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Dawestrema cycloancistrium (Monogenea) from the head pores of arapaimas

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ABSTRACT: *Arapaima gigas* is one of the main cultured fish species in South America, and monogenean parasites of this species cause large economic losses to fish farmers. During surveys of the parasites of cultured arapaimas from Mexiana Island in the Amazon River Delta, Rio Branco, in northwestern Brazilian Amazonia, and Yurimaguas, Peru, the monogenean *Dawestrema cycloancistrium* was found in the gills of *A. gigas* as well as in previously unreported sites, i.e. the head pores and chambers. The aim of this study was to investigate the transmission route of this parasite and its geographical distribution as well as to describe its morphology as observed by light and confocal imaging. Phalloidin labeling confirmed the presence of 2 prostatic reservoirs and showed muscular branches of fibers supporting haptoral sclerites. In arapaimas, the head connects to the gill chambers via 2 perforated scales located at the dorsolateral sides of the distal part of the head. The scales connect to thin channels and chambers situated in the proximal part of the head. These chambers are filled with cephalic mucus, which flows out to the environment through terminal pores. Adults and egg masses of monogeneans were found on the gills and inside the head pores and cavities of fish along with cephalic mucus. This indicates a specialized method of parasite transmission from adult fish to fingerlings during parental care (holding offspring in the mouth) or via head secretions, providing evidence of a new adapted mechanism of dispersion.

KEY WORDS: *Arapaima gigas* · Pirarucu · Parasite · Freshwater fish · Monogenea · Amazonia · Brazil · Peru

INTRODUCTION

The arapaima *Arapaima gigas* (Schinz, 1822) (Arapaimidae), also known as pirarucu, is a fish endemic to the Amazon basin that has gills but is an obligate air breather and surfaces frequently to gulp air to supply its modified swim bladder. Arapaimas are cultured in South America, where their parasitic monogeneans cause large economic losses (Araújo et al. 2009, Valladão et al. 2016).

During surveys into the parasites of arapaimas from Mexiana Island in the Amazon River Delta, Rio Branco in northwestern Brazilian Amazonia, and Yurimaguas, Peru, the monogenean *Dawestrema cycloancistrium* Price and Nowling, 1967 (Ancyrocephalidae) was found in the gills and in a previously unreported site, i.e. inside the head pores and chambers of *A. gigas*. The aim of this study was to investigate the transmission route of this parasite and its geographical distribution as well as to describe its morphology based on light and confocal imaging.

MATERIALS AND METHODS

Sample collection

Arapaimas were obtained from commercial fish farms regulated by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA)

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and the Acre State Environmental Institute (IMAC): Fazenda Santo Ambrosio, on Mexiana Island (00° 05′ 30″ S, 49° 34′ 50″ W), Pará state, Brazil (n = 30), obtained in January and March 2008 (Pirarucu Management Project, IBAMA 005-2007); Fazenda Boa Esperança (9°45′26″S, 68° 04′ 26″ W) in Bujari, Acre state, Brazil (n = 64), in October 2013 to June 2015 (IMAC 484/2011); and from a fish farm in the Yurimaguas region of Peru (05° 54′ 00″ S, 76° 05′ 00″ W) (n = 24). The study was licensed by IBAMA (no. 15898-1 and 39106/2013) and was conducted in accordance with the guidelines of the Brazilian College of Animal Experimentation (COBEA).

Light microscopy

Monogeneans were recovered from gills, and those from fluids of the head chambers were collected using a syringe. Specimens were washed in physiological saline and then fixed in 70% alcohol. For light microscopy, the monogeneans were stained in Gomori's trichrome (Humanson 1967), and some were mounted in Hoyer's or Berlese medium (Humanson 1967) to study sclerotized structures. Illustrations were made with the aid of a drawing tube. Measurements, made using an ocular micrometer, are given in µm, as the range followed by the mean in parentheses, unless otherwise stated. Specimens were deposited in the Helminthological Collection of Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

Confocal laser scanning electron microscopy (CLSM)

Specimens were fixed in 4% formalin and incubated overnight with phalloidin conjugated with FITC 1:700 in phosphate-buffered saline (PBS; Sigma) and mounted on semi-permanent slides in PBS with 2.5% 1,4-diazabicyclo[2.2.2]octane (triethylenediamine) and 50% glycerol, pH 7.2 (after Borges et al. 2017). Slides were observed using an LSM 510 Meta-Zeiss microscope and LP560 filter, with laser excitation of 543 nm.

Analysis of head channels and chambers of fish

To explore the route between the gills and head pores of recently dead fish from Yurimaguas, colored dentistry wax was inserted into the head pores of a dead fingerling. Subsequently, an X-ray was taken to map the channels filled with wax, and then the tissue covering the chambers was manually removed.

RESULTS

Arapaima gigas: head channels and chambers

In arapaimas, the head connects to the gill chambers through 2 perforated scales located at the dorsolateral sides of the distal part of the head (Fig. 1A–D). These holes connect to thin channels that enter into 2 main cavities that connect with a web of thin channels and chambers situated in the proximal and lateral parts of the head (Fig. 1A,B,E,F). These channels and cavities are filled with cephalic mucus, which flows out to the environment through terminal pores (Fig. 1E). The mouth also connects with the gill chambers.

Dawestrema cycloancistrium Price and Nowling, 1967

Sites of infection: Mainly in gills but also in the head pores and cavities.

Localities: Fazenda Boa Esperança (9° 45′ 26″ S, 68° 04′ 26″ W), Bujari, Acre state, Brazil; Fazenda Santo Ambrosio (00° 05′ 30″ S, 49° 34′ 50″ W), Mexiana Island (Amazon River Delta), Pará state, a new geographical location in Brazil; and a fish farm at Yurimaguas, Peru (05° 54′ 00″ S, 76° 05′ 00″ W), a new locality.

Deposition of specimens: Oswaldo Cruz Institute, Rio de Janeiro (CHIOC 38.656 a–c and 38657 a–c).

Adults and egg masses of *D. cycloancistrium* were found in the gills and inside the head pores and cephalic mucus-filled cavities of *A. gigas*, which represent previously unreported sites.

The study of cultured arapaimas from 3 different geographical localities showed that the overall prevalence of *D. cycloancistrium* from Mexiana Island in the Amazon Delta was 78%, with an intensity of 1 to 151 (mean 13) monogeneans per fish. The most heavily infested *A. gigas*, harboring 151 monogeneans, was 70 cm long, whereas the smallest infected fish, 7 cm long, contained only a single monogenean. Inside the head cavities of 1 female and 2 male arapaimas, we found 7, 2, and 2 specimens, respectively, of *D. cycloancistrium*. In Rio Branco, Acre state, the prevalence of *D. cycloan-*

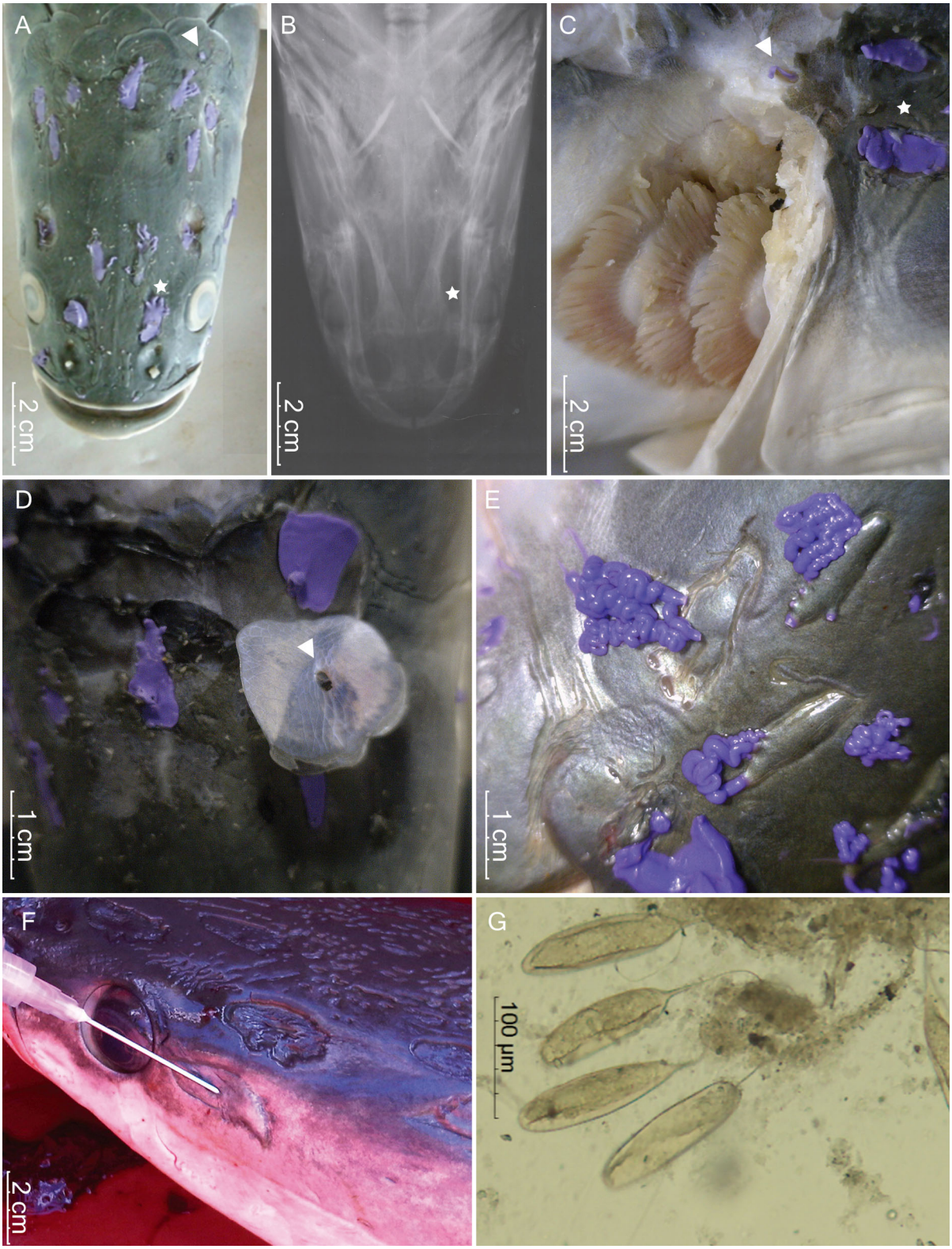

Fig. 1. *Arapaima gigas* from fish farms. (A) Head showing perforated scale (white arrowhead), thin channels, and chambers (star). (B) X-ray of the head showing main cavities and chambers (star). (C) Gills connected to perforated scales (white arrowhead) and cephalic chambers (star). (D) Perforated scales (white arrowhead) at the dorsolateral side of the head. (E) Channels and cavities that open into the environment through terminal pores. (F) Extraction of mucus from cephalic chambers. (G) *Dawestrema* eggs found inside cephalic cavities

cistrium was 56.3%, with an intensity of 1 to 2640 (mean 394) monogeneans per fish. The most heavily infested individual, harboring 2640 monogeneans, was 68.7 cm long. In the Yurimaguas region (Peru), 5 out of 9 fingerlings analyzed were parasitized by *D. cycloancistrium* (only in the gills), with an intensity of 2 to 26 parasites per fish. The analysis of the fluid extracted from the cephalic pores and cavities of 15 adult arapaimas confirmed the presence of *Dawestrema* eggs in 1 female and 1 male. In addition, in another male, eggs as well as 2 adult monogeneans were found.

Morphological data

Light microscopy

Description: Based on 13 specimens from Mexiana Island. Cuticle smooth. Cephalic lobes poorly developed. Two pairs of eyespots. Mouth subterminal. Body length 920–1800 (1446) × 65–300 (136) (Fig. 2A). Pharynx 40–70 × 40–70 (51 × 51). Gut confluent. Haptor 80–150 (109) × 65–195 (109). Dorsal anchor total length 42–45 (44) × 17–22 (20) (Fig. 2B). Ventral anchor robust, 25–36 (31) × 13–20 (16) (Fig. 2C). Bars dissimilar. Dorsal bar 25–44 (32) long (Fig. 2D). Ventral bar 19–25 (22); median sclerotized process 35–45 (38) long (Fig. 2E). Hooks (14), 14–21 (16) long, forming a circle well anterior to haptoral armature (Fig. 2A). Testis oval 37–80 (51) × 19–50 (29). Vas deferens looped around left cecum. Seminal vesicle large. Two prostatic reservoirs well visible. Copulatory complex includes a tubular cirrus with expanded base that coils 6–7 times, largest diameter of coil 32–38 (35) and accessory piece 75–112 (83) long (Fig. 2F). Ovary 60–100 × 30–100 (77 × 51). Vagina ventrolateral, sclerotized, 41–50 (45) long with 4 terminal rami (Fig. 2G). Vaginal duct lightly sclerotized forming ca. 3 coils joining small seminal receptacle. Vaginal pore ventro-lateral, sinistral. Vitellaria well developed and distributed laterally from esophagus to end of gut. Uterus at mid-body extending to genital pore at anterior third of body. Eggs numerous, elongate 168–207 × 51–61 (188 × 570), with terminal filament (Fig. 2H).

CLSM

The high-resolution confocal imaging with phalloidin labeling revealed a complex arrangement of muscular fibers associated with the tegument, pharynx, reproductive system, and haptor (Fig. 3A–E). Longitudinal, diagonal, and circular fibers are located along the tegument, supporting organs and aperture as the genital atrium (Fig. 3B). The muscular pharynx is formed by circular fibers crossed by longitudinal ones (Fig. 3D). Two pyriform prostatic reservoirs, part of an enlarged seminal vesicle, and coiled male copulatory complex are clearly visible (Fig. 3C,D). The ovary surface was labeled (Fig. 3D), and the uterus, formed by longitudinal muscular fibers, enlarges near the genital pore (Fig. 3C,D). Muscular branches are visible in the body end connecting to the haptoral sclerites (Fig. 3E,F).

DISCUSSION

Dawestrema cycloancistrium was previously reported parasitizing the gills of arapaimas in Brazil in the states of Amazonas (Price & Nowlin 1967, Kritsky et al. 1985, Araújo et al. 2009), Mato Grosso (dos Santos et al. 2008), Amapá (Marinho et al. 2013), Acre (Silva et al. in press), and Pará (Malheiros et al. 2016). In Peru, there are also reports of high levels of infection by *D. cycloancistrium* (Ianncone & Luque 1991, Delgado et al. 2013, Serrano-Martínez et al. 2015). According to Braga et al. (2014), the distribution pattern of Monogenea is strongly influenced by the evolutive history of their hosts. This was initially believed to explain the restricted occurrence of *D. cycloancistrium* along the Amazon basin, which is the natural habitat of *Arapaima gigas*.

D. cycloancistrium was first described by Price & Nowlin (1967) from the gills of *A. gigas* from the Amazon River and tributaries in Brazil, and was redescribed by Kritsky et al. (1985) based on the examination of types and material collected from wild arapaimas from the Solimões River, Amazonas. Kritsky et al. (1985) reported the presence of a single elongated prostatic reservoir that was thin walled and frequently twisted, differing from the original description in which the presence of 2 prostatic reservoirs was noted. Our confocal analysis showed the presence of 2 instead of 1 prostatic reservoir, thus confirming the original description of Price & Nowlin (1967).

During development, *A. gigas* undergoes a transition from being an exclusive water breather (gills) to becoming an air breather (swim bladder). These changes occur approximately on the ninth day after the larvae hatch, with intense proliferation of cells in the gill lamellae (Brauner et al. 2004, Fernandes et al. 2012, Ramos et al. 2013).

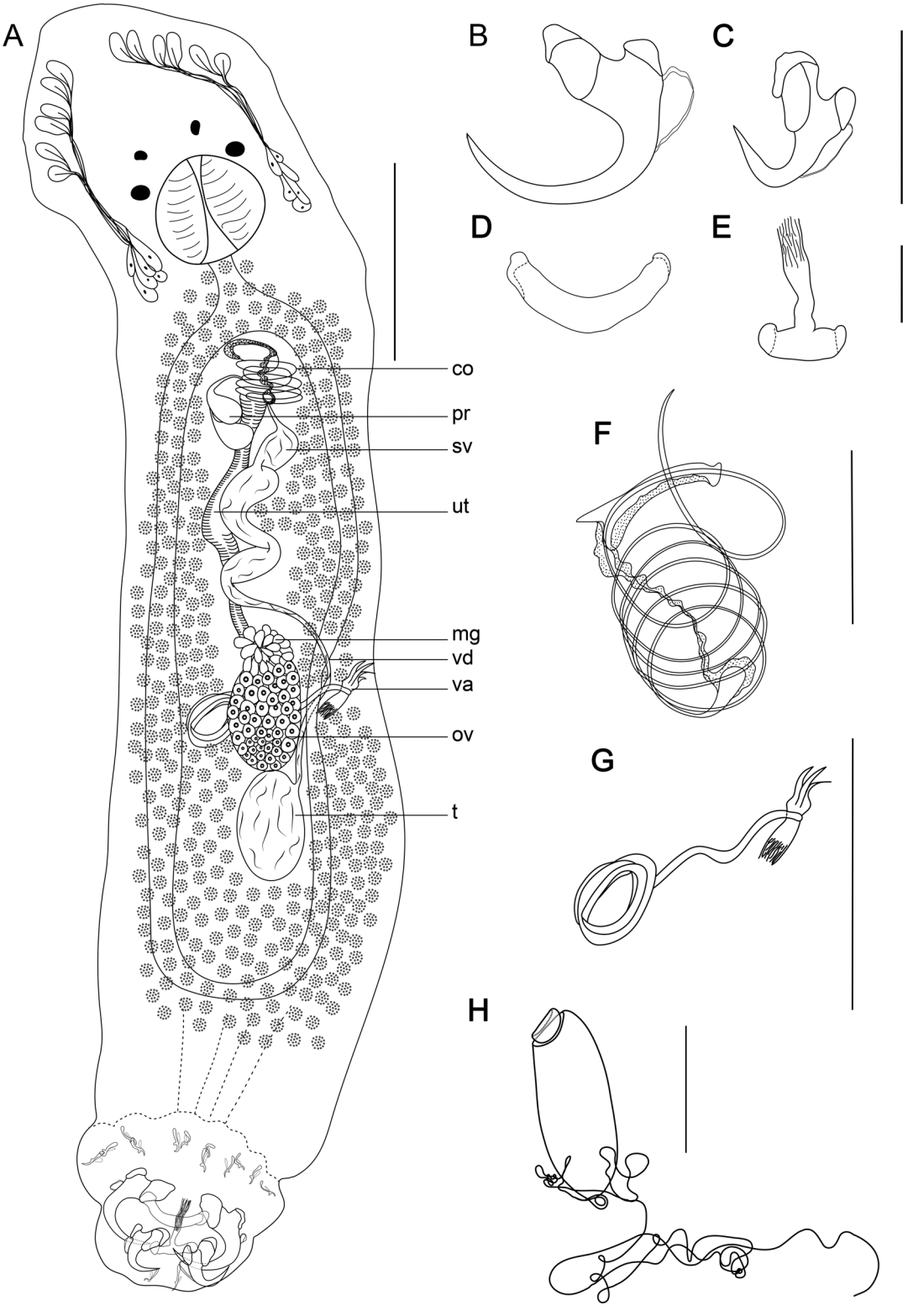

Fig. 2. (A) Composite drawing of *Dawestrema cycloancistrium* (whole organism). co: copulatory organ; pr: prostatic reservoirs; sv: seminal vesicle; ut: uterus; mg: Mehlis' gland; vd: vas deferens; va: vagina; ov: ovary; t: testis. (B) Dorsal anchor. (C) Ventral anchor. (D) Dorsal bar. (E) Ventral bar. (F) Copulatory complex. (G) Vagina. (H) Egg. Scale bars = (A, G–H) 100 µm; (B,C) 50 µm; (D,E) 25 µm; (F) 50 µm

Fig. 3. Confocal images of *Dawestrema cycloancistrium*. (A) Whole organism. (B) Longitudinal, diagonal, and circular fibers encircling genital pore. (C) Reproductive organs, ventral view (co: copulatory organ; pr: prostatic reservoir; sv: seminal vesicle; ut: uterus). (D) Pharynx (ph) and reproductive organs (co: copulatory organ; ut: uterus; pr: prostatic reservoirs; sv: seminal vesicle; ov: ovary). (E,F) Muscular branches at body end connecting to the haptoral sclerites. Scale bars = 50 µm

Both male and female arapaimas have head pores from which mucous substances are excreted. It is assumed that this cephalic liquid is part of a parental care strategy, used to congregate offspring mainly around the male's head. Little is known about the origin, composition, and function of this substance, but it is likely related to chemical communication among arapaimas in different stages of life. Amaral (2009) detected steroids (17β-estradiol, testosterone, 11keto-testosterone, and 17α-hydroxyprogesterone) in the so-called cephalic liquid, both in males and females, including sexually immature fish. After comparing the plasmatic levels of these hormones during the sexual cycle, Amaral (2009) suggested that the cephalic liquid could be a way to excrete these steroids, and could also act as pheromones. To avoid predation, arapaimas can also hold fry and fingerlings inside the mouth for short periods (Campos-Silva & Peres 2016).

The route between the gills and head pores was observed via perforated scales which connected to a web of channels and chambers. It is worth noting that *D. cycloancistrium* is known to parasitize the gills, but we also found eggs and adults inside the head chambers of arapaimas from different localities (Yurimaguas, Peru, and Amazon Delta, Brazil). Considering that arapaimas are ancient fish that, despite having gills, are obligate air breathers and surface frequently to gulp air, it is possible that the monogenean eggs, which float during these movements, could be transferred via the channels to the chambers and pores. This likely indicates a specialized method of *D. cycloancistrium* transmission from adult fish to fingerlings during parental care (holding offspring in the mouth) or via head secretions, and seems to provide better evidence of a new adapted mechanism of dispersion than of pressure for space.

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