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Original Article

Association of *Epistylis* spp. (Ciliophora: Peritrichia) with parasitic crustaceans in farmed piava *Megaleporinus obtusidens* (Characiformes: Anostomidae)

Associação de *Epistylis* spp. (Ciliophora: Peritrichia) com crustáceos parasitas em piava *Megaleporinus obtusidens* de cultivo (Characiformes: Anostomidae)

Gabriela Pala[1]; Thaís Heloísa Vaz Farias[2]; Lindomar de Oliveira Alves[2]; Fabiana Pilarski[2]; Estevam Guilherme Lux Hoppe[1]*

[1] Laboratório de Enfermidades Parasitárias, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – UNESP, Jaboticabal, SP, Brasil

[2] Laboratório de Microbiologia e Parasitologia de Organismos Aquáticos, Centro de Aquicultura, Universidade Estadual Paulista – UNESP, Jaboticabal, SP, Brasil

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Abstract

Parasitic diseases have caused significant problems to global aquaculture production. These studies will further our knowledge of this complex problem and help implement adequate prevention measures and control strategies. The present study aimed to investigate the presence of parasites in *Megaleporinus obtusidens* and to describe the epidemiology and pathology of parasitic infections in these fish. Five moribund fish were sent for parasitological examination. The integument and gills were scrapped off with a glass slide, and samples were examined under a light microscope. Parasitic crustaceans found in these specimens were submitted for scanning electron microscopy and histological analyses. The crustaceans *Dolops carvalhoi* and *Lernaea cyprinacea* and the *Epistylis* spp. were present in all fish examined. *Epistylis* spp. were also seen on the entire surface of the crustacean integument. Microscopic lesions observed in the parasitized gills included hyperplasia and hypertrophy of the lamellar epithelium, an inflammatory infiltrate, telangiectasia, foci of hemorrhage and necrosis, fusion of the secondary lamellae, and detachment of the lamellar epithelium. Crustacean parasites are important mechanical vectors of *Epistylis* infection and disseminate the disease in fish farming operations. *Epistylis* spp. infection affects the health of fish and has significant ecological and economical impact on aquaculture.

Keywords: Aquaculture, epistyliasis, ectoparasites, epibionts, mechanical vector.

Resumo

Doenças parasitárias causam problemas significativos a produção mundial de peixes. Esse estudo aprofundará nosso conhecimento neste complexo problema e ajudará implementar estratégias de prevenção e controle. O objetivo deste trabalho foi analisar os parasitas encontrados em *Megaleporinus obtusidens* de piscicultura extensiva e descrever as relações epidemiológicas e patológicas entre eles. Cinco peixes moribundos foram enviados para análise parasitológica. O tegumento e as brânquias foram raspados com lâminas de vidro e examinados em microscópio óptico. Os crustáceos parasitas foram processados para análises histologicas e de microscopia eletrônica de varredura. Todos os peixes analisados foram infestados pelos crustáceos *Dolops carvalhoi*, *Lernaea cyprinacea* e pelo *Epistylis* spp. *Epistylis* spp. foram também encontrados na superfície de todo tegumento dos crustáceos parasitas. As brânquias parasitadas apresentaram hiperplasia e hipertrofia do epitélio lamelar, infiltrado inflamatório, telangectasia, focos hemorrágicos e necróticos, extensas áreas com fusão de lamelas secundárias e desprendimento de epitélio lamelar. Os crustáceos parasitas são vetores mecânicos importantes da epistilíase, disseminando o microorganismo nas criações de peixes. A infestação por *Epistylis* spp. afeta a saúde dos peixes e tem impacto ecológico e econômico significativo na aquacultura.

Palavras-chave: Aquicultura, epistilíase, ectoparasitas, epibiontes, vetor mecânico.

***Corresponding author:** Estevam Guilherme Lux Hoppe. Laboratório de Enfermidades Parasitárias, Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – UNESP, Via de Acesso Paulo Donato Castellane, s/n, Bairro Rural, CEP 14884-900, Jaboticabal, SP, Brasil.
e-mail: e.hoppe@gmail.com

Introduction

Farmed fish are often exposed to inadequate environmental factors including excessive organic matter and high population density which result in reduced dissolved oxygen (GHIRALDELLI et al., 2006; ZANOLO & YAMAMURA, 2006). Unfavorable conditions induce a stress response in fish (TEROVA et al., 2005) which predisposes them to a number of diseases including parasitic infestations (ENGERING et al., 2013). High parasite loads can cause irreversible damage to both integument and gills of infected fish (PAPERNA, 1991).

Crustaceans are one of the most significant pathogens in aquaculture especially those of the subclasses Branchiura and Copepoda (JITHENDRAN et al., 2008). and occur in fish farms and in natural habitats of fish as well (LUQUE et al., 2013; TAVARES-DIAS et al., 2015), *Argulus* and *Dolops* are the genera of branchiuran crustaceans most frequently found in fish (MARTINS et al., 2002; TAVARES-DIAS et al., 2007). These crustaceans are blood-sucking parasites and are capable of attaching to the integument and gills of fish (THATCHER & BRITES-NETO, 1994).These arthropods move rapidly, and break through the epithelium of the fish skin using their oral appendages (CARVALHO et al., 2004). *Lernaea* and *Lamproglena* are the genera of copepods most often described in fish (LUQUE & TAVARES, 2007). These crustaceans attach firmly to the integument and gills of their fish hosts causing marked epithelial damage and severe vascular injury (INNAL et al., 2017).

Mortality outbreaks were reported in production fish parasitized by crustaceans (CARNEVIA & SPERANZA, 2003), such as reports of epizootics in Atlantic salmon infested by sea lice (Copepoda, Caligidae), in which they cause intense spoliation, loss of epithelium, hemorrhages, increased secretion of mucus, and necrosis of the affected tissue (COSTELLO, 2006). Disease outbreaks and mortality caused by crustaceans have been reported in farmed fish (CARNEVIA & SPERANZA, 2003). In epizootics of sea lice (Copepoda, Caligidae) infestation in Atlantic salmon, affected fish had severe spoliation, loss of the skin epithelium, hemorrhage, increased mucus secretion, and tissue necrosis (COSTELLO, 2006). In addition to the damage caused by branchiuran crustaceans and copepods on their fish hosts, these arthropods can also act as disease vectors. *Caligus rogercresseyi* (sea louse) was recently described as a mechanical vector of Infectious salmon anemia virus (NYLUND et al., 1993; OELCKERS et al., 2014).

Parasitic crustaceans may also serve as substrate for epibionts such as *Epistylis* spp. (SILVA et al., 2011; AZEVEDO et al., 2014; CÔRREA et al., 2016). These protozoa are often considered commensal organisms in fish farms. However, in adverse environmental conditions they can cause damage to the integument of *Oreochromis niloticus* (Nile tilapia) (VALLADÃO et al., 2015) and *Pseudoplatystoma reticulatum* (barred sorubim)/*Pseudoplatystoma corruscans* (spotted sorubim) hybrids (PÁDUA et al., 2016).

The objectives of the present study are to report the association between *Epistylis* spp. and parasitic crustaceans in the headstander *Megaleporinus obtusidens* (known as piava or piapara in Brazil) and to describe the lesions produced by ciliate and crustacean co-infections in this fish host.

Materials and Methods

Fish and study area

Megaleporinus obtusidens (Characiformes: Anostomidae) (known as piava or piapara in Brazil) is an omnivore native fish with a wide geographical distribution, and occurs in the watersheds of the southern and southeastern regions of the country (HARTZ et al., 2000).

This species of fish is ideal for intensive cultivation, and has good consumer acceptance due to the taste of its meat and its size as well; it can weigh up to 7.5 kg (GLUSCZAK et al. 2006; ADORIAN et al. 2017).

In this study, fish examined for the presence of parasites originated from an extensive fishing establishment located in the county of Araraquara, State of São Paulo, southeast Brazil. They were cultivated in earth ponds and marketed to several fish-and-pay fishing camps in the area. The producer claimed that several fish from this farm died without any apparent cause, and that affected fish were coughing at the water entrance of the tanks.

Parasite analysis

Five moribund adult piavas were captured. In order to avoid detachment of parasites from the skin, fish were euthanized immediately after being captured; the spinal cord was severed with a knife or scalpel. Mucus was collected from the skin and gills of these fish by gently scrapping off their body and branchial surfaces with a glass slide. Mucus samples were examined under the light microscope. Parasitic crustaceans that were found in these specimens were gently removed from the surface of the glass slide with anatomical tweezers. Some parasites were stored in 70% alcohol while others were fixed in 2% glutaraldehyde for subsequent scanning electron microscopy analysis. Identification of parasitic crustaceans was based on the morphological characters of these arthropods and according to the information published by Robinson & Avenant-Oldewage (1996) and Thatcher (1991). Images were captured using a Nikon microscope E200® equipped with a Motic 5.0 image capture system. The ecological descriptors are in accord those published by Bush et al. (1997).

Histopathological analysis

Gill specimens were fixed in 10% formaldehyde and then preserved in 70% alcohol.

These tissue samples were dehydrated in alcoholic solutions of increasing concentrations and then embedded in paraffin. Sections of 5 µm were obtained using a microtome, and were stained with hematoxylin and eosin. All lesions were photographed under a light microscope.

Scanning electron microscopy analysis

Crustacean and gill samples were submitted for scanning electron microscopy analysis. Specimens were fixed in glutaraldehyde with 2% sodium cacodylate buffer and washed overnight in osmium tetroxide. Samples were dehydrated in alcoholic solutions of increasing concentrations. Critical point drying was used to remove water from these tissues. After that, samples were carefully placed in aluminum specimen stubs and bathed with gold. Images were captured using a JEOL JSM- 5410 electron microscope.

Results

Parasite identification

All fish were parasitized with the crustaceans *Dolops carvalhoi* (YAMAGUTI, 1963) and *Lernaea cyprinacea* (EIRAS et al., 2010) and by the epibiont *Epistylis* spp. with prevalence rates of 100% for all three parasites. The mean parasite intensity of *Dolops carvalhoi* was 155.6 ± 61.31 (106-229). We were unable to count *Lernaea cyprinacea* since these copecods are fairly small and difficult to visualize with the naked eye, and fish were considered too large [average weight 2.22 kg ± 0.37 (1.8-2.7) and total length 60.6 cm ± 8.11 (54-74)] to be examined under the stereomicroscope.

Host-parasite relationship

Large numbers of the ciliate *Epistylis* spp. were found attached to the integumentary surface of both *D. carvalhoi* and *L. cyprinacea* (Figure 1). *Epistylis* spp. was also attached to the secondary lamellae of each gill filament from these fish. Tissue damage of varying severity was present in all fish infested with parasites. Microscopic lesions observed in the parasitized gills included hyperplasia and hypertrophy of the lamellar epithelium, an inflammatory infiltrate composed of eosinophils, telangiectasia, foci of hemorrhage and necrosis, extensive fusion of the secondary lamellae, and detachment of the lamellar epithelium (epitheilai lifting) (Figure 2).

Discussion

Fish ectoparasites are important mechanical vectors in aquatic environments, and are capable of transferring pathogens from infected fish to healthy fish or between wild fish and farmed fish (XU et al., 2007). Crustaceans are mechanical vectors of a number of pathogens in aquatic ecosystems including viruses, bacteria, and parasites. Parasitic crustaceans can have a major economic impact on commercial fish culture, and can pose a significant threat to species diversity within an aquatic habitat (OVERSTREET et al., 2009). Gnathiid isopods, for example, are vectors of haemoggregarines which are haemoprotozoans that can cause mild chronic infection in fish from coral reefs (DAVIES & SMIT, 2001). The pathology of hemogreggarine infections in fish, albeit largely unknown, may have marked deleterious effects on fish farms (SMIT et al., 2006). The interaction between the branchiurian *Argulus foliaceus* (fish louse) and *Rhabdovirus carpio,* which causes spring viraemia of carp,

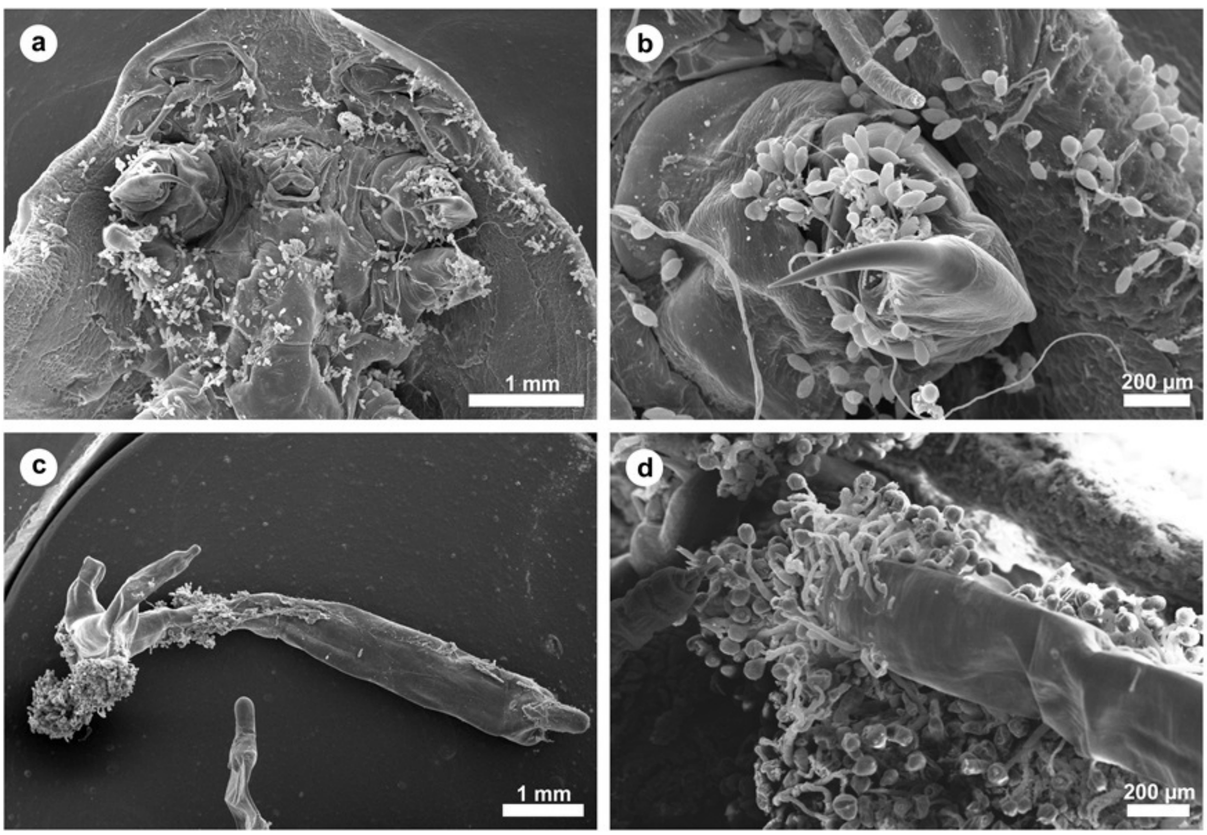

Figure 1. a) *Dolops carvalhoi* in ventral position with the intense presence of the adhesion of the protozoan *Epistylis* spp., scale bar: 1 mm. b) In detail a hook of the crustacean *Dolops carvalhoi* demonstrating the presence of *Epistylis* spp., scale bar: 200 µm. c) Panoramic view of a *Lernaea cyprinacea*, scale bar: 1 mm. d) Adhesion of *Epistylis* spp. in the cephalothorax of the crustacean *Lernaea cyprinacea*, scale bar: 200 µm.

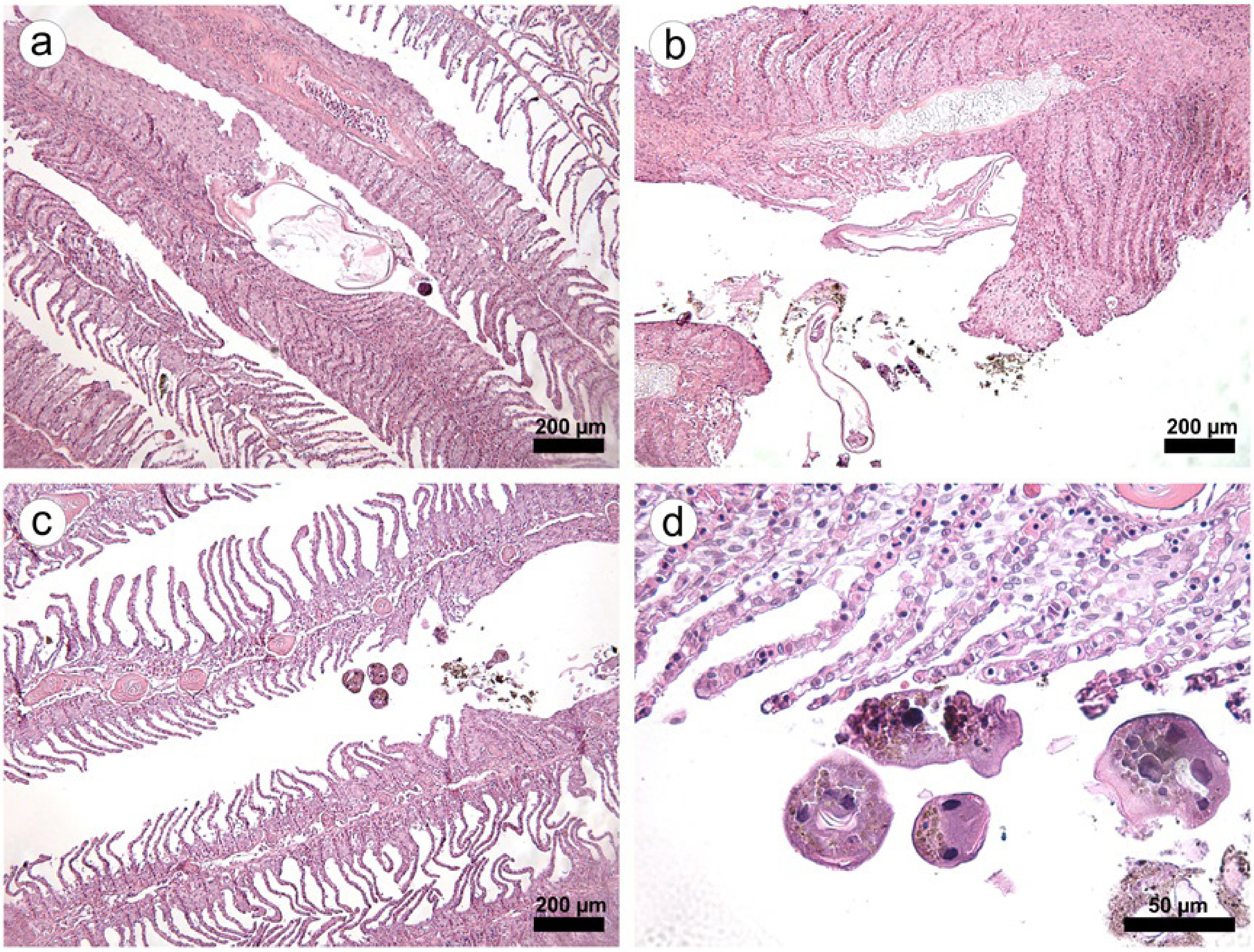

Figure 2. a) *Lernaea cyprinacea* adhered to a branchial filament, causing intense hypertrophy and hyperplasia epithelial, scale bar: 200 µm. b) Crustacean parasite and *Epistylis* spp. causing lamellar damage, with areas of hemorrhage and necrosis, and severe inflammatory infiltrate, scale bar: 200 µm. c) *Epistylis* spp. near areas of epithelial hyperplasia and scaling, scale bar: 200 µm. d) In detail, *Epistylis* spp. parasitizing branchial filament, scale bar: 50 µm.

is another example of a crustacean acting as a vector for diseases in fish. Crustaceans carrying *Rhabdovirus carpio* attach to the body surface of *Cyprinus carpio* (common carp) and transmit the virus to these fish (AHNE, 1985). High mortality rates have been recorded for spring viremia of carp (DIKKEBOOM et al., 2004).

To date, there are no publications reporting that the crustacean *D. carvalhoi* is capable of transmitting pathogens to fish even though other parasitic crustaceans may act as basibionts for *Epistylis* spp. including *Ergasilus chelangulatus* (AZEVEDO et al., 2014). In the present study, *L. cyprinacea* was found in fish skin mucus samples examined by light microscopy. Free-living crustaceans may also act as basibionts for ciliates. An example of such interaction is the association between the shrimp *Macrobrachium rosenbergii* (MANDAL et al., 2015) and the planktonic copepods *Pseudodiaptomus stuhlmanni* (JONES et al., 2016), *Thermocyclops minutus*, and *Notodiaptomus amazonicus* (CABRAL et al., 2016).

The ciliate epibionts *Epistylis* spp. are considered commensal protozoan organisms (VALLADÃO et al., 2015) but have been associated with a decrease in food conversion efficiency and increased susceptibility to predation in crustaceans (VISSE, 2007). Furthermore, *Epistylis* spp. contributed to mortality of the copepod *Boeckella triarticulata* in an environment with limited food availability (XU & BURNS, 1991). Fish parasitized by crustaceans for prolonged periods of time may develop chronic stress. Fish are more susceptible to infections, including epistiliasis, due to stress-induced immunosuppression (PICKERING & POTTINGER, 1989; TORT, 2011; WALKER et al., 2004). Parasitic crustaceans act as carriers for *Epistylis* spp., and disseminate this protozoan organism in fish farms.

This ciliate can cause a variety of lesions in fish such as damage to the scale epithelium including hemorrhage and necrosis (WELICKY et al., 2017). *Epistylis* spp. causes injury to the integument of the Nile tilapia *Oreochromis niloticus* which is characterized by the presence of a subepithelial infiltrate of lymphocytes and mast cells and epithelial hyperplasia and hypertrophy (VALLADÃO et al., 2015). Microscopic lesions that are seen in the integument of cultured cichlid fish and siluriformes (catfish) in association with the cilitate *Epistylis* include hydropic degeneration, multifocal necrosis, proliferation of skin mucous (globet) cells, and the presence of a mixed inflammatory infiltrates composed of granulocytes and mononuclear cells (PÁDUA et al., 2016). Studies have shown that *Epistylis* spp. is involved in severe outbreaks of *Aeromonas hydrophila* infection in farmed fish with high mortality. The ciliate is capable of propagating bacteria which adhere to their peduncles (HAZEN et al., 1978; MILLER & CHAPMAN, 1976).

The gill lesions found in our study are consistent with those previously described by other researchers in cases of macroparasite infection of fish (BYRNE et al., 2002; MARQUES & CABRAL, 2007). Branchial lesions in which the parasite is present are severe whereas those in adjacent areas without the parasites are mild. Since branchial lesions are usually focal, most of the unaffected gill surface area is still functional which allows fish to survive. Death ultimately occurs only in those cases of heavy parasite infestation in which extensive and severe gill lesions are present.

It is imperative to actively monitor parasitic diseases in farmed fish as they can cause significant problems to global aquaculture production resulting in major economic losses. In farmed fish, parasitic crustaceans cause erosions on the integument and gills and can also spread many pathogens including *Epistylis* spp. Further studies are needed to assess the impact of *Epistylis* spp. on fish health. The mechanisms involved in the attachment of parasitic crustaceans on the integument of fish should be investigated in order to further our understanding of the interactions between these arthropods and their fish hosts. Crustaceans parasites are important mechanical vectors of *Epistylis* spp. infection and Epistylis-Dolops-Lernaea complex affects the health of fish.

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