Journal of Fish Diseases 2012, **35**, 615–617 doi:10.1111/j.1365-2761.2012.01365.x

Short Communication

'Branchiomycosis in tambaqui, *Colossoma macropomum* (Cuvier), from the eastern Brazilian Amazon'

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Keywords: Amazon, *Branchiomyces*, *Colossoma macropomum*, gill disease, mycosis.

The tambaqui, *Colossoma macropomum* (Cuvier), plays an important economic role in fish farming in the Amazon region and in Brazil (Ismiño-Orbe, Araujo-Lima & Gomes 2003). It is the second largest fish species in the Amazon region, weighing as much as 30 kg (Gouding & Carvalho 1982).

Fungi of the *Branchiomyces* genus (*B. demigrans* and *B. sanguinis*) are the aetiological agents of branchiomycosis, an acute respiratory disease that has been described in several fish species worldwide (Khoo 2000). Overpopulation, increased temperature (above 20 °C), increased concentrations of non-ionized ammonia and the presence of algae in the aquatic environment create favourable conditions for the outbreaks of this disease (Paperna & Smirnova 1997; Hawke & Khoo 2004).

Lesions caused by *Branchiomyces* are generally circulatory in nature and primarily affect the gills. The intravascular growth of the fungus causes thrombi to form, resulting in areas of bleeding and infarcts (Khoo 2000; Paperna & Di Cave 2001; Ramaiah 2006). The histopathological diagnosis can be made using Grocott's stain, haematoxylin–eosin (H&E) stain, Gomori's methenamine silver stain and periodic acid-Schiff staining (PAS) (Khoo *et al.* 1998; Khoo 2000; Duc & Hatai 2009).

This study aimed to describe the macroscopic and microscopic lesions caused by a *Branchiomyces* sp. infection found in tambaqui after an outbreak occurred in May 2010 in the breeding tank at Mangal das Garças Zoological Park, located in the municipality of Belém, State of Pará, Brazilian Amazon.

A total of eight fish were autopsied, including three specimens of tambaqui, two black prochilodus, *Prochilodus nigricans* (Spix & Agassiz), two silver dollars, *Metynnis hypsauchen* (Müller & Troschel), and one red lip, *Satanoperca acuticeps* (Heckel). Following macroscopic examination, several tissue samples were collected from the gills, blood vessels, liver and kidneys of each fish. The samples were fixed in 10% buffered formalin, embedded in Paraplast® and processed using routine techniques for histological analysis. The slides were stained with H&E and Grocott's stains and analysed under light microscopy.

The results indicated that all autopsied animals were in good nutritional condition and had no external injuries; however, the specimens of *C. macropomum* showed significant gill lesions. In Case 1, the gills were embedded with grayish-white mucus located primarily in the first lamellae. The

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Journal of Fish Diseases

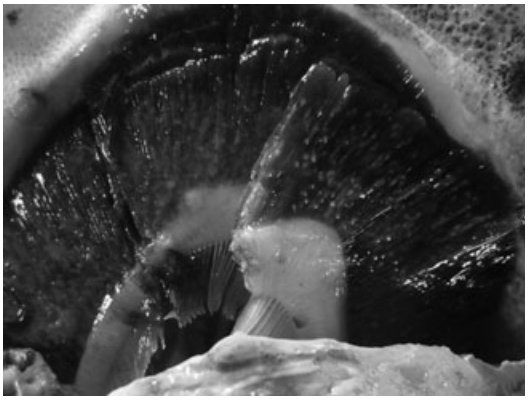

Figure 1 *Colossoma macropomum* gill hyperaemia and exudate, with the formation of white plaques.

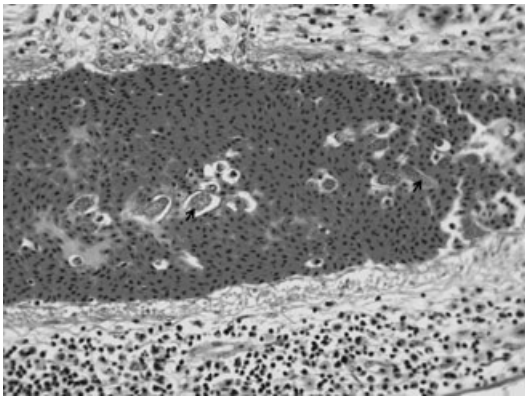

Figure 3 Hyphae proliferating in gill blood vessels (arrows), including the endothelium (*) (H&E, ×200).

mucus had a granular appearance and formed small whitish plaques (Fig. 1). This type of secretion was spread more diffusely in the other lamellae. Although flakes and macroscopic, 1- to 2-mm grayish-white spots were observed on the gill filaments, no filament loss was noted, consistent with Ramaiah (2006). Cases 2 and 3 exhibited grayish gill filaments but less abundant mucus and lamellar foci.

The microscopic observation of hyphae within blood vessels can be performed using at least 150 times magnification to view slides of crushed fresh tissue obtained from gill filaments (Khoo *et al.* 1998; Ramaiah 2006). By viewing smears containing gill mucus stained using the panoptic method, it is therefore possible to identify filamentous structures of fungal hyphae that are morphologically consistent with *Branchiomyces* sp. in specimens of *C. macropomum*.

Histopathologically, as indicated by Hawke & Khoo (2004), the hyphae found inside the blood vessels were associated with vasculitis and the infiltration of mononuclear cells (Figs 2 & 3). The lesions described in this study are consistent with those reported by Khoo (2000), indicating that gills infected with *Branchiomyces* sp. exhibit haemorrhaging and infarction because the hyphae produce regions of granulomatous inflammation that haemorrhage when the fungi leave the intravascular space (Fig. 4; Hawke & Khoo 2004). Grocott staining also revealed the presence of sporulated hyphae (Figs 5 & 6), indicating an acute stage of infection (Ramaiah 2006).

Upon internal organ examination, Case 1 exhibited a normal-coloured liver. However, in Cases 2

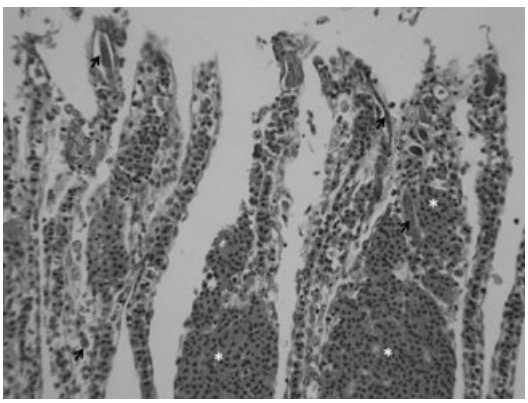

Figure 2 Gill filaments devoid of lamellae. The afferent artery of the gill filament exhibits blood stasis in several segments (*). Hyphae proliferating in the blood vessels of gill filaments (arrows) (H&E, ×200).

Figure 4 Hyphae, some of which are sporulated, proliferating in gill blood vessels (arrows). Note oedematous perivascular tissue, with several inflammatory cells and erythrocytes. (H&E, ×400).

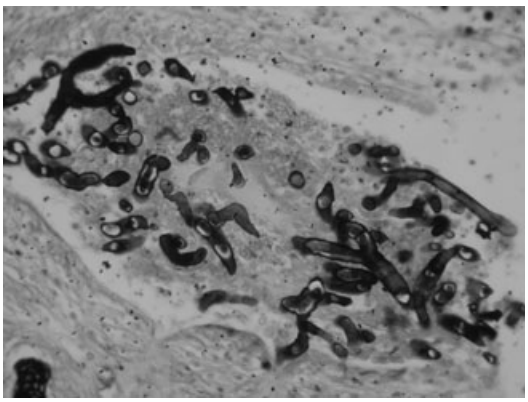

Figure 5 Hyphae, some of which are sporulated, proliferating in gill blood vessels (Grocott, ×400).

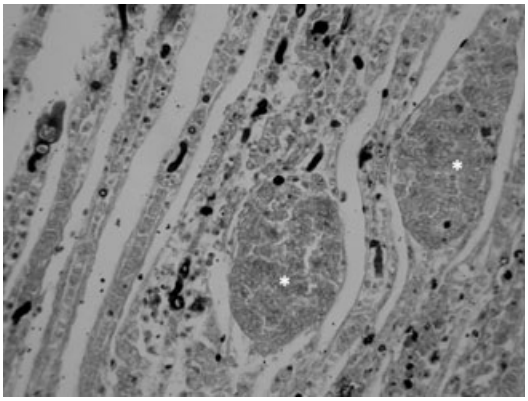

Figure 6 Gill filaments showing vessels with hyphae. Thrombosis in afferent arteries of the gill filament (*) (Grocott, ×200).

and 3, several of the liver lobes had a pronounced dark red colour accompanied by a firm consistency and a significant amount of blood flow upon cutting, which are unique characteristics of haemorrhagic infarction. In Case 3, the infarcted region affected half of the liver; however, the aetiology of the liver infarct could not be determined through microscopic examination. The kidneys of each *C. macropomum* specimen were enlarged and congested and exhibited an intense red colour.

In both cases of haemorrhagic infarction of the liver, the process was not histologically associated with the *Branchiomyces* sp. infection, which agrees with reports in the literature that have indicated that such lesions tend to be restricted to the gills (Khoo 2000; Ramaiah 2006).

The systemic and gill alterations associated with *Branchiomyces* sp. infections were only observed in the *C. macropomum* specimens. The other autopsied fish specimens showed no macroscopic or microscopic lesions compatible with a *Branchiomyces* sp. infection, and the direct examination of the gill secretions did not indicate the presence of fungal hyphae.

In this study, we concluded that the fungus *Branchiomyces* sp. is strongly associated with a gill lesion condition whose histopathology is characterized by severe circulatory alterations produced by the fungus. The fungus has been previously described in another fish species in the Brazilian Amazon (*Baryancistrus* sp.; Paperna & Di Cave 2001). However, our results provide the first report of a *Branchiomyces* sp. infection in *C. macropomum* from the Brazilian Amazon. Investigations of the occurrence of this fungus in the region should therefore take a complementary approach.

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Received: 3 January 2012
Revision received: 8 March 2012
Accepted: 8 March 2012

