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First report of scuticociliatosis caused by *Uronema* sp. in ornamental reef fish imported into Brazil

Primeiro relato de scuticociliatose causada por *Uronema* sp. em peixes ornamentais de recife importados para o Brasil

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Abstract

Scuticociliatosis, which is caused by an opportunistic ciliate protozoan, is responsible for significant economic losses in marine ornamental fish. This study reports the occurrence of *Uronema* sp., which was found to be parasitizing three species of marine reef fish imported into Brazil and maintained in quarantine: Vanderbilt's Chromis (*Chromis vanderbilti*), blue-green damselfish (*Chromis viridis*), and sea goldie (*Pseudanthias squamipinnis*). During the quarantine period, some fish presented with behavioral disorders and hemorrhages and ulcerative lesions on the body surface. Histopathological analysis showed hemorrhages, inflammation comprising mononuclear and granular cells in the skeletal muscle, and necrosis of the skin and the secondary lamellae of the gills, and parasites were also observed in the renal capsule. The absence of transboundary measures available to prevent the occurrence of ornamental fish diseases is also discussed.

Keywords: Ornamental fish, *Chromis*, *Pseudanthias*, Scuticociliatia, transboundary disease, histopathology.

Resumo

Scuticociliatose, causada por protozoários ciliados oportunistas, é responsável por perdas econômicas significativas em peixes ornamentais marinhos. O presente estudo relata a ocorrência de *Uronema* sp., parasitando três espécies de peixes ornamentais de recife importados para o Brasil e mantidos em quarentena: Vanderbilt's Chromis (*Chromis vanderbilti*), blue-green damselfish (*Chromis viridis*), e sea goldie (*Pseudanthias squamipinnis*). Durante o período de quarentena, alguns peixes apresentaram alterações comportamentais tais com hemorragias e lesões ulcerativas na superfície da pele. A histopatologia mostrou hemorragias, inflamação com células mononucleares e granulares na musculatura, e necrose da pele e lamelas secundárias das brânquias, e parasitas também foram observados na cápsula renal. A ausência de medidas transfronteiriças disponíveis para prevenir a ocorrência de doenças de peixes ornamentais também é discutida.

Palavras-chave: Peixes ornamentais, *Chromis*, *Pseudanthias*, Scuticociliatia, doença transfronteiriça, histopatologia.

Introduction

Scuticociliatosis, a severe disease that compromises the health of marine ornamental fish, is caused by marine ciliates belonging to the subclass Scuticociliatia. *Uronema marinum* Dujardin, 1841 was first reported in nine fish species from the Atlantic and Pacific Oceans maintained in the New York Aquarium

(CHEUNG et al., 1980). The disease has a worldwide distribution and causes lesions on the body surface and gills in addition to systemic infection (PIAZZON et al., 2014), being responsible for high mortality rates. The fish species most affected are the olive flounder *Paralichthys olivaceus* (IGLESIAS et al., 2001; JIN et al., 2009; MOUSTAFA et al., 2010a), turbot *Scophthalmus maximus* (IGLESIAS et al., 2001; WHANG et al., 2013), sea bass *Dicentrarchus labrax* (WHANG et al., 2013), Southern bluefin tuna *Thunnus maccoyii* (MUNDAY et al., 1997; GARZA et al., 2017), grouper *Polyprion oxygeneios*, yellowtail kingfish *Seriola lalandi* (SMITH et al.,

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2009), and silver pomfret *Pampus argenteus* (AZAD et al., 2007). The most pathogenic scuticociliates are *Pseudocohnilembus persalinus* Evans & Thompson, 1964, *Pseudocohnilembus longisetus* Evans & Thompson, 1964, *U. marinum*, and *Miamiensis avidus* Thompson & Moewus, 1964 (GARZA et al., 2017; MOUSTAFA et al., 2010b; WHANG et al., 2013).

Uronema Lagerheim, 1887 is an opportunistic, free-living ciliate protozoan that parasitizes mostly the body surface, gills, and fins of marine fish. It tolerates a wide range of salinity, of 15-35 ppt (CROSBIE & MUNDAY, 1999) and causes severe disease in ornamental fish, from which fish farmers can suffer heavy economic losses if the disease is not treated sufficiently rapidly (CHEUNG et al., 1980; GILL & CALLINAN, 1997; NOGA, 2010).

Pathogenicity caused by this parasite is more severe in environments with high levels of bacteria, nutrients, and organic matter (URRUTXURTU et al., 2003). It can be found parasitizing both farmed and ornamental marine fish (AZAD et al., 2007; DECLERCQ et al., 2014).

The parasite produces proteases responsible for digestion of the host's tissues and proteins (AL-MARZOUK & AZAD, 2007). It also provokes asymptomatic changes and frequent macroscopic and ulcerative lesions on the body surface and fins, exophthalmia, and a swollen visceral cavity. Infected fish may also present with internal changes such as brain liquefaction, hemorrhages, and muscle ulceration (AZAD et al., 2007; IGLESIAS et al., 2001; JIN et al., 2009; MOUSTAFA et al., 2010a, b; ROSSTEUSCHER et al., 2008), these being readily observed on histopathological exam.

Microscopic observation shows the parasites feeding mostly on cell tissue of the gills and muscles (IGLESIAS et al., 2001; JIN et al., 2009; PIAZZON et al., 2014; ROSSTEUSCHER et al., 2008).

This study reports the occurrence of scuticociliatosis followed by an acute infection in three ornamental marine reef fish species imported into Brazil, Vanderbilt's Chromis (*Chromis vanderbilti*), blue-green damselfish (*Chromis viridis*), and sea goldie (*Pseudanthias squamipinnis*), as well as chronic lesions and recovery after treatment of *C. viridis*.

Materials and Methods

Ornamental marine fish *C. vanderbilti* ($n = 3$), *C. viridis* ($n = 10$), and *P. squamipinnis* ($n = 3$) were obtained from quarantine following importation into São Paulo State, Southeast Brazil. The fish had been captured from their natural environment in Kenia, Indian Ocean and were maintained in aquaria (2.8 m³, with a recirculating system) and fed five times a day with the commercial diet (Tetra® Marine flakes and granules). The monitored water temperature was maintained at 26 °C, salinity 29 ppt, dissolved oxygen 5 mg L⁻¹, pH 8.2, and total ammonia <0.1 mgL⁻¹.

Six *C. viridis* and two *P. squamipinnis* had arrived dead in the transport bags and, 24 h following acclimation in quarantine, other fish presented with behavioral disorders such as gasping, swimming on water surface, starvation and lethargy and depigmented skin

lesions (Figure 1a); the latter culminated, in <48 h, in hemorrhagic and ulcerative lesions and fish death.

The diseased fish were examined by a qualified veterinary service as part of the disease diagnostic investigation. Scrapings of the body surface and gills and internal examination were performed.

Five specimens of *C. viridis* and one of *P. squamipinnis* with lesions on the body surface were euthanized by brain concussion and fixed in buffered 10% formalin solution for histopathology. Fragments of lesions, gills, and internal organs were carefully removed and processed according to an accepted histological process, embedded in paraffin as posterior cross sections of 5 µm, and stained with hematoxylin-eosin. The slides were examined and photomicrographs taken using an Olympus BX60® (Olympus Optical Co., Ltd., Tokyo, Japan) equipped with an image analyzer (Image Pro Plus 6.1 for Windows, Copyright© 1993-2006, Media Cybernetics, Inc.).

Other fish from the same imported group (20 *Chromis vanderbilti*, 150 *Chromis viridis*, and 20 *Pseudanthias squamipinnis*) showed mild clinical signs but with no visible lesions, and these were maintained in a bath, incorporating a recirculating system, with 37% formalin at a concentration of 0.125 mL L⁻¹ of water for 60 min, with constant aeration. After treatment, they were transferred to the hospital aquarium (total volume 0.45 m³) where they received prophylactic treatment with neomycin sulfate at 6 g 100 L⁻¹ for 5 days. During this period, the fish were treated with two further baths of formalin (0.125 mL L⁻¹ of water for 60 min, 3 and 7 days after harvesting). After treatment, 10 specimens of *C. viridis* were collected for skin scrapings analysis. Some fish showing clinical recovery and healing of ulcerative lesions presented with blackish skin nodules, and lesions of one *C. viridis* were collected for histopathological analysis.

Results

Fresh mounts of skin scrapings from all diseased fish showed a heavy parasite load of a pyriform-shaped ciliate protozoan suggestive of *Uronema* sp. (Figure 1a). No other type of parasite was found.

Histopathological analysis showed invasion by scuticociliates in the skin layers, compromising the skeletal muscle. Hemorrhages, inflammation composed by mononuclear and granular cells in the skeletal muscle, cutaneous necrosis, muscle fiber edema, and necrotizing myositis were observed. In the gills, the parasites had caused necrosis of the secondary lamellae, and an inflammatory infiltrate of eosinophilic granulocytes was observed; this latter feature was also observed in the renal capsule.

Seven days post treatment, the scrapings from 10 *C. viridis* individuals showed no parasites. These fish were then isolated and monitored for observation of blackish nodules secondary to tissue repair (Figure 2a). The presence of these nodules renders such fish commercially worthless. These fish showed granulomatous lesions on the skin (Figure 2b, c) and adjacent skeletal muscle (Figure 2d), the lesions in most cases containing deposits of brownish pigment and with a necrotic center.

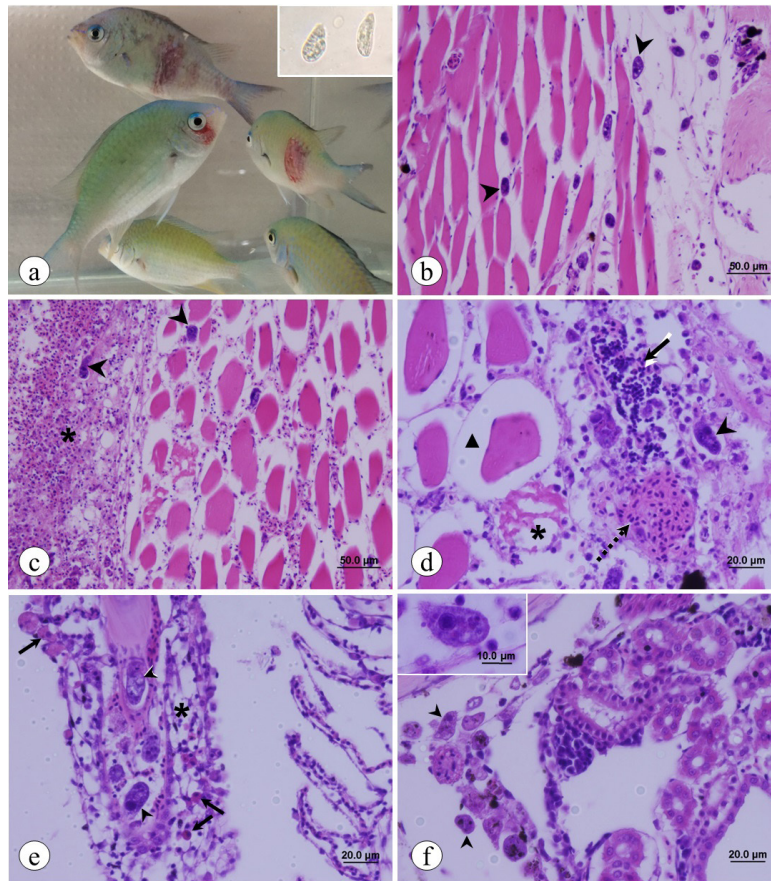


Figure 1. *Chromis viridis* showing hemorrhagic ulcerative lesions on the skin (a); presence of scuticociliates invading the subcutaneous tissue and skeletal muscle (a-b, arrow heads), with necrosis, hemorrhages and inflammatory infiltrate in the subcutaneous tissue (c - asterisk); edema of the muscular fiber (d - ▲), necrotizing myositis (d - asterisk) with mononuclear inflammatory focus (d - continuous arrow) and hemorrhages (d - dotted arrow); protozoans invading the gill tissue (e - arrow head) with vacuolar degeneration, necrosis (e - asterisk) and inflammatory infiltrate composed by eosinophilic granulocytes (e - continuous arrows); parasites invading the renal capsule (f - arrow head). Stained with hematoxylin-eosin (b-f).

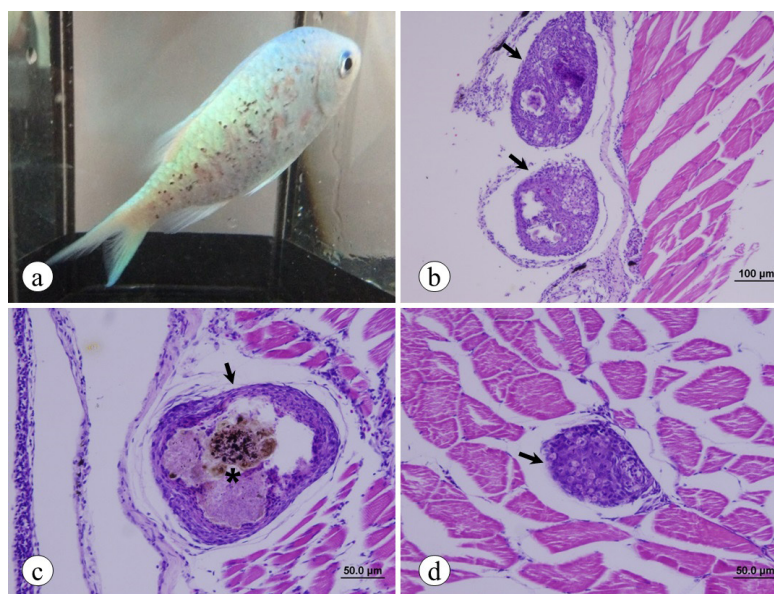


Figure 2. *Chromis viridis* showing blackish nodules on the skin and complete healing of the ulcerative lesions (a). Dermatitis and reminiscent granulomatous myositis (b-d) with some granuloma containing deposits of brownish pigmentation into the necrotic site (c - asterisk). Stained with hematoxylin-eosin (b-d).

Discussion

Improved water quality and earlier diagnostic and therapeutic measures with freshwater baths followed by formalin treatment enhance the prognosis of this condition (personal communication). *In vitro* studies showed the efficacy of 100-200 mg L⁻¹ formalin baths for 120 and 60 min against ciliate protozoans; weak (25-50 mg L⁻¹) and strong (167-250 mg L⁻¹) formalin baths are commonly used to treat ornamental fish (CROSBIE & MUNDAY, 1999).

In contrast, in the absence of early diagnosis and treatment, the disease can develop rapidly, affecting not only the body surface but also the muscles, visceral cavity, kidney, pancreas, liver, swim bladder, and brain, causing deep ulceration and death (GILL & CALLINAN, 1997; IGLESIAS et al., 2001; AZAD et al., 2007; JIN et al., 2009). Recovered fish lose their natural color and present with blackish areas on the body surface, rendering them worthless commercially.

Scuticociliatosis is frequently accompanied by bacterial infection, as previously reported in the seahorse *Hippocampus kuda* (DECLERCQ et al., 2014). Mortality in Japanese flounder can reach 70-80%, as reported by Moustafa et al. (2010a). The lesions herein observed such as hemorrhages, inflammatory reaction in the skeletal muscle and necrosis of the secondary lamellae were similar to those previously related (MOUSTAFA et al., 2010a).

The lack of epidemiological data on fish diseases and asymptomatic carriers, allied to the absence of control by post-frontier importers, increases the risk of diseases in native fish and subsequent economic losses. Importer facilities must adopt biosecurity measures to prevent the introduction and dissemination of diseases. The quarantine period for fish in Brazil is only 7 days (BRASIL, 2008), which is not sufficient in most cases to detect the etiological agent(s). Australia and New Zealand carry out rigorous risk analysis and quarantine for exotic animals, comprising three weeks of quarantine and regular diagnostic procedures (WHITTINGTON & CHONG, 2007).

Taking into account the severity of scuticociliatosis in *C. viridis* and the transboundary potential of *Uronema* sp., it is necessary not only to improve the best management practices but also to implement biosecurity with rapid, accurate diagnosis to minimize the risk of economic losses and introduction of new diseases to fish farms.

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