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Dermocystidium sp. in the gills of farmed *Oreochromis niloticus* in Brazil

LILIAN D. STECKERT, LUCAS CARDOSO, KAREN R. TANCREDO, MAURÍCIO L. MARTINS and GABRIELA T. JERÔNIMO

AQUOS – Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Rodovia Admar Gonzaga, 1346, Itacorubi, 88040-900 Florianópolis, SC, Brazil

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Abstract: The genus *Dermocystidium* is very comprehensive in the host and site of infection, however this is the first report of the occurrence of *Dermocystidium* sp. in the gills of Nile tilapia. This study was carried out in a fish farming located in the state of Santa Catarina, Brazil. No mortalities were reported in the facility studied and the animals were clinically healthy. During the histopathological analysis of the gills, 8.33% of the fish presented spores of *Dermocystidium* sp. in the gill tissue. The spores reported herein had a mean length and width of 6.206 x 5.233 μm and a refractile body diameter of 1.965 μm and were studied by histopathology and Transmission Electron Microscopy. This study highlights the importance of a new branchial pathogen in farmed tilapia, as well as to its pathogenic potential, considering the outbreaks of mortalities associated with other fish species.

Key words: fish farming, dermatocystidiosis, Nile tilapia, histology, electron microscopy, spores.

INTRODUCTION

The genus *Dermocystidium* comprises pathogens of the order Dermocistida, class Ichthyosporea (Langenmayer et al. 2015). Numerous different species of *Dermocystidium* have been described, infecting a variety of fish and producing gill infections, skin lesions and visceral diseases worldwide (Feist et al. 2004, Zhang and Wang 2005).

Among the main species of fish of interest to aquaculture that have been affected by *Dermocystidium* spp., is the common carp with *Dermocystidium koi* (Hoshina and Sahara 1950), *Dermocystidium cyprini* (Cervinka et al. 1974, Lotman et al. 2000), rainbow trout with *Dermocystidium macrophagi* (Van de Moer et al. 1987), common perch with *Dermocystidium percae* (Pekkarinen and Lotman 2003), several salmonids with *Dermocystidium salmonis* (Olson et al. 1991, Olson and Holt 1995), kinguio, catfish and Nile tilapia with unidentified species of *Dermocystidium* spp. (Zhang and Wang 2005, Mahmoud et al. 2009, Shaheen 2000). Still in tilapia, El-Mansy (2008) identified *D. aegyptiacus* in the intestinal epithelium of farmed *O. niloticus* in Egypt.

Gill infection by *Dermocystidium* is pathogenic and causes mortality in farmed salmonids (Olson et al. 1991), carps (Cervinka et al. 1974) and eels (Wootten and McVicar 1982, Molnár and Sovenyi

Correspondence to: Gabriela Tomas Jerônimo
E-mail: gabrielatj@gmail.com
ORCid: https://orcid.org/0000-0002-2071-4428

1984). In Brazil, this pathogen was reported by Eiras and Silva-Souza (2000) in *Trichomycterus* sp., and more recently by Fujimoto et al. (2017) in cultures of hybrid fish tambatinga; however, this is the first report of *Dermocystidium* sp. infecting gills of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758).

MATERIALS AND METHODS

The procedures adopted for this study were approved by the Committee on Ethics in the Use of Animals of Federal University of Santa Catarina - CEUA Nº PP00928. Sixty adult tilapia (average weight 480.9 ± 210.2 g and average length 28.1 ± 4.2 cm) were captured from 12 fish farms located in the state of Santa Catarina, southern Brazil. The study was conducted between May and December 2015. The fish were anesthetized with eugenol (75 mg L^{-1}) and euthanized by rapid cerebral concussion. Then, the first right branchial arch was removed, divided and fixed in 10% buffered formalin and 2.5% glutaraldehyde.

The samples previously fixed in 10% buffered formalin were dehydrated in progressive graduation of alcohol, diaphanized in xylol and embedded in paraffin. Using a microtome PAT-MR10 (The Pathologist®, Brazil), samples were sectioned in 3 μm and stained with Harris haematoxylin and eosin (HH & E), mounted on permanent blades with Entellan® and analyzed by DIC (Differential Interference Contrast) microscope model Axio Imager A2 (Zeiss®, Germany).

For analysis in transmission microscopy, the gills were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2 for 24 hours. Post-fixed with osmium tetroxide solution, dehydrated in increasing solution of ethanol and transferred to ethanol: spurr resin. Ultra thin (60 nm) sections were cut with a diamond blade and stained with uranyl acetate and lead citrate for microscopic observation (JEOL JEM-1011). The identification of the parasite was performed through morphological characteristics of the spores, such as refractile body, vacuoles and cytoplasm. To determine the approximate size of the spores, were performed by light microscopy at a magnification of 100x, the measurements of length (μm), width (μm) and refractile body diameter (μm) of 53 spores randomly found on the histological sections of the infected animals.

The spores (Figure 1) found in this study are spherical, with central refractile body and presented an average size of 6.206 x 5.233 μm (Table I). The size of the spores is similar to that reported by Wootten and McVicar (1982) who observed spheres of 4 to 7 μm spherical, measuring 25 cells, in gills of *Anguilla anguilla*. Based on the histological and ultrastructural findings, gill dermocystitis was diagnosed.

The presence of spores was observed through routine histopathological examination (Figure 1) and supported by Transmission Electron Microscopy (Figure 2).

According to Bruno et al. (2006) *Dermocystidium* species that infect fish, are located in epithelial tissue of the skin, fins, gills or visceral organs. Infections usually appear as small white, round or oval nodules, or cysts in the affected tissue. In the histological sections, species attributed to the genus *Dermocystidium* are characterized by a

TABLE I
Measurements (μm) of the spores of *Dermocystidium* sp. (n = 53) collected in the gills of *Oreochromis niloticus*, cultivated in Santa Catarina, southern Brazil. (Mean ± standard deviation).

Indices	Length	Width	Refractile body perimeter
Mean	6.206±1.149	5.233±1.105	1.965±299.640
Interval	3.314–7.803	2.794–7.403	1.177–2.943

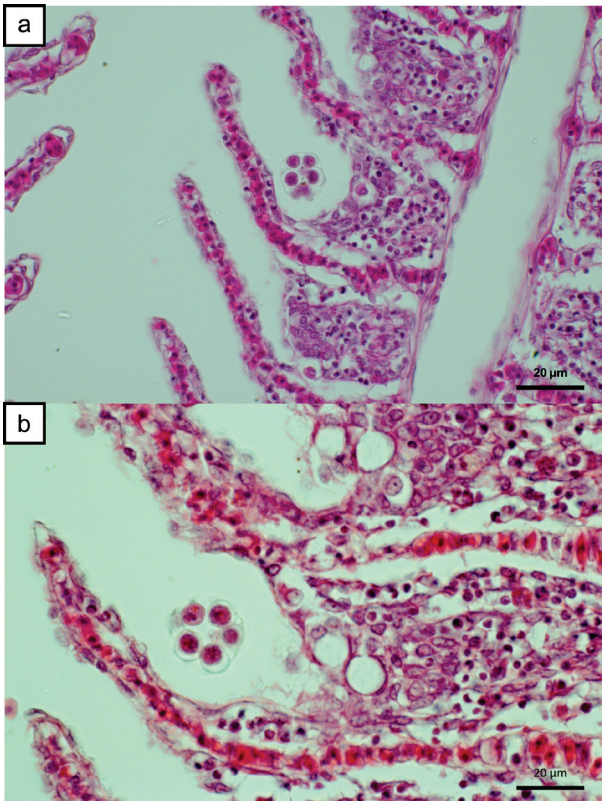

Figure 1 - Photomicrographs of the gill tissue. **a.** interlamellar epithelial hyperplasia along the gill filaments (bar = 20 µm). **b.** Spores of *Dermocystidium* sp. between the secondary lamellae of gills of Nile tilapia, evidencing the hyaline cytoplasm (HH & E staining, 20 µm bar).

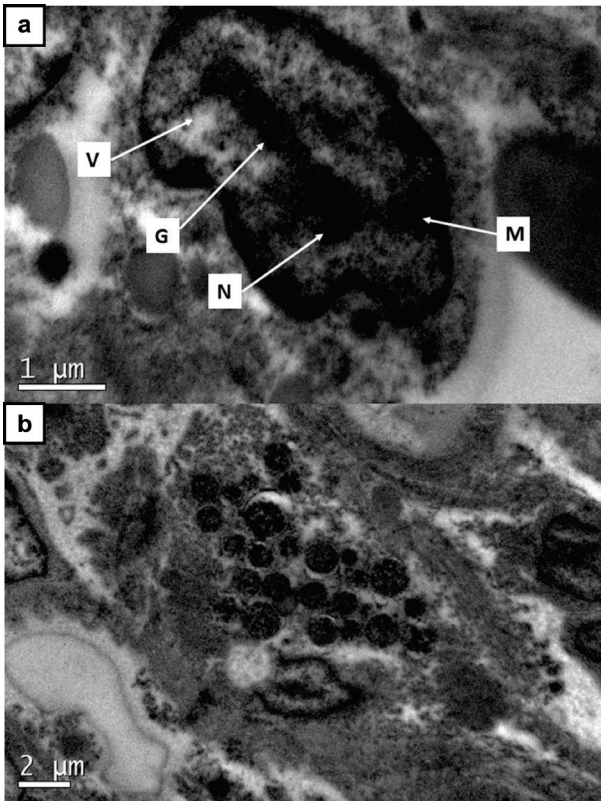

Figure 2 - a. Transmission Electron Microscopy of *Dermocystidium* spores showing the structures. V – vacuole; G – Golgi-complex; N – nucleus; M – mitochondria. (bar = 1 µm). **b.** Transmission Electron Microscopy of *Dermocystidium* spores showing the hyaline cytoplasm (bar = 2 µm).

spherical spore stage, with a large central vacuole or solid refractile body and the cytoplasm with the nucleus restricted to a narrow peripheral layer. In most species, the spores are relatively uniform in size (3 to 12 µm in size, depending on the species).

The histological sections of the present study showed gill alterations such as interlamellar epithelial hyperplasia (Figure 1a), secondary lamella epithelial hyperplasia and fusion of secondary lamellae. According to Bruno (2001) epithelial hyperplasia and the fusion of the gill lamellae are common alterations in the infection by *Dermocystidium*.

During collection, it was not possible to observe the fresh cysts in the gills and the diagnosis was made through histopathological analysis. Feist et al. (2004) report in their samples that several ruptured cysts were observed in the *Cottus gobio* epithelium, allowing the release of spores into the environment. The aforementioned authors believe that this is the usual mechanism of spore dispersion.

CONCLUSIONS

Based on the few morphological descriptions of *Dermocystidium* sp. found in the literature, it was not possible to identify the species. The authors report that it is difficult to compare the size of the spores, the shape and stage of development of the cysts, and the lack of sufficient data for the studied fish species (Feist et al. 2004).

Corroborating the results of the present study, Wootten and McVicar (1982) did not observe mortality that could be attributed directly to the

infection by *Dermocystidium* sp. in farmed of *Anguilla anguilla*. Presumably, tilapia is a rustic fish, able to ensure adequate oxygen through the unaffected parts of the gills or skin. This contrasts with the severe disease outbreak and mortality in chinook salmon *Oncorhynchus tshawytscha* (Walbaum) and carp *Cyprinus carpio* (Pauley 1967, Allen et al. 1968, Cervinka et al. 1974) caused by *Dermocystidium* in the gills.

The occurrence of *Dermocystidium* sp. in the gills of Nile tilapia, recorded for the first time in asymptomatic fish, may be due to an infection detected at an early stage. Therefore, it is attentive to a possible emerging pathogen in farmed tilapia in Brazil. It is suggested the monitoring of fish farms to complete the identification of the agent, as well as epidemiological investigation and its pathogenicity in Nile tilapia.

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AUTHOR CONTRIBUTIONS

L.D.S., M.L.M. and G.T.J. conceived and planned the experiment. L.C. aid in histological analysis and aid in obtaining photomicrographs of the material analyzed; L.D.S. and K.R.T. scanning and electron microscopy, sample preparation, analysis, writing. L.D.S., M.L.M. and G.T.J. data analysis, writing and revision of the manuscript.

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