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Short Communication

Scuticociliatosis caused by *Uronema* sp. in ten different ornamental aquarium reef fish in Brazil

Scuticociliatose causado por Uronema sp. em dez diferentes peixes ornamentais de recife no Brasil

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Abstract

Scuticociliatosis, caused by an opportunistic ciliate protozoan, is responsible for significant economic losses in marine ornamental fish. This study reports the occurrence of *Uronema* spp., parasitizing ten species of marine reef fish at an ornamental fish wholesaler: Blue green damselfish (*Chromis viridis*), Vanderbilt's Chromis (*Chromis vanderbilti*), Pennant coralfish (*Heniochus acuminatus*), Threespot angelfish (*Apolemichthys trimaculatus*), Goldspotted angelfish (*Apolemichthys xanthopunctatus*), Sea goldie (*Pseudanthias squamipinnis*), Orchid dottyback (*Pseudochromis fridmani*), Threadfin butterflyfish (*Chaetodon auriga*), Vagabond butterflyfish (*Chaetodon vagabundus*), and Bluecheek butterflyfish (*Chaetodon semilarvatus*). Diseased fish showed disorders such as hemorrhages and ulcerative lesions on the body surface. Histopathological analysis of the muscle, liver, gut, kidney, spleen, gills, and stomach revealed hemorrhages and degeneration of muscle fiber, vacuolar degeneration of hepatocyte, inflammatory process and granuloma in the liver, atrophy of intestinal villi, inflammatory process and granuloma in the kidney, melanomacrophage centers, as well as inflammatory process in the spleen, epithelial cells hyperplasia and granuloma formation in the gills, and vacuolar degeneration and eosinophils in the stomach. Due to the severity of the disease, it is necessary to implement biosecurity measures with rapid and accurate diagnosis to minimize the risk of economic losses caused by *Uronema* spp.

Keywords: Ornamental reef fish, fish health, Scuticociliatia, histopathology.

Resumo

Scuticociliatose, causada pelo protozoário ciliado oportunista, é responsável por significativas perdas econômicas em peixes marinhos ornamentais. O estudo relata a ocorrência de *Uronema* spp., parasitando dez espécies de peixes de recife em um distribuidor de peixes ornamentais: "Blue green damselfish" (*Chromis viridis*), "Vanderbilt's Chromis" (*Chromis vanderbilti*), "Pennant coralfish" (*Heniochus acuminatus*), "Threespot angelfish" (*Apolemichthys trimaculatus*), "Goldspotted angelfish" (*Apolemichthys xanthopunctatus*), "Sea goldie" (*Pseudanthias squamipinnis*), "Orchid dottyback" (*Pseudochromis fridmani*), "Threadfin butterflyfish" (*Chaetodon auriga*), "Vagabond butterflyfish"

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(*Chaetodon vagabundus*), e "Bluecheek butterflyfish" (*Chaetodon semilarvatus*). Peixes doentes apresentaram distúrbios como hemorragias e lesões ulcerativas na superfície do corpo. A análise histopatológica do músculo, fígado, intestino, rim, baço, brânquias e estômago revelou hemorragias e degeneração das fibras musculares, degeneração vacuolar de hepatócitos, processo inflamatório e granuloma no fígado, atrofia das vilosidades intestinais, processo inflamatório e granuloma no rim, centros de melanomacrófagos e processo inflamatório no baço, hiperplasia das células epiteliais, bem como formação de granuloma nas brânquias e degeneração vacuolar e eosinófilos no estômago. Devido à gravidade da doença, é necessário implementar medidas de biossegurança com diagnóstico rápido e preciso para minimizar o risco de perdas econômicas causadas por *Uronema* spp.

Palavras-chave: Peixes ornamentais de recife, saúde animal, Scuticociliatia, histopatologia.

Introduction

Scuticociliatosis, a severe disease that compromises marine ornamental fish, is caused by marine ciliates of 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 is responsible for high fish mortality rates (Piazzon et al., 2014). The parasite produces proteases which digest the host's tissues and proteins (Al-Marzouk & Azad, 2007). Clinical changes include macroscopic and ulcerative lesions on the fins and body surface, exophthalmia, and in the advanced stage, a swollen visceral cavity. Infected fish may also present with internal changes that could be observed on histopathological examination, such as brain liquefaction, hemorrhages, and muscle ulceration. Microscopic observation shows the parasites feeding mostly on the cell tissue of the gills and muscles ulceration (Iglesias et al., 2001; Azad et al., 2007; Rossteuscher et al., 2008; Jin et al., 2009; Moustafa et al., 2010a, b). Microscopic observation shows the parasites feeding mostly on the 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 acute infection in ten different ornamental marine reef fish species: Blue green damselfish (*Chromis viridis*), Vanderbilt's Chromis (*Chromis vanderbilti*), Pennant coralfish (*Heniochus acuminatus*), Threespot angelfish (*Apolemichthys trimaculatus*), Goldspotted angelfish (*Apolemichthys xanthopunctatus*), Sea goldie (*Pseudanthias squamipinnis*), Orchid dottyback (*Pseudochromis fridmani*), Threadfin butterflyfish (*Chaetodon auriga*), Vagabond butterflyfish (*Chaetodon vagabundus*), and Bluecheek butterflyfish (*Chaetodon semilarvatus*), and the lesion caused by the parasite in the histopathologic analyses in Blue green damselfish, Sea goldie, Orchid dottyback, and Goldspotted angelfish.

Materials and Methods

Animals

Ten ornamental marine reef fish species C. viridis (n = 1), C. vanderbilti (n = 1), H. acuminatus (n = 1), A. trimaculatus (n = 1), A. xanthopunctatus (n = 1) P. squamipinnis (n = 1), P. fridmani (n = 1), Chaetodon auriga (n = 1), C. vagabundus (n=1), C. semilarvatus (n = 1), with skin ulcerations (Figure 1) were studied. The animals were collected from an ornamental fish wholesaler from São Paulo city, SP, Brazil, which reported a mortality rate of 20% in fish with similar lesions.

Figure 1. (a) *Chromis viridis*, (b) *Chromis vanderbilti*, (c) *Apolemichthus xanthopunctatus*, (d) *Pseudanthias squamipinis*, (e) *Pseudochromis fridmani* and (f) *Chaetodon semilarvatus* with ulceration in the skin (arrow).

Diseased fish were examined by a qualified veterinary service as part of the disease diagnostic investigation and then anesthetized in a eugenol solution 75 mg L^{-1} (Roubach et al., 2005) For 2 to 6 min and euthanized by the spinal cord section technique (Noga, 2010). Scrapings of the body surface, gills, and internal organs were examined.

Bacterial isolation and identification

Samples were taken from the eye, skin ulcers, kidneys, and gills, and plated in MacConkey, CHROMagar Orientation™, and sheep blood agar 5% (Difco-BBL, Sparks, MD, USA). The agar plates were incubated under aerobic and anaerobic conditions for 24 h at 37°C. Selected colonies were screened by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) identification. MALDI-TOF MS sample preparation, data processing, and analysis were performed as described by Hijazin et al. (2012).

For MALDI-TOF MS identification, the spectra were loaded into MALDI BioTyper™ 3.0 and compared with the manufacturer's library. Standard Bruker interpretative criteria were applied. Scores ≥ 2.0 were accepted for species assignment and scores ≥ 1.7 but ≤ 2.0 for genus identification.

Histopathology

The gill, gut, kidney, liver, skin, stomach, and spleen of four fish (*C. viridis*, *P. squamipinnis*, *P. fridmani* and *A. xanthopunctatus*) were fixed in buffered 10% formalin solution for histopathological analysis. Fragments of lesions 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 analyzed in a computerized system for image analysis (Qwin Lite 3.1, Leica Microsystems) in a microscope DM 5000 B equipped with a differential interference contrast (DIC) system.

Results

Fresh mounts of skin scrapings from all diseased fish showed heavy parasitic load of a pyriform-shaped ciliate protozoan suggestive of *Uronema* spp. No other species of parasite was found, except bacteria.

Nine infection profiles were detected in the fish lesions. Seven samples were positive for *Shewanella putrefaciens*, one for *Plesiomonas shigelloides*, and one for *Vibrio alginolyticus*.

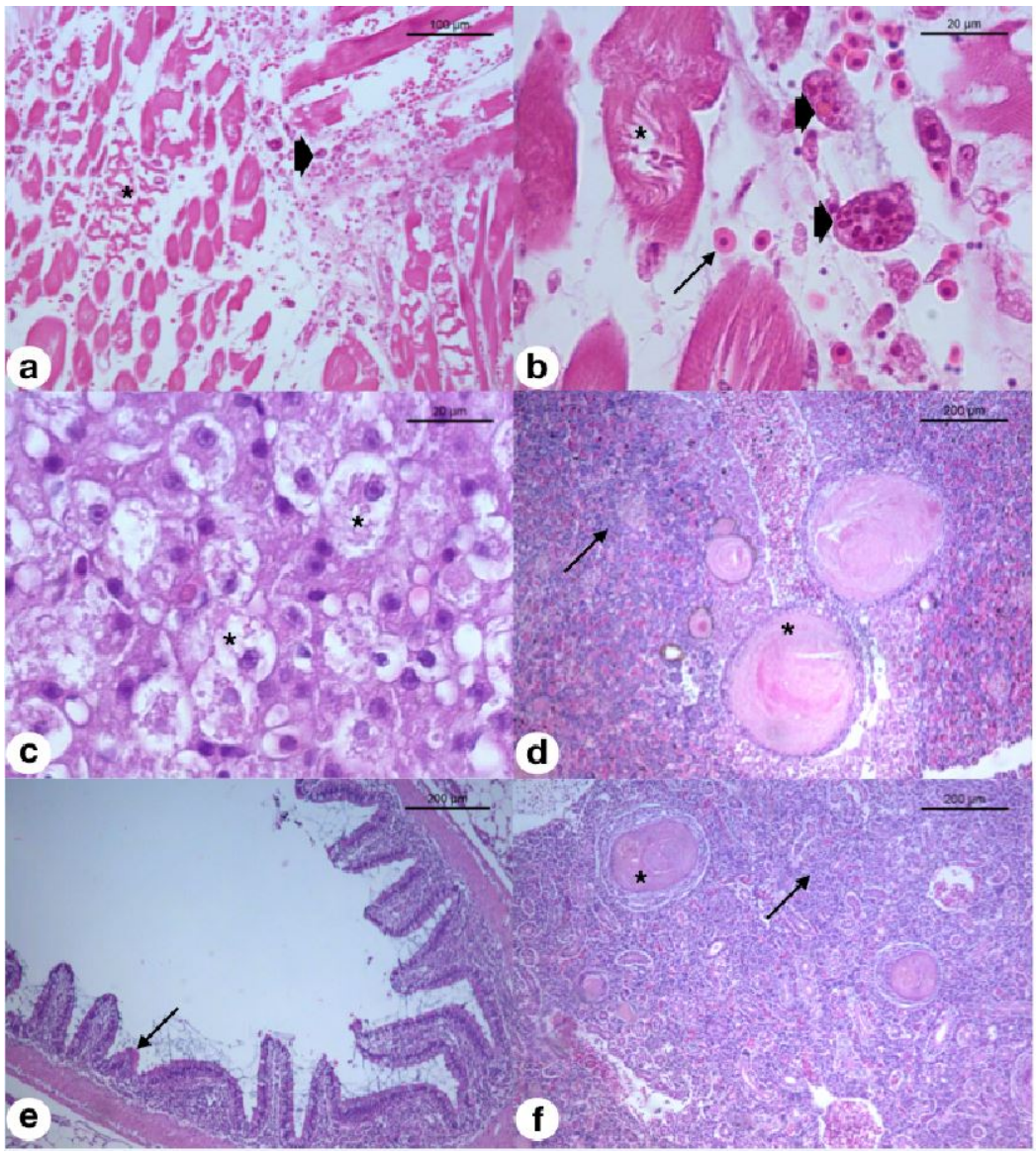

Figure 2. Histological alterations caused by *Uronema* spp. in the muscle, liver, gut, and kidney. (a-b) Presence of scuticociliates (large arrow), discrete hemorrhage (thin arrow), and degeneration of muscle fiber (asterisk); (c) Vacuolar degeneration of hepatocytes (asterisk); (d) Inflammatory process (arrow) and granuloma (asterisk) in the liver; (e) Atrophy of intestinal villi; (f) Inflammatory process (arrow) and granuloma (asterisk) in the kidney. Coloration: Hematoxylin and eosin.

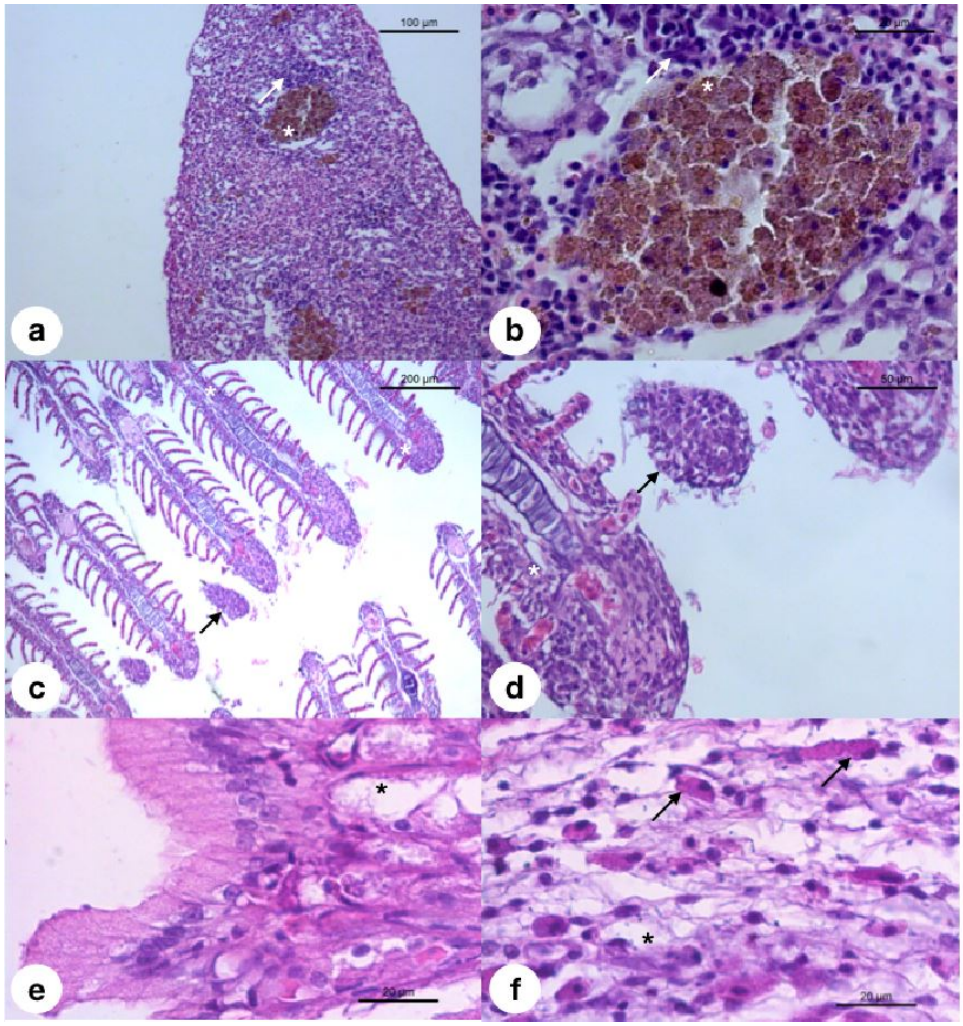

Figure 3. Histological lesions caused by *Uronema* spp. in the spleen, gills and stomach. (a-b) Melanomacrophage centers (asterisk) and inflammatory process (arrow) in the spleen; (c-d) Epithelial cells hyperplasia (asterisk) and granuloma formation (arrow) in the gills; (e-f) Vacuolar degeneration (asterisk) and eosinophils (arrow) in the stomach. Coloration: Hematoxylin and eosin.

Histopathological analysis showed moderate inflammatory processes composed of lymphocytes and macrophages in the gills and gut, and both moderate and massive inflammation in the liver, kidney, and muscle (Figures 2 and 3). Scuticociliates were observed between the secondary lamellae of the gills; in addition, mild to moderate eosinophilia, discrete hemorrhage, hyperplasia of epithelial cells, and lamellae fusion were noted. Bacterial colonization of the gut, liver, and muscle resulted in coagulative necrosis in the liver and stomach, congestion in the gut and kidney, and degeneration of muscle fibers and epithelial cells of the kidney tubules and glomerulus. Further analysis showed dystrophic calcification in the liver, presence of eosinophils in the stomach and muscle, granuloma in the liver and kidney, and hemorrhage in the liver, gut, muscle, and spleen. Hemosiderin deposits were found in the liver; hyperplasia of goblet cells of the gut and lysis, atrophy, and metaplasia of the intestinal villi were also observed. Proteinaceous material was discovered in the lumen of kidney tubules; vacuolar degeneration in the liver, gut, spleen, and stomach, and invasion of scuticociliates in the gut, muscle, and stomach were observed.

Discussion

In a study by Cheung et al. (1980), were described the fish species and sites of infection caused by *Uronema* in *Pimelometopon pulchrum* (kidney and muscle), *Tautogolabrus adspersus* (mesentery and muscle), *Hippocampus erectus* (gill and muscle), *H. kuda* (blood vessels, neural canal, urinary bladder, kidney, and connective tissue), *Hypsypops rubicunda* (muscle and skin), *Chaetodon unimaculatus* (muscle), *C. auriga* (muscle), *Chelmon rostratus* (muscle), and *Heniochus acuminatus* (muscle and heart). In our study, infection caused by *Uronema* spp. was detected in other ornamental fish species, such as *A. xanthopunctatus* (gill, gut, and stomach), *C. viridis* (muscle), *P.* (muscle), *P. fridmani* (muscle), *C. vanderbilti*, *A. trimaculatus, C. vagabundus,* and *C. semilarvatus* (skin). This was related to previous descriptions in *Pampus argenteus* (Azad et al., 2007), *Paralichthys olivaceus* (Kwon et al., 2003), *Polyprion oxygeneios* (Smith et al., 2009), *Scophthalmus maximus* (Sterud et al., 2000) *Dicentrarchus labrax* (Dragesco et al., 1995); *Thunnus maccoyii* (Munday et al., 1997), and *Seriola lalandi* (Smith et al., 2009). However, this is the first report of scuticociliatosis for *A. xanthopunctatus*, and *P. fridmani* using histopathological analyses and fresh mounts of skin scrapings and *C. vanderbilti*, *A. trimaculatus C. vagabundus* and *C. semilarvatus* (fresh mounts of skin scrapings) in Brazil.

Our study found the same lesions caused by *Uronema* spp. in fish previously described by Cheung et al. (1980), such as hemorrhage, numerous ciliates, and necrosis of the muscle, aneurysms, and epithelial detachment of gill lamellae. Furthermore, we report moderate to extensive inflammatory processes not observed by Cheung et al. (1980), showing the reaction of the host to parasitic infection. Another aspect that differs from the previous study is that we found bacterial colonization and granulomas, suggesting a secondary infection. *Shewanella putrefaciens,* the bacteria found in most of the lesions, is opportunistic and associated with saltwater or marine fish. It is frequently isolated from animals exhibiting ocular lesions, skin ulcer, and septicemia corroborating our results (Pękala et al., 2015).

Azad et al. (2007) also reported necrotic degeneration of the gill epithelium, eroded dermal epithelium and liquefied necrotic degeneration by inflammatory cells (monocytes, macrophages, and thrombocytes) of the underlying musculature, clogging of the kidney tubules, severe necrosis of the tubular and collecting ducts epithelium, sloughed intestinal mucosal epithelium with cell debris in the intestinal lumen, degeneration of the stomach musculature, necrotic degeneration of the epithelium of intestinal microvilli, and complete loss of mucosal epithelium of the stomach, which were also found in the current study.

Cardoso et al. (2017) recently described histopathological lesions similar to our findings here found. These authors reported invasion of 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, necrosis of the secondary lamellae and inflammatory infiltration of eosinophilic granulocytes in the gills, and inflammatory infiltration of eosinophilic granulocytes in the renal capsule. Severe degeneration of the internal organs, which was observed in this study, can be caused by proteases produced by *Uronema* sp. (Azad et al., 2007), such as metalloproteases, which have a high potential of destroying the host tissue (Lee et al., 2003).

The high incidence of lesions observed is probably due to decreased immune response caused by stress, bad handling, temperature, or changes in water quality parameters as previously described in other studies (Cheung et al., 1980; Munday et al., 1997; Iglesias et al., 2001).

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 (Azad et al., 2007; Gill & Callinan, 1997; Iglesias et al., 2001; Jin et al., 2009). Therefore, considering the severity of

scuticociliatosis in different species of ornamental reef fish, 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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