucu fish farming still lacks mastery of technologies for formulating feed for carnivores, hence the importance of studying food structures and food capture. The study of the functional anatomy of the digestive system of fish, in particular the oropharyngeal cavity, is of great importance, mainly because it allows inferences about the eating habit, mechanisms of capture, selection, and processing of food carried out by different species (Khanna, 1962; Prejs, 1981; Rodrigues; Menin, 2006; Moritz and Lalèyè, 2018).

In view of the exposed assumptions, the aim of this study was to describe the morphological of the oropharyngeal cavity and the pirarucu tongue, as well as morphological adaptations, using macroscopic and scanning electron microscopy (SEM) analyses.

MATERIAL AND METHODS

The project was submitted and approved by the Research Ethics Committee on the use of animals of the Universidade Federal de Rondônia (UNIR), with protocol no. 043/2019.

For this purpose, samples were collected from the oropharyngeal cavity of six specimens of pirarucu (*A. gigas*). The fish were juveniles weighing about 120.0±5.0 g. The fishes sampled belonged to the Center for Research in Aquaculture Carlos Eduardo Matiazze, at the UNIR, Presidente Médici, Câmpus Universitário, RO, Brazil. The juveniles were created in an intensive system of external ponds and fed with pelleted commercial feed. The fish were caught by artisanal fishing, and after removal of the viscera, the oropharyngeal cavity and the tongue were dried and washed in running water and then fixed in 10% buffered formalin.

To observe macroscopically the different structures, the organs were dissected and exposed on a dark background. Later, they were photographed with the aid of a professional photographic camera — NIKON 7557 Coolpix P950 Digital Camera. After complete fixation, these were processed for SEM. The resolutions established for observation in SEM were 100× and 800×, according to the standardization of observation suggested by Pinto et al. (2022).

The tissue fragments were dehydrated in an increasing series of alcohols in concentrations from 70 to 100%, dried in a LEICA EM CPD 300 critical point apparatus, glued with carbon glue on metallic aluminum bases (stub) and metallized ("sputting") with gold in the EMITECH K550 metallizer, and analyzed and documented in an SEM LEO 435VP (Melo et al., 2019) at the Advanced Center for Diagnostic Imaging — Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (CADI/FMVZ/USP).

RESULTS

Macroscopically, we observed that the oropharyngeal cavity consists of five pairs of branchial arches, apical portion of the tongue not adhered, floor of the tongue, lower pharyngeal area, and upper pharyngeal plate. In the frontal view of the oropharyngeal cavity, we observed that the five pairs of branchial arches have different sizes. In the last arch at the top, they have a pair of pharyngeal plates. The branchial arches are formed by two branches: the lower, longer, and the upper, shorter.

The apical area of the tongue presented a rounded shape, free base, and light gray color, different from the floor of the tongue that is stuck and yellowish (Figure 1A). In SEM, we observed that the apex surface mucosa, apparently smooth, was covered by squamous cells and numerous small openings scattered over the surface (Figure 1B) and the floor of the tongue of *A. gigas* (Figure 1C). However, in SEM, we observed at the basal portion/at the base of the tongue, a rough texture and countless projectile-shaped denticles distributed through the mucosa (Figure 1D), being more pointed at the margins of the floor of the tongue compared to the center, suggesting that the crushing of the food occurs in the center and the possible wear of its apex (Figure 1D).

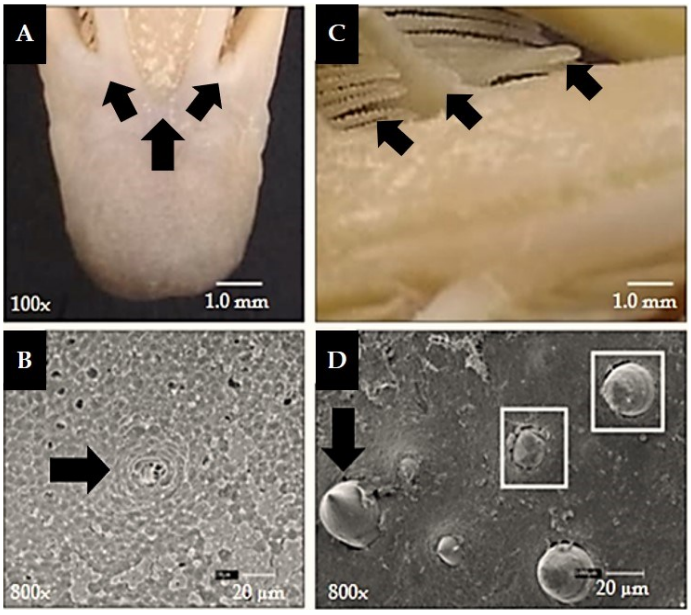

Figure 1. Photomicrograph of images of the apical region and floor of the tongue of *A. gigas*: (A) whole tongue, (B) the mucosa with smooth scaly texture, with the distribution of small openings (arrows), (C) the floor of the tongue, (D) photomicrograph of the dispersion of the denticles, showing wear (squares) and the sharp projections of the denticles that are marginalized.

The lower pharyngeal area shows a slight concavity delimiting the esophagus and the floor of the tongue (Figure 2A), surrounded by the branchial traces, described as important in feeding, and it can direct the food during swallowing. In SEM, we observed that the mucosa was formed by typical scaling cells and the presence of projections with a projectile shape (Figures 2B and 2C), leaving the mucosa texture rough, in addition to the presence of numerous sensory papillae (Figures 2D and 2E).

The pharyngeal plate located on the upper pharyngeal roof has a cylindrical shape, with its convex contact surface, previously limited by its oral cavity, the base being directed toward the esophagus. We observed on its lateral surface, short, rigid, and arrow-shaped branchial gills, which play an aid role in feeding (Figure 2F). In the most rostral portion of the plate, scattered, small, and pointed spicules are observed on its surface, while in the most caudal portion of the plate, the surface appears smooth. Thus, we can infer that the functions of the plate include the processes of food apprehension, crushing, maceration, and rapid clearance of the pharynx.

In SEM analysis, we observed that the mucosa has a smooth texture in its caudal and rough portion in its cranial portion, small openings, and scattered and undeveloped papillae, suggesting that the rough region has the function of kneading while the smooth one facilitates the entry of food by sliding it into the esophagus (Figures 2G and 2H).

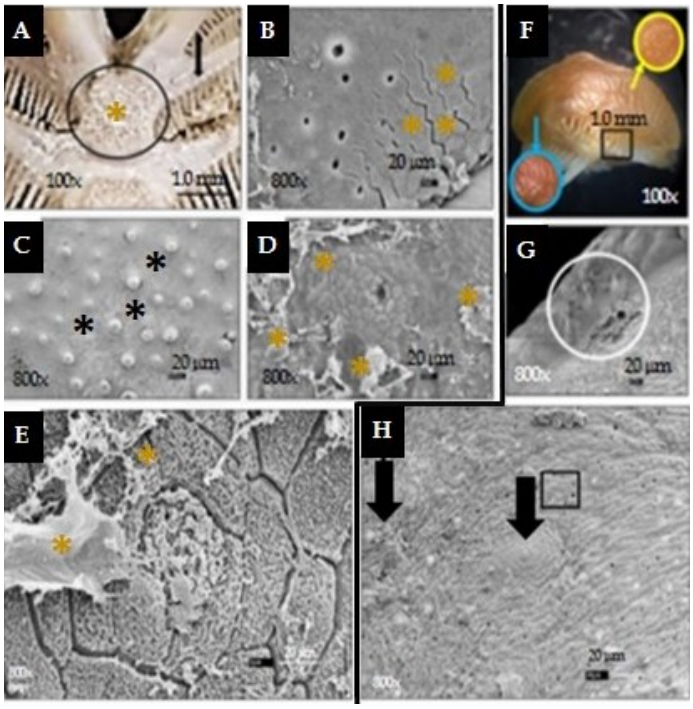

Figure 2. (A) The lower pharyngeal area of *A. gigas* (asterisk encircled), with the branchial arches around it (arrow). (B and C) The rough mucosa with scaling and projectile-shaped denticles (asterisks). (D and E) The dispersion of the sensory papillae. (F) Photograph of the upper pharyngeal plate of *A. gigas* highlighting the brachial traces by the square on its side, short and poorly distributed spicules in the cranial portion in yellow, and smooth surface in its most caudal portion in blue. (G) Distribution of the spicules (circle). (H) The dispersion of the papillae (arrows) and small openings as pores (square).

DISCUSSION

The anatomical information about the oropharyngeal cavity of the pirarucu corroborates the descriptions of Menin and Mimura (1991). In this study, we observed that the pharyngeal dental appliance is located in the caudal portion of the pharyngeal cavity, being composed of the lower pharyngeal area and the upper pharyngeal plate pair, located on the pharyngeal floor and roof, similar to that observed in *Chagunius chagunio* by Chawla and Tyor (2014).

Concerning the superficial mucosa of the apex, apparently smooth, which was covered by squamous cells and innumerable small openings spread over the surface, carnivorous species generally have smooth mucosa, which facilitates the passage of prey and provides rapid clearance of the cavity, corroborating the findings in *Hoplias malabaricus* by Menin and Mimura (1991), and in *Salminus brasiliensis* (Chawla and Tyor, 2014). Although the tongue does not aid in swallowing, some studies have reported the occurrence of taste buds, attributing it as an essential structure in the taste system (Bakary, 2012; Malabarba and

Malabarba, 2020), However, the degree to which taste receptors are developed morphologically in the tongue of different fish species is related to the environment and, in particular, to the variety of foods (Meyer-Rochow, 1981).

The floor of the tongue of *A. gigas* (Figure 1C), as described in other individuals of the Teleostei class (Kent, 1954; Nagar and Khan, 1958; Khanna, 1962; Hassan, 2013; Rosa et al., 2020), is thin and fixed in the oral cavity, being incapable of wide movements, which allows us to infer that it does not help in swallowing food.

Concerning the lower pharyngeal region, showing a slight concavity delimiting the esophagus and the floor of the tongue, it is described as important in feeding, being able to direct the food during swallowing (Peretti and Andrian, 2008; Bakary, 2012; Rønnestad et al., 2013).

Rodrigues and Menin (2006) and Rosa et al. (2020) described that, in the oral cavity of *Pseudoplatystoma corruscans*, the lower right and left pharyngeal areas were triangular in shape and separated by a narrow region of mucosa in the sagittal plane. In our findings, we observed a small region supposed to fit the upper pharyngeal plate.

Regarding the pirarucu mucosa, it was formed by typical scaling cells and the presence of projectile-shaped projections. Similar descriptions were found in the scientific literature for *H. malabaricus* (Menin and Mimura, 1991) and *S. brasiliensis* (Chawla and Tyor, 2014). Although the morphological findings are similar to the findings described by other authors such as Al-Hussaini and Kholy (1953), Nikolsky (1963), Hassan (2013), Rosa et al. (2020), and Pastana et al. (2020), in each species observed, the plaque performed only one specific function according to its feeding, differing in our specimen, where, through its adaptations and abrasive feeding, we can infer that it performs all the described functions.

The morphology of the oropharyngeal area of the *A. gigas* digestive tract indicated carnivorous eating habits (Moritz and Lalèyè, 2018). The oropharyngeal cavity is adapted for the capture, apprehension, crushing, maceration of its prey, and rapid swallowing to clear the oropharyngeal cavity (Moritz and Lalèyè, 2018; Baldisserotto et al., 2020).

CONCLUSION

Anatomically, the oropharyngeal cavity of the pirarucu is composed of five pairs of branchial arches, apical portion of the tongue, floor of the tongue, lower pharyngeal area, and upper pharyngeal plate. In SEM, we observed that the mucosa of the apex of the tongue and the upper pharyngeal roof have a smooth texture and are covered by squamous cells with numerous small openings scattered over the surface. The portions of the floor of the tongue and the lower pharyngeal area, on the other hand, have adaptations in the form of a projectile and numerous sensory papillae, giving a rough texture to the region. Thus, the oropharyngeal cavity of *A. gigas* is adapted for the capture, apprehension, and swallowing of its prey, with signs of carnivory.

CONFLICT OF INTERESTS

Nothing to declare.

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AUTHORS' CONTRIBUTIONS

Melo, L.F.: Investigation, Methodology, Writing — original draft. Aquino, J.B.: Formal Analysis, Funding acquisition, Investigation. Pinto, K.S.: Project administration, Conceptualization, Investigation. Dantas Filho, J.V.: Formal Analysis, Writing — original draft, Investigation. Miglino, M.A.: Visualization, Formal Analysis, Investigation. Schons, S.V.: Supervision, Formal Analysis, Visualization. Rici, R.E.G., Investigation, Visualization, Conceptualization — review & editing.

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