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Gross anatomy and histology of the intestine of *Aspidoras gabrieli* indicate adaptations for air-breathing behavior

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Resumo: Nós descrevemos características morfológicas do trato digestivo do calictídeo endêmico *Aspidoras gabrieli* na Serra dos Carajás, sudeste da Amazônia. A espécie exibe um trato digestivo tubular dividido em intestino digestivo e intestino aéreo, separados por uma leve constrição. O intestino aéreo é mais longo, mas com paredes mais finas do que o intestino digestivo. Microscopicamente, o intestino aéreo apresenta características de tecidos respiratórios, como células pseudoestratificadas com células achatadas na mucosa, lâmina própria encurtada, ausência de dobras e maior número de células caliciformes. Essas características podem permitir que *A. gabrieli* respire no ar e, assim, habite ambientes hipóxicos.

Palavras chave:. Callichthyidae, morfologia, ictiologia, peixes

Abstract: We describe morphological features of the digestive tract of the endemic callichthyid *Aspidoras gabrieli* in Serra dos Carajás, southeastern Amazon. The species displays a tubular digestive tract divided into the digestive and aerial gut, separated by a mild constriction. The aerial gut is longer, but with thinner walls than the digestive gut. Microscopically, the aerial gut exhibits traits of respiratory tissues, such as pseudostratified cells with flattened cells in the mucosa, shortened lamina propria, lack of folds, and higher number of goblet cells. These traits may allow *A. gabrieli* to air-breath and thus inhabit hypoxic environments.

Key words:. Callichthyidae, morphology, ichthyology, fishes

Aquatic environments with oxygen depletion conditions are common in the Neotropical region, limiting the distribution of fish that do not display morphological and behavioral adaptations to survive under these conditions (Lowe-McConnell 1999). Loricarioidea, the most species-rich group of Neotropical catfishes, developed a peculiar solution to the oxygen depletion problem. Gee (1976) suggested that most of the species in this superfamily exhibit modifications in the digestive system that allow breathing atmospheric air. Several authors have studied this adaptation in Loricarioidea (*e.g.*, Gee & Graham 1978; Armbruster 1998), but it is unknown how widespread it is even in well-studied groups, such as Callichthyidae.

Callichthyidae is one of the largest families within Loricarioidea and among Neotropical catfishes (Reis 1998). Callichthyids display small to medium size and broad geographic distribution, from the Pacific coastal streams of Panama to the streams of the Río de la Plata basin (Reis 1998). Callichthyids inhabit different environments, from large rivers with fast water flow and highly oxygenated waters to small-sized streams and ponds with acid waters (Britto 2014), which favor numerous adaptations to aerial breath.

Corydoras Lacépède 1803 and *Hoplosternum* Gill, 1858 exhibit thin and highly vascularized walls in the posterior portion of the intestinal tract, which facilitate using atmospheric air to breathe and hydrostatic equilibrium (Gee 1976; Gee & Graham 1978). Nonetheless, the information is still lacking for six of the eight callichthyid genera. This study describes the gross and microscopic morphology of the intestine of *Aspidoras gabrieli*, an endemic species of callichthyid from Serra dos Carajás (Tocantins River basin).

Specimens were sampled in the Buritizal stream (S 6º 04' 58", W 50º 08' 05"), a second-order stream in the lower Tocantins River basin within the Carajás National Forest. The stream shows low turbidity and pristine riparian vegetation. The streambed has large rocks and decomposing leaves and trunks, and it is very heterogeneous, encompassing both riffles and pool areas. The stream exhibits flooded areas into the forest, up to 40 cm depth, on a muddy substrate and turbid water during the rainy season. *A. gabrieli* occurs in both flooded and riffle environments.

Individuals were collected with seine nets in September 2005 (rainy season) in flooded areas with an average depth of 30 cm, black waters, and oxygen saturation of 20%. Fish were anesthetized with Eugenol, fixed in 10% formalin, and transferred to ethanol 70°GL. In the laboratory, 60 specimens of *A. gabrieli* were measured to standard length (L_S) and dissected following Ferreira & Britto (2018). The total length of the intestinal tract, length of the digestive gut, and length of the aerial gut were also measured with 0.1 mm precision calipers under a stereoscopic microscope. Histological sections of the first and last third of the gut of five specimens were prepared following routine histological techniques with infiltration and embedding in resin, stained with hematoxylin-eosin, and using Entellan as mounting medium. Histological measurements were performed on the digital images of the histological slides using an image editor software (Adobe Photoshop CS 5.1) based on known distances. Voucher specimens are deposited at the ichthyological collection of the National Museum of Brazil (UFRJ), voucher number MNRJ 51724.

Analyzed specimens ranged from 19 to 34 mm (26 ± 3) of Ls. The intestinal coefficient ranged from 0.37 to 0.75 mm (0,58 ± 0,08). *A. gabrieli* displays a tubular-shaped intestine with a larger circumference and intestinal walls thinner in the posterior portion of the intestinal tract than in the anterior portion (Fig. 1). The digestive gut is linked to the pyloric region of the stomach by the first intestinal loop, which comprises a descending curve that circumvents the right flank and *fundus* of the stomach in the cranial direction. The proximal and middle segments form the first intestinal loop. The proximal segment is linked to the pyloric region of the stomach and has an intestinal wall thicker than the other segments. This segment extends along its entire anterior portion, anteriorly contacting the liver and dorsally contacting the esophagus. A mild constriction marks the beginning of the middle segment, which exhibits a uniform diameter and thin intestinal walls. Another mild constriction marks the transition to the aerial gut, which presents thin and translucent intestinal walls. This portion of the gut represents more the 50% of the total length of the intestinal tract.

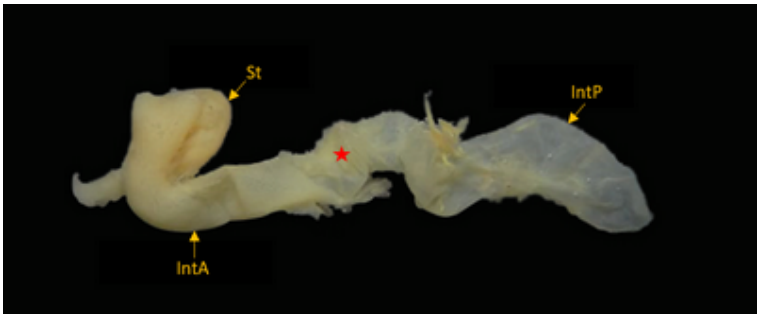

Figure 1: Extended digestive tube of *Aspidoras gabrieli.* St: stomach; IntP: posterior intestine (aerial gut); IntA: anterior intestine (digestive gut); Star mark: approximate change point separating anterior and posterior intestine.

Histological characterization demonstrates morphological differences in the digestive and aerial gut of *A. gabrieli* (Fig. 2). The tunica mucosa of the digestive gut shows layers of enterocytes, goblet cells, muscles, villi, and immune system cells in the lamina propria. The mucosa of the aerial gut presents pseudostratified epithelium with flattened cells and goblet cells. The tunica muscularis in the aerial gut was thicker (10.8 ± 0.8 µm) than in the digestive gut (7.9 ± 0.5 µm), while the epithelial layer was thinner (7.9 ± 0.2 *vs.* 9.2 ± 0.5 µm).

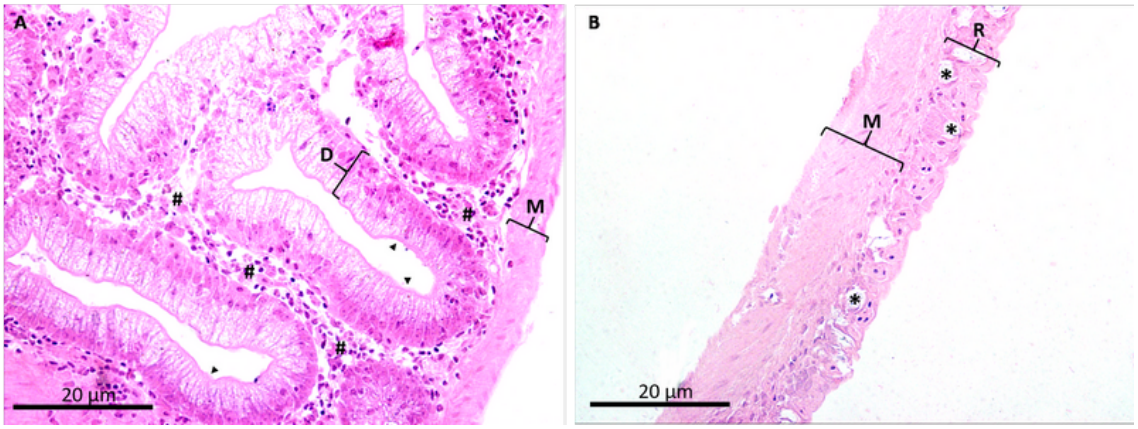

Figure 2: Digestive (A) and aerial (B) guts of *Aspidoras gabrieli*. In A, the digestive gut is lined by simple columnar epithelial cells layer (D) with the nucleus in the basal portion of these cells. The apical domain contains digitiform projections characterizing a brush border (arrowheads) responsible to absorb nutrients. Below these simple columnar epithelial cells, there is a layer of lymphoid tissue (#) followed by a muscular layer (M). In B, the aerial gut is lined by a pseudostratified columnar epithelial cell layer (R) with goblet cells (*) followed by a muscular layer (M). The calibration bar represents 20 µm. Slides were prepared using resin as infiltration and embedding medium, and hematoxylin-eosin for staining.

Modifications in the digestive tract that allow aerial breathing are found in other callichthyids and include macroscopic and microscopic characteristics similar to those of *Aspidoras gabrieli*. Macroscopically, these modifications encompass thin surfaces that can be kept humid (Graham 1997). The aerial gut of *Aspidoras gabrieli* is proportionally longer than the digestive one. Similar proportions were recorded for *Hoplosternum littorale* (Persaud 2000), which inhabits both highly oxygenated and hypoxic habitats. On the other hand, *Corydoras* has a shorter aerial gut (Persaud 2000) and is restricted to well-oxygenated waters (*e.g.,* Plaul et al. 2016).

Histologically, the aerial gut shows characteristics for accessory organs to breathe. As shown in Lieschke & Trede (2009), villi in the gut and enterocytes in the submucosa are characteristic structures for nutrient uptake in the digestive gut, as well as immune cells and, to some extent, goblet cells. In contrast, the aerial gut showed pseudostratified epithelium with flattened cells associated with goblet cells, shortening of the lamina propria, and the lack of folds, features related to gas exchange structures (Lieschke & Trede 2009). This suggests that this portion of the gut can gas exchange with adjacent capillaries. Besides, the modified aerial gut may be associated with floatability, as it may compensate for the encapsulation of the swim bladder in the cranial region (Gee & Graham 1978). Without the aerial gut located near the center of mass of the body, the individual would present asymmetry in the floatability.

The gross morphology and histology of the intestine of *A. gabrieli* indicate a respiratory function of its posterior portion, which would allow essential ecological strategies for the species. The occurrence of the intestinal adaptation pointed by the reported morphology widens the potential of this family for studies about adaptations to hypoxia. New insights on the morphology underpinning responses to hypoxia may be provided by analyzing fresh individuals and applying other histological techniques. However, it should be integrated with behavioral, physiological, and functional morphology perspectives.

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