

Protozoan infections in farmed fish from Brazil: diagnosis and pathogenesis

Infecções por protozoários em peixes cultivados no Brasil: diagnóstico e patogênese

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Abstract

The Phylum Protozoa brings together several organisms evolutionarily different that may act as ecto or endoparasites of fishes over the world being responsible for diseases, which, in turn, may lead to economical and social impacts in different countries. Apart from the recent advances for the diagnosis of fish diseases in Brazil, little is known on the protozoan parasites and their relationship with environment and host. This revision presents the most important protozoan parasites found in farmed fish from Brazil, not only with emphasis on its diagnosis, biology, transmission and host-parasite relationship, but also on some information that may be useful to researchers in determining the correct diagnosis in fish farms.

Keywords: Fish parasites, disease, ciliate, dinoflagellate, pathogenicity.

Resumo

O filo Protozoa reúne diversos organismos evolutivamente distintos que podem atuar como ecto ou endoparasitos de peixes em todo o mundo, sendo responsáveis por doenças as quais, por sua vez, podem ocasionar impactos econômico e social nos diferentes países. Apesar dos recentes avanços no campo de diagnóstico de doenças em peixes no Brasil, ainda pouco se conhece sobre a fauna de protozoários parasitos de peixes e suas relações com o ambiente e hospedeiro. Esta revisão apresenta os mais importantes protozoários parasitos encontrados em peixes cultivados no Brasil, não apenas com ênfase no seu diagnóstico, biologia, transmissão e relação hospedeiro-parasito, mas também algumas informações que podem ser úteis para pesquisadores para o correto diagnóstico em pisciculturas.

Palavras-chave: Parasitos de peixes, enfermidade, ciliado, dinoflagelado, patogenicidade.

Introduction

The Phylum Protozoa gathers several organisms evolutionarily different that may act as ecto and endoparasites in fish (LOM & DYKOVÁ, 1992), as well as in other vertebrates and also invertebrates. They are the causative agents of diseases in the global aquaculture causing, among other things, damage and reduced growth of the host fish (MORAES & MARTINS, 2004), favoring secondary bacterial infections (XU et al., 2012) and mortality, all leading to constraints in global aquaculture production.

In this way, parasitic protozoan diseases are responsible not only for great losses to the commercial fishing industry, but also for a

negative social impact in developing countries where aquaculture activities contribute to food production of high nutritional value to the needy population (BONDAD-REANTASO et al., 2005).

A number of prophylactic and curative measures have been suggested, although many of the recommended chemicals may be over-used or misused by aquacultural workers, leading to parasite's potential drug resistance and negative impact on the aquatic environment. In this way, it must be highlighted the importance of disease impact in order to elaborate efficient strategies for early diagnosis and fast intervention in management practices in fish farms so as to obtain a stable and sustainable production (PÁDUA & CRUZ, 2014).

This revision presents the most important protozoan parasites found in farmed fish in Brazil, with emphasis on its diagnosis,

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distribution, biology, transmission routes and host-parasite relationship that may be useful to researchers in determining the correct diagnosis of fish pathogens.

Ciliophora

These unicellular protozoans possess mobile cilia involving the external body surface in some stage of their life cycle. Cytostome, macronucleus and micronucleus present. Reproduction by binary fission and conjugation. *Apiosoma*, *Balantidium*, *Chilodonella*, *Epistylis*, *Ichthyophthirius multifiliis*, *Nyctotherus*, *Rhynchodinium paradoxum*, *Tetrahymena* and Trichodinidae are the main representatives.

Apiosoma Blanchard, 1885

It comprises sessile peritrichid ciliated protozoans in the adult stage, with a conical body shape provided by contractile and nutritive vacuoles, infundibulum (oral cavity), scopula (from which the parasite attaches to host surface), peristomial disc, macronucleus and micronucleus (LI et al., 2008; EL-TANTAWY et al., 2013).

Like other sessile peritrichids such as *Epistylis* and *Heteropolaria* Foissner et Schubert, 1977, they use fish only for attachment and do not invade the epithelial cells, thus feeding by filtration of suspended material in the water. This phenomenon is termed epibiosis, in which the ciliate acts as epibiont and the host as basibiont (substrate organism) (PÁDUA et al., 2012b).

Life cycle

Apiosoma reproduces by binary fission and conjugation (LOM & DYKOVÁ, 1992). Apart from these strategies, they can develop non-sessile forms denominated telotroch (free-swimming migratory stage which detaches from the parent colony in order to search for new hosts for attachment) in the water.

Transmission

It is transmitted by the free-swimming infective telotrochs.

Diagnosis

Microscopic examination of fresh-mounted scraps of fish's skin, fins and gills is the main technique for diagnosis of *Apiosoma* (Figure 1a,b). As the parasite presents a robust and long (40-70 µm) body shape, it can be therefore easily diagnosed even in infections with low parasite densities. For a detailed view of the morphological features used for specific identification, the following staining techniques can be used: silver nitrate impregnation (Figure 1c), protargol and Giemsa (Figure 1d), Heidenhain, Ehrlich or Harris haematoxylin, as well as neutral red (LI et al., 2008). The main characteristics used for identification are body length and width; presence of scopula; body shape; position, length and width of

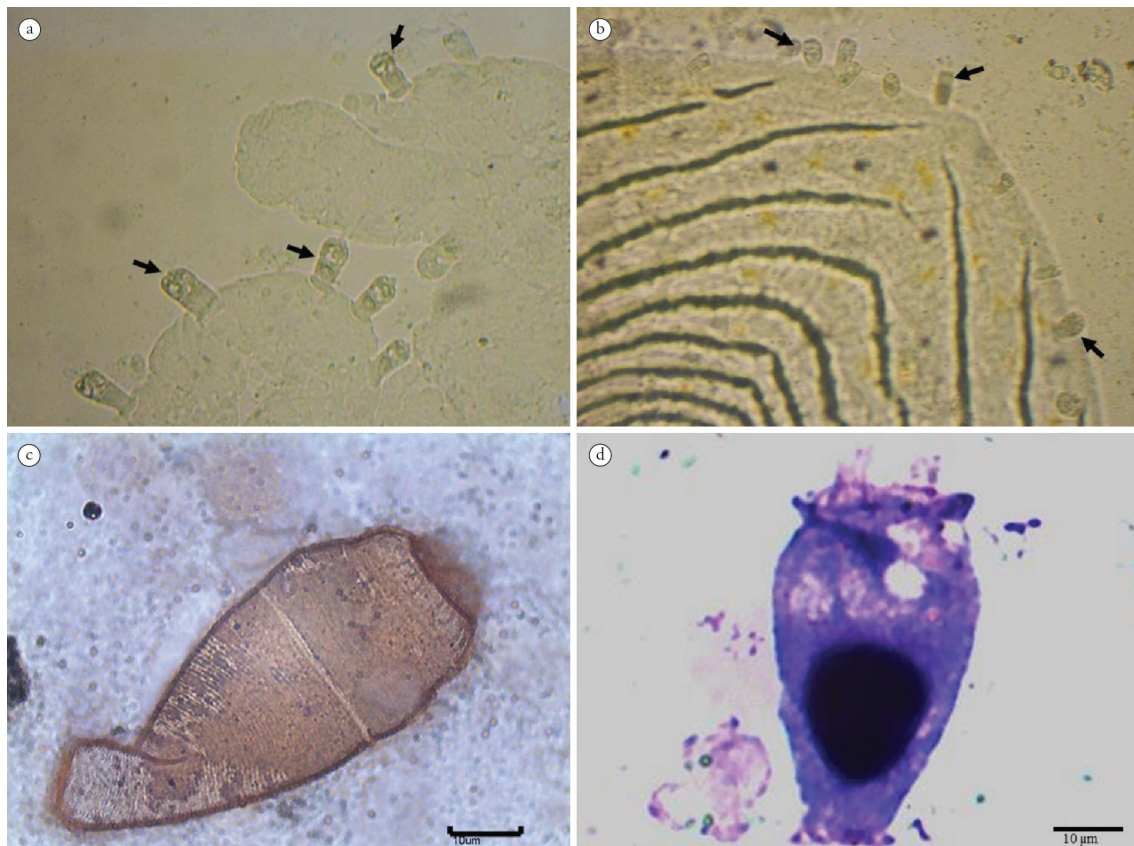


Figure 1. *Apiosoma* attached on the epithelium (a) and scale (b) of Nile tilapia larva *Oreochromis niloticus* in fresh-mounted slide. Silver nitrate impregnated specimen to observe the ciliary (c) and stained with Giemsa (d) showing the nuclear apparatus.

macronucleus; micronucleus diameter; position of contractile vacuole; peristomial disc diameter and peduncle width (LI et al., 2008; EL-TANTAWY et al., 2013).

Pathogenesis and clinical signs

As the parasite attaches on the host by scopula and do not invade epithelial cells, the pathological alterations are discrete or even less evident. When heavily infested on the fish gills, however, these parasites cause reduced breathing surface to gas exchanges on the gills. In this case, fish will experience respiratory distress and may be seen near the surface of the water, gasping for air (DURBOROW, 2003). Differently, it was observed displacement of scales possibly due to secondary infections on tilapia fingerlings highly infested on the skin (PÁDUA, S.B. personal communication).

Clinical signs may be seen in diseased fish but are not specific and are frequently associated with respiratory difficulty and hyperventilation. Skin color alterations like darkness can also be observed. Li et al. (2008) detected loss of equilibrium, low swimming on the water surface and anorexia. As with most protozoans, environmental debasement and crowded conditions cause them to become more damaging (DURBOROW, 2003).

***Chilodonella* Strand, 1926**

Leaf-shaped ciliated protozoans, oval, dorsoventrally flattened, slightly asymmetric (Figure 2a-c) and mobiles. Macro and micronucleus well evidenced (Figure 2c); the ventral surface has two longitudinal rows of ciliary kineties (Figure 2b,d) (PÁDUA et al.,

2013a). *Chilodonella* species are free-living but some of them parasitize the skin, gills and fins of both freshwater, marine and estuary fish (PÁDUA et al., 2013a). Only two species have been observed causing damage in fish: *Chilodonella hexasticha* Kiernik, 1909, mainly found in tropical fishes and *C. piscicola* (Zacharias, 1894) Jankowski, 1980 (syn. *C. cyprini* Moroff, 1902) mainly parasitizing fishes from subtropical and temperate waters. In Brazil, Pádua et al. (2013a) have reported for the first time *C. hexasticha* causing outbreak mortality in Nile tilapia (*Oreochromis niloticus*), pacu (*Piaractus mesopotamicus*), and tuiuba (*Gymnotus aff. inaequilabiatus*).

Life cycle

Chilodonellids show monoxenic life cycle with transversal division on the host (Figure 2d), besides sexual reproduction by conjugation (PYNE et al., 1974; PÁDUA et al., 2013a). Some researchers suggest that chilodonellids have one life stage capable of forming resistant cysts for parasite maintenance, but this strategy is not yet fully understood.

Transmission

The transmission of chilodonellosis occurs especially by direct contact among infested and healthy fish. The parasite disseminates in fish farming via routine utensils and water in fish transporting, which can be considered as the most important dispersion factors.

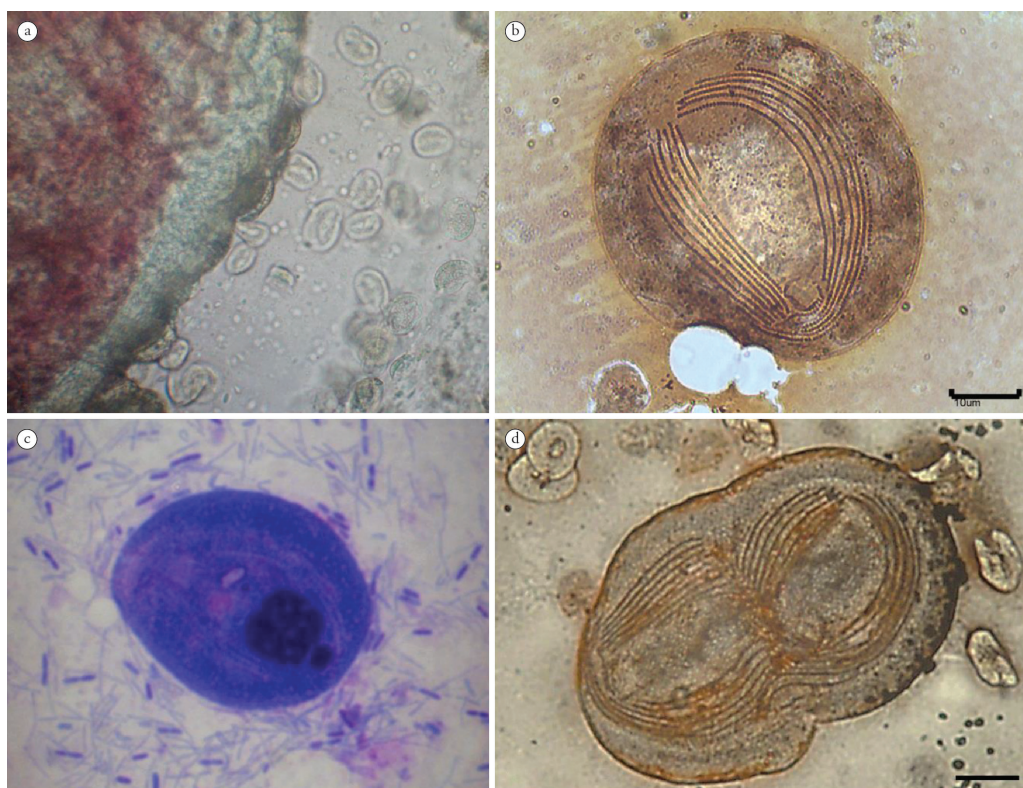


Figure 2. *Chilodonella hexasticha* observed in fresh-mounted slide from the gills of Nile tilapia *Oreochromis niloticus* (a), with detail of ciliary kineties in silver nitrate impregnation (b), nuclear apparatus and great amount of bacteria when stained by Giemsa (c), transversal fission of the parasite after silver nitrate impregnation (d). Bar: 10 µm (b,d).

Diagnosis

Diagnosis can be made from microscopic examination of fresh-mounted scraps of the skin, fins and gills of suspected fish. In fresh-mounted slides the rapid movement of the parasite, generally to a single direction can be easily detected. Silver nitrate impregnation technique allied to Giemsa or haematoxylin staining procedures are fundamental to observe the main taxonomic characters for specific identification (PÁDUA et al., 2013a). The numbers of ciliary kinetics constitute the most important taxonomic character that distinguishes *C. hexasticha* from *C. piscicola*. In this way, *C. piscicola* has more numerous and less spaced ciliary kinetics (KAZUBSKI & MIGALA, 1974).

Histological techniques also allow the researcher to find out the definitive diagnosis. However, not all details can be observed in infested tissue from histological sections, allowing only its determination at the generic level.

Pathogenesis and clinical signs

Infestations by *Chilodonella* cause severe lesions when compared to other ciliates with similar lifestyle, as for example trichodinids. Pathological changes caused by *Chilodonella* are related to its abrasive action on the host epithelium, being the gill filaments the most sensitive organ to the parasite attack. In acute cases, an increase in the mucus production with consequent congestion of the gills maybe found. Consequently in severely infested fish, epithelial proliferation, necrosis and desquamation culminating in blood capillary rupture and mononuclear inflammatory cell infiltrate can be found (PÁDUA et al., 2013a). Since host health

and defense system are strongly affected, the disease is frequently accompanied by bacterial opportunistic secondary infections (Figure 1c) that provoke systemic infection and host death.

Clinical manifestation comprises non-specific signs such as respiratory difficulty, loss of equilibrium and appetite. Apart from these alterations, whitish lesions on the gills, scaleness, darkened skin, skin and fins ulcers, and haemorrhagic areas are common in mixed infection with bacteria.

Epistylis Ehrenberg, 1830

Representatives of *Epistylis* are colonial ciliates with bell-shaped body, provided by a long peduncle (not contractile) in which, in the apex, is located the zooid cell with a nucleus, contractile vacuoles and cilia (LOM & DYKOVÁ, 1992). Similar to *Apiosoma*, this sessile peritrichid uses fish as a substrate for attachment (epibiosis) and feeds on suspended particles in the water (PÁDUA et al., 2012a). When present in a population under high stocking densities associated with bacteria that colonizes its peduncle, this ciliate might cause host damage and then be named as parasite. In Brazil, epistyliasis has been characterized as an emergent disease with greater impact in farmed catfish (ISHIKAWA et al., 2012; PÁDUA et al., 2012a,b, 2013b).

Life cycle

These ciliates rely on binary fission of the zooids (Figure 3b) for asexual propagation, but they may reproduce by conjugation on its sexual reproduction (ISHIKAWA et al., 2012). Similar to *Apiosoma*, it occurs as non-sessile telotrochs in the water column (ISHIKAWA et al., 2012).

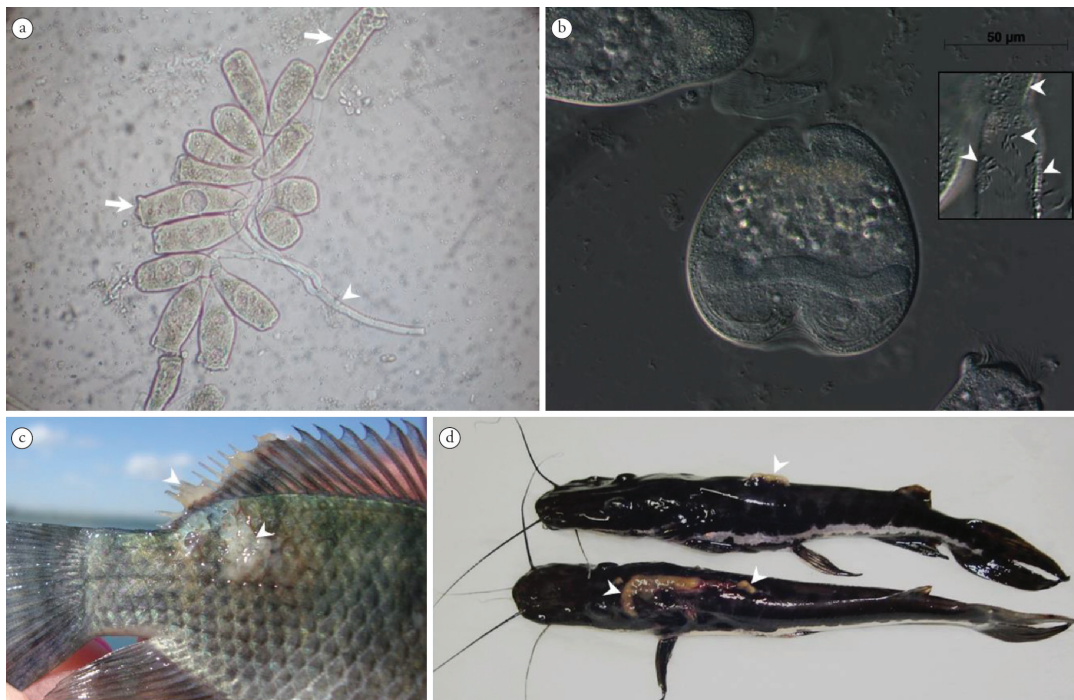


Figure 3. *Epistylis* sp. (a) in fresh-mounted slide under light microscope. Zooid in binary fission (b) and detail of bacteria (arrow heads) attached on the peduncle of the ciliate. Nile tilapia *Oreochromis niloticus* (c) with fin erosion and loss of scale (arrowheads) associated with *Epistylis* colonization, and hybrid surubim *Pseudoplatystoma* sp. (d) showing total erosion of dorsal fin and colonies of *Epistylis* sp. on the dorsal fin and head (arrow heads).

Transmission

Epistylia is transmitted from infective telotrochs searching for new hosts for attachment to develop new colonies. In addition, Pádua et al. (2013b) suggested that zooplanktonic microcrustaceans may act as vectors or reservoir hosts for *Epistylis* in farming conditions. In fact, it is common to find these ciliates fixed on the body surface of those organisms increasing thus disease dissemination (VISSE, 2007). Besides, it is a routine practice for fish farming to add manure to the ponds in order to improve the primary productivity of the water resulting in increased phytoplankton and zooplankton production for fish feeding. Nevertheless, as a result of the addition of nutrients into the water, the sanitary risk of an increase in the number of disease-vectors protozoans is imminent.

Diagnosis

Macroscopical observation of epistylia from whitish to yellowish colonies on the fish surface, fins, operculum, head and mouth should be accompanied by fresh-mounted slides observed in microscope (Figure 3a). This is especially true because observation of parasite colonies at first view (naked eye) can lead to misleading or confusing diagnosis since it can be easily confused with fungal infection. From microscopic observation, contraction movements of the zooids afford the definitive diagnosis. As complementary material, the histopathological analysis provides a more comprehensive view of the disease and its effect on tissues but do not preserve the important characteristics found in fresh-mounted material. In early infestation it is not possible to detect the colonies by the naked eye (PÁDUA et al., 2013b), being necessary to examine scraps from skin, fins and gills.

The identification at specific level from *in vivo* analysis must be complemented in samples impregnated with silver proteinate (protargol). Length and width of zooid, cell and colony shape, peristomial lips diameter, arrangement of buccal ciliary, nucleus shape and length and width of peduncle are important characteristics to be measured (LI et al., 2012).

Differential diagnosis

To confirm *Epistylis* on fish, microscopical analysis is fundamental, once the fungus *Saprolegnia* sp. can also develop colonies visible to the naked eye. It can be differentiated from the other sessile peritrichids, *Apiosoma* and *Ambiphrya*, by the formation of branched colonies. On the other hand, other ciliates not commonly found to be parasitizing fish such as *Zoothamnium* and *Carchesium* also develop branched colonies similar to *Epistylis*. Nevertheless, they differ from the latter in their peduncle contraction movement.

Pathogenesis and clinical signs

Its pathology is strongly associated with the presence of bacteria on the peduncle (Figure 3b, arrow heads). As they do not invade directly the host cells in the attachment process, neither pathological alterations in infested tissue nor substances are released on the host surface (HAZEN et al., 1978). Moreover, these authors confirmed the involvement of *Aeromonas hydrophila* as the

bacterium responsible in colonizing the parasite and secreting lytic enzymes that degrade the adjacent tissue. In this case, haemorrhagic alterations associated with peritrichid colonies in channel catfish can determine the *red-sore disease*. The colonization of parasite depends on the fish species and developmental stage of fish as well as the production system conditions.

In highly infested fish by *Epistylis* sp. it can be seen fin erosion and skin ulcers related to bacterial enzymes activity (Figure 3c,d). On histological sections, degeneration and epithelial necrosis adjacent to the colonies, intense desquamation, as well as increased mucus production and inflammatory infiltrate are also related (PÁDUA et al., 2012a). The fish death occurs generally after secondary invasion by opportunistic bacteria thus causing systemic infection.

Ichthyophthirius multifiliis Fouquet, 1876

The causative agent of ichthyophthiriasis or white spot disease is one of the most important fish parasites of worldwide distribution compromising skin, fins, gills and eyes of farmed fish. This parasite is not host specific and any freshwater fish can potentially transmit the parasite (EIRAS, 2013a). Similar to that found for channel catfish (*Ictalurus punctatus*) in the United States (XU & KLESZIUS, 2004), in Brazil it is considered an important parasitic disease in farmed hybrid surubim catfish (*Pseudoplatystoma* sp.) (ISHIKAWA et al., 2012), including the Central-North region of Brazil where water temperature variations are less frequent.

During a parasitological survey of ornamental fishes from North Brazil, Tavares-Dias et al. (2009) identified *I. multifiliis* in the gills of *Paracheirodon axelrodi*, *Hyphessobrycon copelandi* and *Dianema urostriatum*. Several fish species are susceptible to *I. multifiliis* including the silver catfish jundiá (*Rhamdia quelen*) (MARTINS et al., 2013) and the amazonian hybrid pintado (*Pseudoplatystoma* sp. x *Leiarius marmoratus*) are susceptible to *I. multifiliis*.

Life cycle

The life cycle of *I. multifiliis* is monoxenic and involves only a fish to be completed. Similar to *Amyloodinium* and *Piscinoodinium*, its life cycle has three stages as follows:

- I. Theront: infective and mobile form measuring 30 to 50 µm; it needs to find a host otherwise it will die. Theronts are provided by a structure named *perforatorium* used in fish cell invasion.
- II. Trophont: adult mobile stage found in fish epithelium, can reach 800 to 1,000 µm in diameter.
- III. Tomont: free form of the parasite provided by a cyst for protection. Attaches to plants and substrate to divide asexually and originate 500 to 1000 daughter cells named tomites that will differentiate in infective theronts and so search for a new host.

Transmission

Its transmission occurs by co-habitation (XU et al., 2007) with infested fish or directly from the theronts. Fishery utensils used in fish farms and water transport are potential vectors of ichthyophthiriasis. The release of theronts into the water is strongly associated with temperature. At water temperatures above

24°C, the life cycle is favored and completed rapidly. Differently, temperatures below 10°C or above 28°C can inhibit the parasite life cycle (ISHIKAWA et al., 2012). An exception, however, must be commented when it was observed mortalities of up to 50% in fingerlings of tambaqui *Colossoma macropomum* infected with *I. multifiliis* at Central-North and North regions of Brazil, when water temperatures ranged from 29.5 to 31.5 °C (PÁDUA, S.B. personal communication). In this way, studies on different strains of *I. multifiliis* to verify its susceptibility under uncommon conditions must be encouraged.

Diagnosis

Ichthyophthiriasis diagnosis is made based on macroscopical observation of trophonts within the host's skin and microscopical analysis of fresh-mounted material (skin, fins and gills scraps) between a slide and a coverslip (Figure 4a). Under an optical microscope, the observation of the mobile pear-shaped theronts and mature trophonts uniformly covered by a layer of external cilia and with a horse-shoe shaped nucleus confirms the diagnosis (Figure 4b). Histopathological analysis can also reveals the parasitosis (Figure 4c,d).

Differential diagnosis

Because of its similarity with the dinoflagellate *Piscinoodinium pillulare*, definitive diagnosis ought to be careful. This dinoflagellate shows similar color and shape to *I. multifiliis*, but does not swim

and has no cilia around the body. Moreover, *P. pillulare* has rounded nucleus instead of the horse-shoe shaped nucleus in *I. multifiliis*.

Pathogenesis and clinical signs

Pathological alterations associated with ichthyophthiriasis are related to theront invasion on the epithelium layers with posterior histophagia stage of trophont. This process provokes inflammatory reaction of the host with intense epithelial proliferation by goblet cells, and in severe cases it can be observed fusion of secondary lamellae, degeneration and epithelial cell necrosis forming several ulcers on the epithelium after the releasing of mature trophonts (PÁDUA et al., 2014).

The main clinical sign is the presence of white spots on the fish surface including skin, fins, eyes, buccal cavity and gills. It is common to observe fish with respiratory difficulty, flashing behavior in ponds and aquaria, and in cage-reared fish flashing on the sieve cages, leading to muddy water in earth ponds (MARTINS et al., 2000; ISHIKAWA et al., 2012).

Trichodinidae Claus, 1874

Trichodinids are mobile ciliates characterized by the presence of a body covered by a slender membrane surrounded by an adoral ciliary spiral, a horse-shoe shaped macronucleus and an adhesive disc provided with a denticulate ring in which the denticles are found (Figure 5c) (BASSON & VAN AS, 2006). In Brazil, the genera *Trichodina*, *Paratrachodina*, *Tripartiella* and *Trichodinella* have been found parasitizing aquatic animals (PÁDUA et al., 2011a), including zooplanktonic microcrustacean (SILVA et al., 2009), gastropod mollusc (PINTO et al., 2006), bivalve mollusc from mangrove

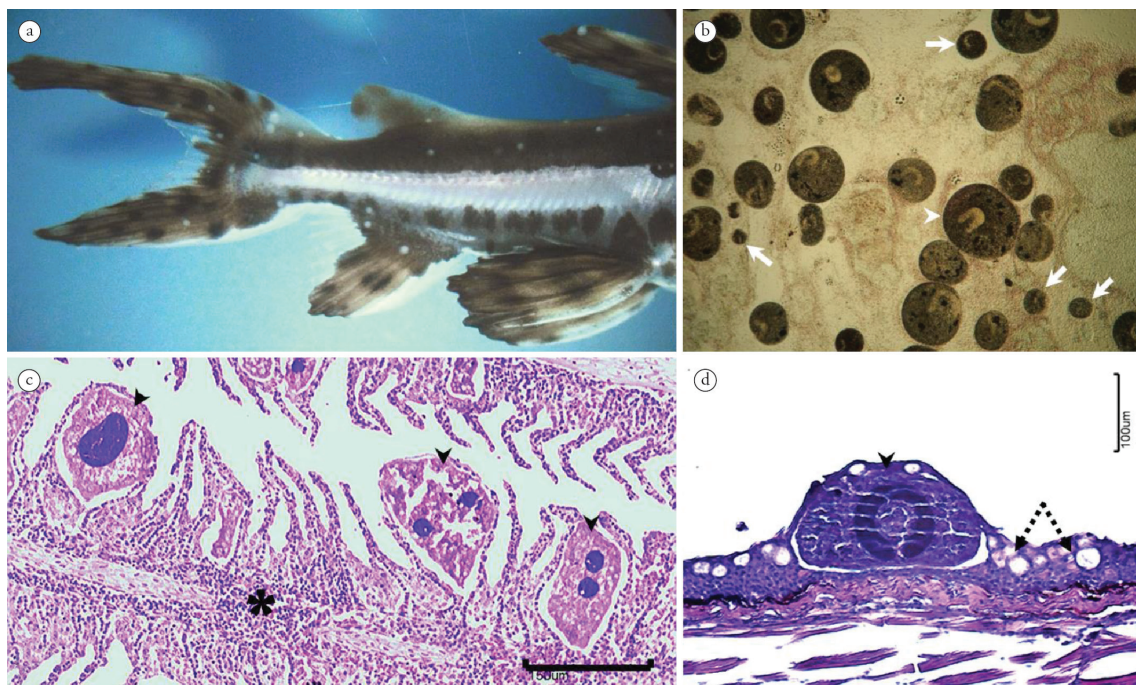


Figure 4. Hybrid surubim *Pseudoplatystoma* sp. presenting white spots on the skin and fins (a). *Ichthyophthirius multifiliis* observed in fresh-mounted slide from the skin: mature trophont and its horse-shoe shaped macronucleus (b - arrowhead) and several immature trophonts in developmental stages (b - continued arrow). Histological section of the gill lamellae (c), with inflammatory infiltrate (c - asterisk), and the parasite inserted in the skin of infected fish (d - arrow head). Proliferation of mucous cells next to the parasite attachment (d - dotted arrows). Bar: 150 µm (c) and 100 µm (d). Figure 4 a was obtained from Pádua et al. (2012b) and Figure 4 d from Pádua et al. (2014).

(SABRY et al., 2013), ornamental fishes (MARTINS et al., 2012), wild fishes (BITTENCOURT et al., 2014), as well as farmed fish (VALLADÃO et al., 2013) and amphibians (FERNANDES et al., 2011). Until 2006 trichodinids from farmed fishes in Brazil were recognized only at their generic level. From that year on, several studies have been performed in order to identify trichodinids at specific level (GHIRALDELLI et al., 2006; PINTO et al., 2009; JERÔNIMO et al., 2011; MARTINS et al., 2010a; MARTINS et al., 2012; MIRANDA et al., 2012; PÁDUA et al., 2012c).

Trichodinids can be found parasitizing both freshwater and marine fishes on the body surface, buccal cavity and gills. Nevertheless, relatively few of them have become endoparasites in the intestine, kidney and urinary bladder of their hosts (LOM & DYKOVÁ, 1992). Proliferation of the group in the environment seems to be associated with bad water quality, total number of bacteria and ecological aspects of the fish species. In this way, the use of trichodinids as an indicator for eutrophication in brackish-water environments was suggested (PALM & DOBBERSTEIN, 1999).

Their reproduction in fish farms has been related to high stocking density, high organic matter contents and increased water temperature (BASSON & VAN AS, 2006; MARTINS et al., 2010b). Differently, some trichodinid species were found to be suppressed with increased water temperature (YEMMEN et al., 2011).

Life cycle

Trichodinids have monoxenic life cycle and reproduce mainly by binary fission (conjugation is also possible) on the host (Figure 5d). In a short period of time they can reproduce rapidly and reach 100% prevalence and up to 299,100 parasites per host as observed by Martins et al. (2010b).

Transmission

Trichodinids can be horizontally transmitted by direct contact or by contaminated water in which the parasite searches for new hosts. Contaminated fish farming utensils are also another important source of trichodinids transmission.

Diagnosis

Trichodinid diagnosis can be made from scraps of skin, fins and gills of diseased fish observed under a stereomicroscope by an expert researcher or under a microscope (Figure 5a,b). In fresh-mounted material between a slide and a coverslip the parasites move rapidly in circle. The most important techniques for specific diagnosis are silver nitrate impregnation (Figure 5c) and staining by Giemsa or haematoxylin (LOM, 1958; VAN AS & BASSON, 1989).

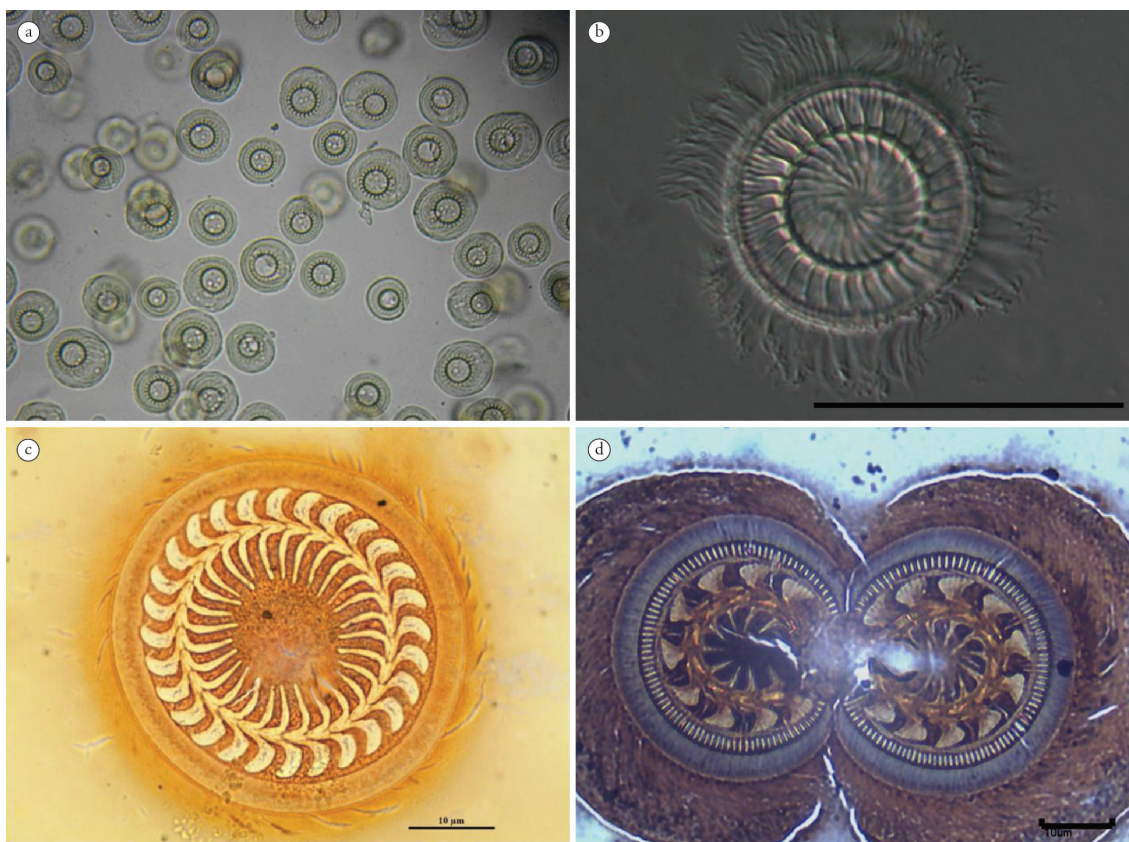


Figure 5. Trichodinids in fresh-mounted smear from the skin (a), *Trichodina centrostrigata* in differential interference contrast microscope (b), adhesive disc of *Trichodina magna* in silver nitrate impregnation (c) and an adhesive disc of a trichodinid during binary fission in silver nitrate impregnation (d). Bar: 50 µm (b) and 10 µm (c,d).

Histological sections can also allow identifying the parasites only at the family level, because the shape of the denticles varies within and between the genera.

Pathogenesis and clinical signs

According to Basson and Van As (2006), in a “firmly attached *Trichodina*, the rim of the border membrane “bites” into the surfaces of the fish’s epithelial cells, and the surface it encircles is forcibly vaulted as by a sucker”; these activities (attachment and rotating movements) may cause serious irritation and damage to the epithelial or epidermal cells of fish.

The disease may occur in acute form mainly in larvae and fingerlings causing ulcers, subepithelial oedema, displacement of the secondary lamellae in the gill filaments, hyperplasia and mononuclear inflammatory infiltration (VALLADÃO et al., 2014). On the other hand, in chronic infestations trichodinids may induce an increase in the mucus cells of the epithelium and gill filaments, discrete hyperplasia with partial or total fusion of the secondary lamellae, inflammatory infiltrate and gill necrosis (VALLADÃO et al., 2013). The micro-lesions caused by the presence of these parasites are next colonized by bacteria (VALLADÃO et al., 2014), which, in turn, are responsible for opportunistic infections and accelerate the progression of the disease thus causing outbreaks of mortality.

Clinical signs are not specific and diseased fish may present darkness of the skin, whitish areas in the gills, hypoxia and flashing on the ponds or aquaria surface (PÁDUA et al., 2011a). Lethargy and erratic swimming on the body surface are frequently found in diseased fish larvae (VALLADÃO et al., 2013).

Flagellata

Flagellate protozoans are mainly characterized by the presence of one or more flagella for movement. The majority of them are ectoparasites while others can be found parasitizing internal organs (EIRAS, 1994). They reproduce by longitudinal binary fission as for kinetoplastids or present a three-phased life cycle such as dinoflagellates. *Amyloodinium ocellatum*, *Piscinoodinium pillulare*, *Trypanosoma*, *Cryptobia* and *Ichthyobodo* are the main representatives.

Amyloodinium ocellatum Brown et Hovasse, 1946

Amyloodinium comprises dinoflagellates of varied shape depending on the life stage. The causative agent of velvet disease *A. ocellatum* is ubiquitous, affects marine farmed fish and may provoke important outbreak mortalities and economical losses in aquaculture systems (LEVY et al., 2007; PEREIRA et al., 2011; MOREIRA et al., 2013). The trophont stage is pear or rounded-shaped, has golden to brownish color, presents chloroplasts and is capable of feeding when adhered on the fish surface or gills by its rhizocysts. The tomont is the reproductive form (it encysts on the substrate) and the dinospore corresponds to the infective free-swimming stage with a longitudinal and transverse flagellum (NOGA, 1987; GUERRA-SANTOS et al., 2012; WOO & ARDELLI, 2014).

Life cycle

The life cycle has no intermediate hosts and presents the following three stages: dinospore, the infective form; trophont, that develops on the host; and tomont, the reproductive stage with consecutive divisions developed on the ponds or aquaria substrates (Figure 6). Reproduction by binary fission occurs when tomonts divide repeatedly producing the free-swimming infective dinospores (FRANCIS-FLOYD & FLOYD, 2011; WOO & ARDELLI, 2014).

Transmission

Obligatory parasite of monoxenic life cycle, *Amyloodinium* has low host specificity parasitizing mainly the gills followed by skin of farmed and wild marine and estuary fishes (REED & FRANCIS-FLOYD, 1994; ABREU et al., 2005; WOO, 2007). It can be transmitted by direct contact with infective dinospores present in the water. In open aquaculture systems, such as cage farming, the parasite can be easily transmitted to wild fish. In this way, Roberts-Thomson et al. (2006) related tomonts of *A. ocellatum* transported by dynamic airflow system until 2 meters from the source of infection. The capacity of transport or dissemination of tomonts was proved by those authors and constitute one more possibility of infection in indoor systems.

Diagnosis

Diagnosis of *A. ocellatum* is made from scrapings of body surface and gills between a glass slide and a coverslip to be observed in stereomicroscope or light microscope (MONTGOMERY-BROCK et al., 2001; ABREU et al., 2005; GUERRA-SANTOS et al., 2012). In addition, microscopic examination of histological

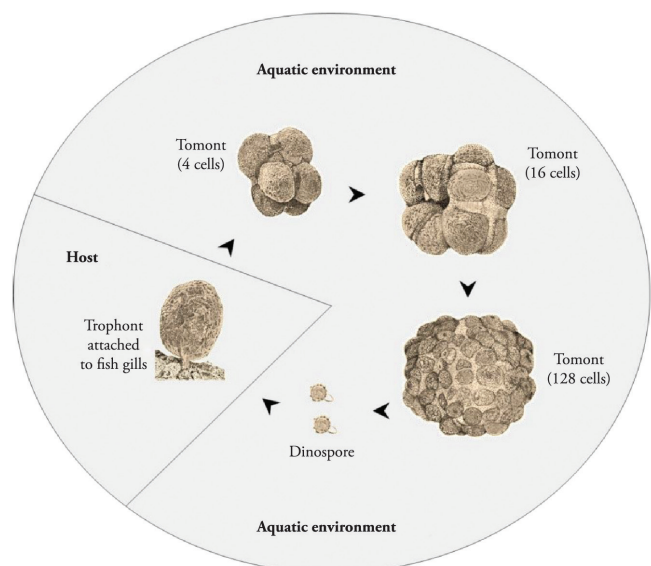


Figure 6. Life-cycle of *Amyloodinium ocellatum*. Modified from Pereira et al. (2011).

sections of parasitized tissue also reveals the presence of the parasite (GUERRA-SANTOS et al., 2012), while the serological exam with the presence of specific antibodies is yet little explored (CECCHINI et al., 2001). Its fixation is made directly in the slides with 5% formalin solution or in a flask with 10% buffered formaline solution for histopathology. The most applied staining techniques are May-grunwald and Giemsa or haematoxylin and eosin (GUERRA-SANTOS, 2011), as well as iodine to evidence the amyloid granules.

In dinospores, the presence of longitudinal and transverse flagella, length and width of cellular body, distribution pattern of epitheca and hypotheca plates and observation of a peduncle, rhizocysts, pores and nodules are the main characteristics for parasite identification (LANDSBERG et al., 1994), as well as genetic information and trophont measurements (ABREU et al., 2005).

Pathogenesis and clinical signs

Tissue alteration on the host is a result of the mechanical action of trophonts during the attachment process by the ryzoids on the epithelial cells. Among the acute pathological changes an increase in mucus production and gill congestion is common (GUERRA-SANTOS et al., 2012), frequently leading to vacuolar degeneration, epithelial rupture, hyperplasia and fusion of the secondary lamellae and necrosis in severely affected fish (CRUZ-LACIERDA et al., 2004; SARAIVA et al., 2011).

Anorexia, erratic swimming, lethargy, "flashing" onto the substrates, skin depigmentation, emaciation and branchial hyperventilation are the main clinical signs (ABREU et al., 2005; FRANCIS-FLOYD & FLOYD, 2011; GUERRA-SANTOS et al., 2012).

Piscinoodinium pillulare Lom, 1981

Causative agent of piscinoodiniasis or velvet disease has similar morphological and developmental characteristics of *A. ocellatum* but is considered an important parasite in freshwater fish (NOGA & LEVY, 2006). With worldwide distribution, this parasite is not host-specific (MARTINS et al., 2001) and is responsible for important sanitary problems in Brazil in native farmed fishes such as tambaqui (*Colossoma macropomum*), pacu (*Piaractus mesopotamicus*), pirapitinga (*Piaractus brachypomus*) besides their hybrids, piaçu (*Leporinus macrocephalus*) and curimbatá (*Prochilodus lineatus*), in hatchery of Amazonian pintado (*Ictalurus punctatus*) and less frequently in Nile tilapia (*Oreochromis niloticus*). This dinoflagellate was also reported in five ornamental freshwater fishes from an exporter's holding facility in the Amazonas State (TAVARES-DIAS et al., 2009). Under a microscope, it can be observed three forms of the dinoflagellate from the scraps of fish: pear-shaped, banana-shaped and the mature rounded parasite of brownish color (MARTINS et al., 2001; FOIN, 2005), although in high infestations different developmental stages can be found. Special care must be taken on the mature trophonts that could be confused with *I. multifiliis* by an inexperienced person.

Life cycle

Its life cycle is similar to *A. ocellatum* (three-phased cycle), with trophont, tomont and dinospore on the host or aquatic environment. Immobile trophonts adhere to hosts by their rhizocysts (KLINGER & FRANCIS-FLOYD, 1998; FOIN, 2005). Tomonts on the substrate undergo successive divisions in order to originate free-swimming dinospores provided with flagella.

Transmission

Piscinoodiniasis can be transmitted by contact with infested fish and dinospores from contaminated water (FOIN, 2005) and also by fish farming utensils with no disinfection after using them. The transport of live fish among fish farms represents an efficient way of transmission of infectious-parasitic diseases once the water can load infective or resistant stages of parasites, bacteria and viruses of sanitary importance.

Diagnosis

Traditional evaluation by scraps from the body surface and gills observed in stereomicroscope or microscope is used to diagnose piscinoodiniasis in fish. The detection of trophonts stage allows the definitive diagnosis in posterior slides fixed in methylic alcohol for 10 min and stained with diluted Giemsa (one drop per 1 mL of distilled water for 120 to 180 min) (Figure 7) or iodine staining to view the amyloid granules (MARTINS et al., 2001). Routine histopathological analysis may also provide definitive diagnosis by viewing the trophonts attached on the gill filaments. As low parasitic infestations do not cause clinical signs and disease in fish, prophylactic measures must be adopted.

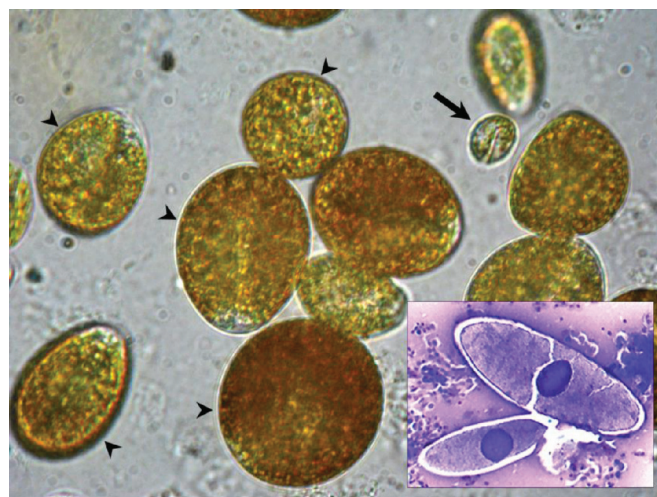


Figure 7. *Piscinoodinium pillulare* from gill scraps of tambaqui *Colossoma macropomum*. Pear-shaped trophonts, rounded (arrowheads) and one dinospore (arrow). In detail, two parasites stained in Giemsa exhibiting oval to rounded nucleus with the absence of micronucleus.

Pathogenesis and clinical signs

Pathological alterations are associated with the mechanical action of the parasite after attachment to the host. In this stage trophonts insert their rhizocysts from the fixing disc and invade the cytoplasm of epithelial cells of the host (LOM & SCHUBERT, 1983).

In low parasitic levels is common to observe an increase in the mucus production and gill congestion. On the other hand, in severe infections where the disease leads to a chronic form it has been observed proliferative alterations, including fusion of the secondary lamellae, mucus cell proliferation, inflammatory cell infiltration associated with degeneration and necrosis of the epithelial cells, subepithelial oedema, haemorrhages and ulcers (MARTINS et al., 2001). Recently, a case of mixed parasitism by *P. pillulare* and a myxozoan parasite in farmed fish was reported by Sant'Ana et al. (2012), when 90% of diseased fish died in 15 days.

Proliferative disturbs in the gills associated with the interlamellar presence of parasites are responsible for hypoxia, loss of equilibrium and erratic movements (SANT'ANA et al., 2012). Consequently, fish search for for more oxygen on the water surface or water inlet in ponds. Heavily infested fish present "flashing" on the substrate as a response of parasite's irritant action. Macroscopical observation of gills heavily infested by *P. pillulare* may reveal

brownish coloration (Figure 8a,b). Trophonts of *P. pillulare* attach mainly on the primary lamellae filling all the interlamellar space between the secondary lamellae (Figure 8c), where the rhizocysts can be found attached to the host epithelial cells (Figure 8d).

Trypanosoma Gruby, 1843

This haemoflagellate parasite is ubiquitous throughout freshwater and marine environments and may cause problems in aquaculture. Members of *Trypanosoma* present a slender body, elongated, cylinder-shaped with more or less thin extremities, free flagellum, undulating membrane besides the nucleus and kinetoplast (Figure 9a) and volutin granules disposed generally in the middle of the body (HUSSEIN et al., 2010).

Life cycle

These protozoans have a heteroxenic life cycle involving an annelid Hirudinea as an intermediate host and vertebrates as definitive hosts. However, little is known about the distinct stages of their life cycle infecting South American fishes and, additionally, they are rarely found in farmed fish. During the infectious process on the host, asexual reproduction by cellular division was described in infected knifefish *Gymnotus* aff. *inaequilabiatus* (PÁDUA et al., 2011b). Tripomastigote, epimastigote, amastigote and several dividing flagellate forms were observed in the stomach of leech

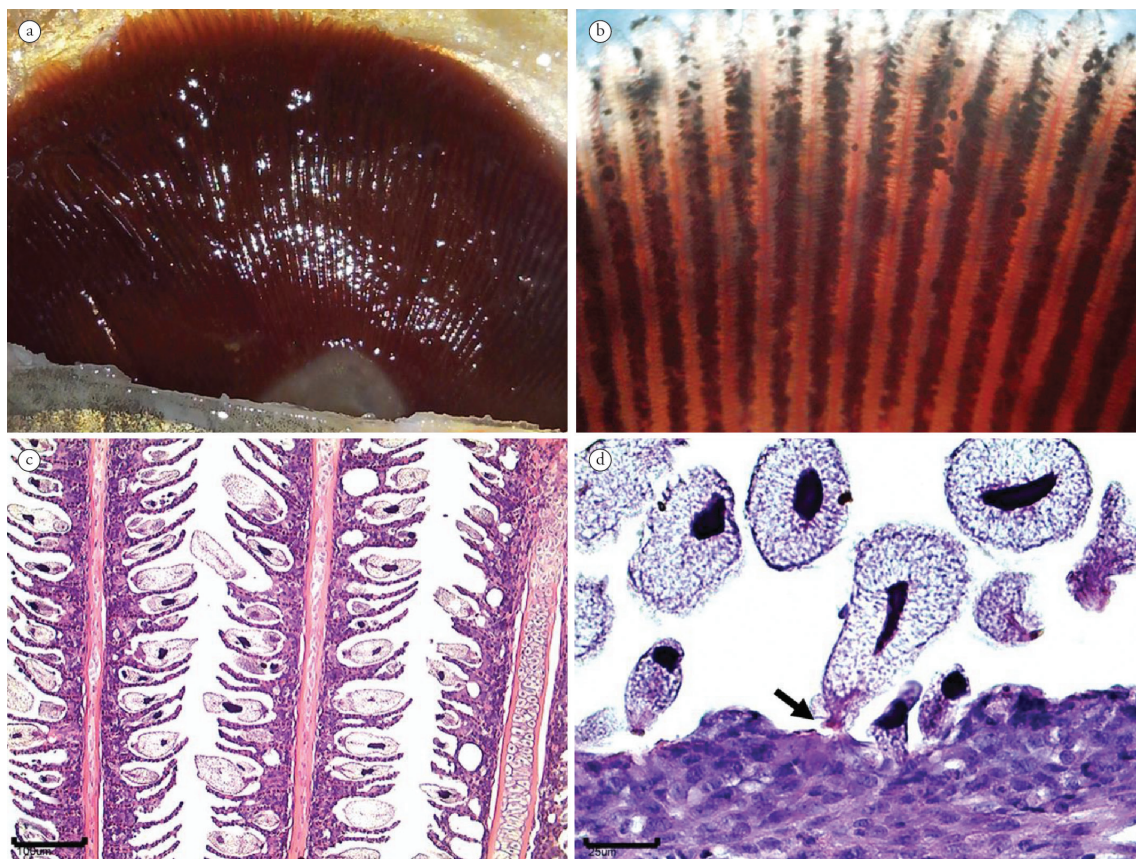


Figure 8. Pathological changes in fish with piscinoodiniasis. Brownish gill of tambaqui *Colossoma macropomum* (a), hundreds of parasites adhered in gill filaments observed in stereomicroscope (b), as well as from histological section (c), rhizocysts penetrating host's skin (d - arrow). Bar: 100 µm (c) and 25 µm (d).

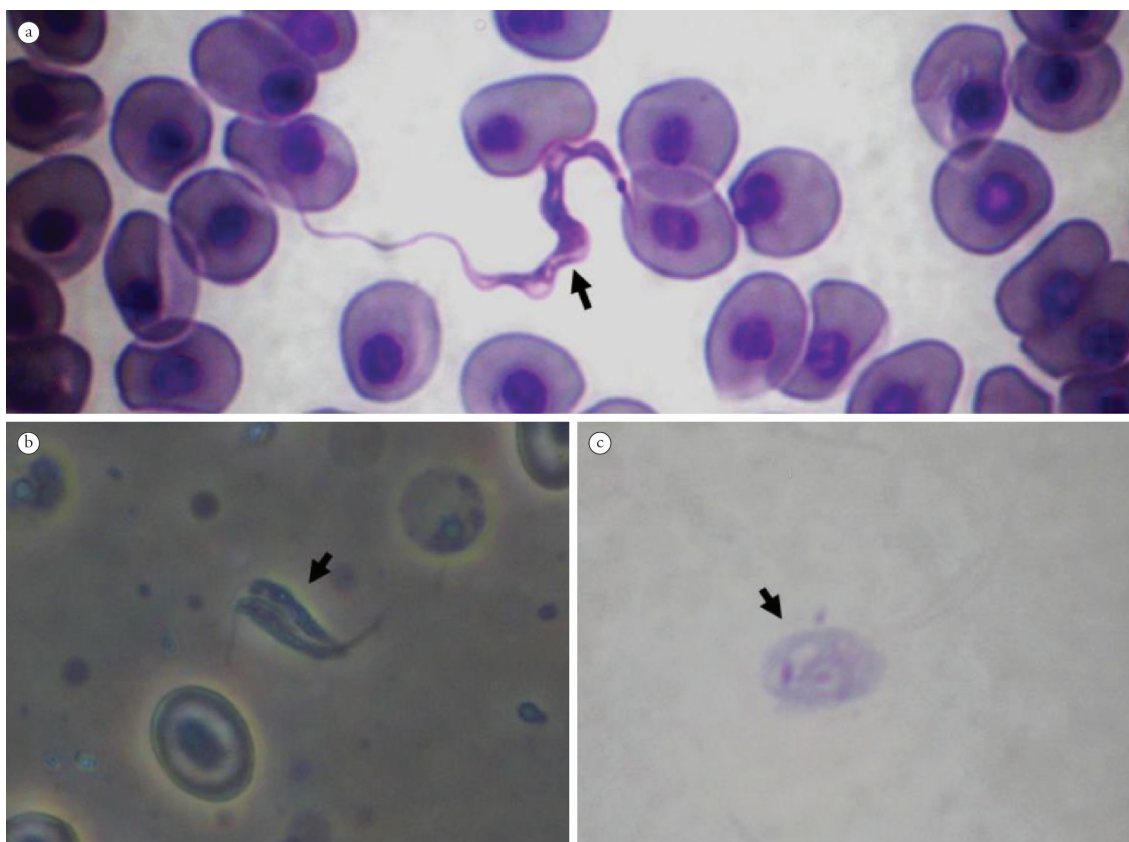


Figure 9. *Trypanosoma* sp. (a) observed from blood extension of tucuna *Gymnotus* aff. *inaequilabiatus*; *Cryptobia* sp. (b) from gill scraps of hybrid tambacu (*C. macropomum* x *P. mesopotamicus*) observed in a phase-contrast microscope and *Ichthyobodo* sp. (c) from skin of Nile tilapia *Oreochromis niloticus* previously stained with Giemsa. Figure 9 a was obtained from Pádua et al. (2011b).

Batrachobdella gemmata, vector of trypanosomiasis to the loriciid catfish *Hypostomus punctatus* (D'AGOSTO & SERRA-FREIRE, 1993).

Transmission

Transmission occurs during the leech parasitism on fishes (BRUNO et al., 2006) and in the case of loriciids, they are found attached to the regions of the body not covered by plates or on the gill filaments inoculating the infective form (D'AGOSTO & SERRA-FREIRE, 1993).

Diagnosis

Blood smears stained with a combination of May Grünwald-Giemsa or May Grünwald-Giemsa-Wright or only Giemsa are simple techniques for morphological characterization of the haemoflagellates. Nevertheless, this is a low-sensitive method and because of that, it is recommended to prepare fresh-mounted slides with a drop of blood between a glass and a coverslip as well as centrifuge the blood in a microhaematocrit tube for parasite concentration in order to provide high-sensitive method for diagnosis (WOO, 1969).

Histological sections of the liver, spleen and kidney stained with Ehrlich haematoxylin and eosin can improve the diagnosis

(HUSSEIN et al., 2010). Parasite identification at species level should be performed by analysis of morphological and morphometric characteristics (total length, width, nucleus length and width, distance from the nucleus centre to the posterior and anterior extremities, flagellum length and number of undulating membrane), besides application of molecular phylogenetic methods (MASLOV et al., 2001; HUSSEIN et al., 2010; FERREIRA & AVENANT-OLDEWAGE, 2013). Molecular tools for DNA detection are poorly explored in diagnosis of trypanosomiasis in fish from Brazil despite their recognized importance.

Pathogenesis and clinical signs

In case of cultured fish condition high levels of parasitism could be responsible for lethargy, gill paleness, splenomegaly, nephromegaly and altered color of the liver.

Cryptobia Leidy, 1846

These biflagellates have a triangulate to elongated body shape, a kinetoplast and a nucleus at the anterior end. Two flagella arise at the anterior end; the anterior flagellum is free while the recurrent one is attached to the body and extends beyond it as a free flagellum responsible for the movement (Figure 9b) (KUPERMAN et al., 2002; BRUNO et al., 2006). It can be found parasitizing either the gills or skin of marine and freshwater fishes around the world and the majority of them are ectocommensals. However, some

species are pathogenic for young fish (KUPERMAN et al., 2002; RANZANI-PAIVA et al., 2005).

Cryptobia branchialis Nie (Chen, 1956) has been registered in different continents (KUPERMAN et al., 2002). In Brazil, several occurrences of *Cryptobia* sp. exist (EIRAS et al., 2012), but so far little is known about the parasite species involved. High infestations by this flagellate are frequently diagnosed on pacu, tambaqui, pirapitinga and their hybrids, as well as Nile tilapia, the hybrid surubim (*Pseudoplatystoma* spp.) and hybrid Amazonian pintado.

Life cycle

Cryptobia presents monoxenic life cycle, with longitudinal fission occurring on the host to posterior detachment from the gills of the host to be free in water column (KUPERMAN et al., 2002).

Transmission

It presents horizontal transmission either by direct contact (host to host) or contaminated water with free-swimming infective forms. Fishery utensils as well as the water used in fish transport might be the source of parasitism in aquaculture facilities.

Diagnosis

Cryptobiosis can be diagnosed by observation of fresh-mounted smears of skin and gills under microscope. Later, infected smears can be stained with, for example, silver albumose (protargol) impregnation in order to provide a correct diagnosis. For fixation prior to impregnation, Hollande's fluid can be used (KOZLOFF, 2004). Observation of flagella, length and body width, nucleus diameter, cell shape and the *aciculum* (a bunch of microtubules to reinforce the parasite pharynx) are some of the most important diagnostic features for *Cryptobia*. Differently, the observation of flagella, disposition and contraction movement are fundamental information to the correct diagnosis. After that, the smears containing the parasites must be stained for posterior analysis under a light microscope (for example, protargol impregnation as recommended by Kozloff (2004)), in order to evidence the flagelli, nucleus, cell shape and the presence of *aciculum*. Bruno et al. (2006) used the techniques of Feulgen or Giemsa staining for observation of the kinetoplast.

A combination of fixative solution containing methylic alcohol for 10 min and later staining with diluted Giemsa (one drop per 1 mL of distilled water, for 120 to 180 min) affords a detailed observation of parasite morphology, although flagella are poorly evidenced. The length and body width, flagelli length, nucleus diameter and position as well as the length and position of kinetoplast, and the cell shape are used for identification at specific level (PUTZ, 1972; KOZLOFF, 2004).

Differential diagnosis

The diagnosis of *Cryptobia* sp. in the gills must be made carefully due to its similarity with *Ichthyobodo* spp. (both have two flagella). Nevertheless, *Ichthyobodo* is mainly found on the skin. In order

to differentiate both parasites, fresh-mounted microscope slides with special aware of flagella's arrangement are useful. *Cryptobia* sp. presents flagella with rapid movements while in *Ichthyobodo* the flagella move in circles.

Pathogenesis and clinical signs

Its pathogenicity is controverted and the findings of Kuperman et al. (2002) suggest that the parasite neither invades the host cells during the attachment process nor cause pathological alterations. However, observations from young tilapia highly infested by the parasite showed a direct relation with an increase in the gill mucus production, gill filaments oedema and reduced respiratory lamellae (KUPERMAN et al., 2002).

Ichthyobodo Pinto, 1928

Causative agent of ichthyobodiasis, these obligatory parasites are small biflagellated kinetoplastids found in skin, fins and gills of wild and farmed marine and freshwater fishes from temperate and tropical waters (ROBERTSON, 1985; LOM & DYKOVÁ, 1992; TODAL et al., 2004). Widely distributed, *Ichthyobodo necator* (Henneguy, 1883) was registered in different fish species from Brazil (EIRAS et al., 2012). Similar to *Cryptobia*, it is pear-shaped (Figure 9c) and when fixed on the host it shows circling or zigzag movements (LOM & DYKOVÁ, 1992).

Life cycle

Ichthyobodo presents monoxenic life cycle, asexual reproduction in which occurs the longitudinal fission of the parasite cell (ROBERTSON, 1985). Cells containing two pairs of flagella can be found moments before binary fission (LOM & DYKOVÁ, 1992).

Transmission

The parasitosis is horizontally transmitted by direct contact among diseased and healthy fish. Free-swimming infective parasites are responsible for disease dissemination as well as contaminated fishery utensils.

Diagnosis

Fresh-mounted smears of skin and gills viewed under microscope constitute the most employed technique for routine diagnosis. *Cryptobia* is more or less pyriform and has two unequal flagella extending posterior-laterally, while *Ichthyobodo* is more elongate-shaped and has two flagella, one posterior and one recurrent which is attached to the body forming the posterior free flagellum.

Smears previously fixed in methylic alcohol and stained with haematoxylin, Feulgen or Giemsa can be used for microscopical analysis (LOM & DYKOVÁ, 1992; TODAL et al., 2004; ISAKSEN, 2013). The distribution pattern and kinetoplast morphology are useful information for specific identification level (MOREIRA et al., 2004).

Histopathological analysis also presents an efficient tool for diagnosis of ichthyobodiasis (URAWA et al., 1991; BRUNO et al.,

2006), but constitutes a non-sensitive technique in low parasitism intensity. Molecular methods of DNA extraction are more efficient (ISAKSEN et al., 2012) but less used and unpractical as a routine diagnostic method.

Pathogenesis and clinical signs

In Brazil this parasite does not cause significant mortality and in most cases low intensity infections were recorded in larvae and young fishes. Among the pathological alterations, it has been observed destruction of the epithelial cells due to parasite attachment (LOM & DYKOVÁ, 1992), as well as degenerative and proliferative epithelial alterations with reduced mucus production (URAWA et al., 1991). Clinical signs are non-specific indicating respiratory difficulty and altered skin and gill color (URAWA et al., 1991; LOM & DYKOVÁ, 1992).

Other Parasites

Entamoeba Casagrandi et Barbagallo, 1897

Entamoeba belongs to the amoebae group, moves and feeds by using extensions of its cytoplasm (pseudopods) and reproduces by binary fission. According to Ponce-Gordo and Martínez-Díaz (2010), the main morphological characteristic, which distinguishes *Entamoeba* from other genera, is the morphology of the nucleus. When cysts are mature, the different species of *Entamoeba* present a characteristic number of nuclei, which can be 1 belonging to *bovis* group, 4 to *histolytica* group, 8 to *coli* group and as *gingivalis* group when the number of cysts is not known. Analysis of *Entamoeba* can be made with histological sections of the affected organ, after fixing in 10% formalin solution and staining with iron haematoxylin or Heidenhain-Mallory Azan (ORIAS & NOBLE, 1971). Smears of the intestine viewed under microscope with a drop of saline solution or iodine (BULLOCK, 1966) are used. Until the present, only *Entamoeba pimelodi* found in the intestine of yellow catfish *Pimelodus clarias* was observed parasitizing freshwater fishes in Brazil (CUNHA & PENIDO, 1926).

Balantidium Clarapède et Lachmann, 1858

Members of *Balantidium* are unicellular and endocommensal parasites in the intestine of their hosts. Some of these ciliates appear to be host-specific, whereas others are generalists (BRUNO et al., 2006). Two developmental stages are present: the trophozoite, flexible and covered by a row of longitudinal cilia reaching 40 to 140 µm and the cyst, ovoid and measuring 40 to 60 µm in diameter (BRUNO et al., 2006). Its horizontal transmission is made by oral and fecal via. In Brazil, *Balantidium piscicola* Geza, 1913 was found parasitizing caranha *Piaractus brachipomus* and mandi catfish *Synodontis clarias* (JUNIOR, 1913; PINTO, 1928).

Theileria Bettencourt, Franco et Borges, 1907

Members of *Theileria* spp. undergo exoerythrocytic merogony in the lymphocytes, erythroblasts and other cells of the internal organs, and this is followed by invasion of the erythrocytes by the merozoites, which may or may not reproduce (LAINSON, 2007). When division takes place, the parasite produces no more than four daughter cells (LEVINE, 1988 *apud* LAINSON, 2007). The elongated bacillary or bayonet forms are characteristic for

Theileria (BARNETT, 2012). In Brazil, *Theileria electrophori* is, so far, the only fish *Theileria* species reported from the viscera of a single juvenile of the electric eel *Electrophorus electricus* from the State of Pará, North Brazil. Air-dried smears of infected organs are fixed in absolute methyl alcohol and stained by Giemsa's method may be useful for accurate diagnosis of *Theileria*.

Calyptospora Overstreet, Hawkins et Fournie, 1984

These intracellular protozoan parasites are found in the liver and intestine of their hosts and have a heteroxenic life cycle transmitted by an infected crustacean ingested by a fish. In fish host, the oocysts (elliptical, ovoid or pear-shaped) present 4 sporocysts covered by a thin veil fixed by the presence of wall projections named sporopodia. Moreover, it presents a suture on the wall that do not divide the cell into two valves (EIRAS, 2013b).

Three species of *Calyptospora* were described parasitizing hepatocytes of Brazilian fishes: *C. serrasalmi* Cheung, Nigrelli and Ruggieri, 1986 from piranha *Serrasalmus niger* (CHEUNG et al., 1986); *C. tucunarensis* Békési and Molnár, 1991 from tucunaré *Cichla ocellaris* (BÉKÉSI & MOLNÁR, 1991) and *C. spinosa* Azevedo, Matos and Matos, 1993 from joaninha *Crenicichla lepidota* (AZEVEDO et al. 1993). Coccidiosis by *Calyptospora* was found by Bonar et al. (2006) in *Arapaima gigas* exported to the United States. Albuquerque & Brasil-Sato (2010) registered *Calyptospora* sp. in the liver and intestines of piaba-facão *Triportheus guentheri* and in the intestines of piaba *Tetragonopterus chalcus*. Additionally, Silva et al. (2012) have reported in piramutaba *Brachyplatystoma vaillantii* and Santiago et al. (2012) in tucunaré *Cichla temensis*. Fresh-mounted smears can be observed in light microscope. For histological analysis the infected organs are fixed in Davidson or 10% formalin solution and stained with hematoxylin and eosin (BÉKÉSI & MOLNÁR, 1991; SANTIAGO et al., 2012). The oocysts can be studied by transmission electronic microscopy (AZEVEDO et al., 1993).

Haemogregarina Danylewsky, 1885, *Cyrlia* Lainson, 1981 and *Desseria* Siddall, 1995

These groups comprise several blood protozoan parasites (DINIZ et al., 2002; EIRAS, 2013b). They are commonly found in both erythrocytes and leukocytes of marine fishes (DAVIES, 1995), except for *Cyrlia* spp., which parasitize only the erythrocytes of freshwater fish. According to Davies et al. (2008), although most fish haemogregarine life cycles are unknown, fishes are likely to act as intermediate hosts, while leeches or gnathiid isopods are probably the definitive ones.

Cyrlia and *Haemogregarina* are both characterized by the presence of an intra-erythrocytic merogony phase in fish host, while in *Desseria* spp. this stage is not found (DAVIES et al., 2004). In Brazil, *Haemogregarina* was found parasitizing lungfish piramboia *Lepidosiren paradoxa*, mullet *Mugil liza* and sole *Paralichthys orbignyanus* (JEPPS, 1927; EIRAS et al., 1995; DAVIES et al., 2008), while *Cyrlia lignieresi* Laveran, 1906 was found in the blood of marbled swamp eel *Synbranchus marmoratus* (LAINSON 1981, 1992; DINIZ et al. 2002).

Eimeria Schneider, 1875

Frequently found parasitizing the intestinal wall of vertebrates including fish (MOLNAR et al., 2012). This group was originally confused with *Calyptospora* but according to Molnar (2006), differently from the latter, *Eimeria* does not have intermediate hosts

in its life cycle. Sporocysts can be recognized by the presence of a “stieda” body (the opening of the sporocyst from where sporozoites goes out) (MOLNAR et al., 2012). According to Eiras (2013b) the degree of the lesions are variable. As for example, *E. sardinae* cause testis deformation and castration in *Sardina pilchardus*. Up to now, *Eimeria* sp. was only observed in the intestine of lungfish *L. paradoxus* in the State of Pará, North Brazil by Lainson & Ribeiro (2006).

Nyctotherus Leidy, 1849 and ***Rhynchodinium paradoxum*** Cunha et Penido, 1927

Ciliated *Nyctotherus* is present in digestive tract of insects, amphibians and fishes. The trophozoite has around 200 µm, is oval and provided with cilia arranged in longitudinal rows and a cytostome located in the middle of the body (THATCHER, 2006; EIRAS, 2013a); cytopharynx - a duct that communicates cytostome with the interior of the parasite - with undulating membrane. Great macronucleus, triangular and located at the anterior end of the body.

According to Thatcher (2006), these protozoans do not cause severe pathology and can be considered as endocommensals (EIRAS, 2013a). In Brazil, it was observed in *P. brachypomus*, *Acestrorhampus* sp. and *P. clarias* (PINTO, 1928).

Rhynchodinium paradoxum shows elongated and cylinder-shaped body, bean-shaped macronucleus located at the anterior end of the body. Cilia are long but absent on the posterior end of the body (THATCHER, 2006). This protozoan was found in the intestine of granulated catfish abotoado *Pterodoras granulosus* (CUNHA & PENIDO, 1927).

Tetrahymena Furgason, 1940

Tetrahymena is considered an important pathogenic agent to ornamental freshwater fish where it may cause severe mortalities (BRUNO et al., 2006) (Figure 10). This protozoan has a pear-shaped body covered by a row of cilia, besides macronucleus and micronucleus. According to Bruno et al. (2006), these ciliates do not form cysts and studies suggest that *Tetrahymena* penetrates the host epithelium (especially where there are wounds) reaching the blood and parasitizing the gills, kidney, eyes and brain (EIRAS, 2013a).

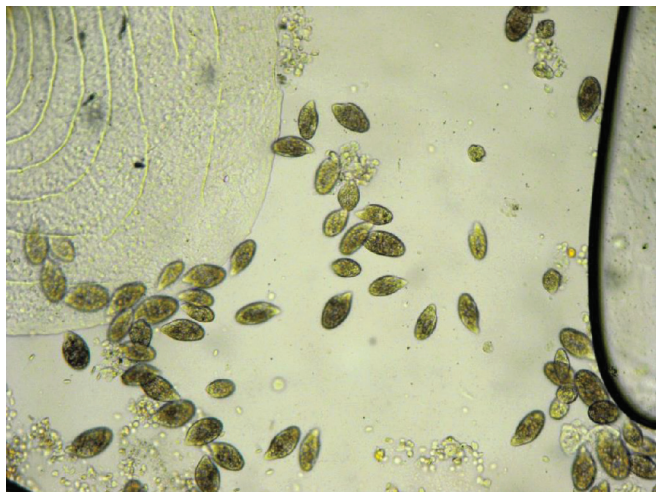


Figure 10. *Tetrahymena* sp. from the skin of guppy (*Poecilia* sp.) observed in light microscope at 40 x magnification.

In Brazil, this parasite was found parasitizing the butterfly fish *Carnegiella strigata* (TAVARES-DIAS et al., 2010), and can be commonly found parasitizing guppies. Cutaneous infestation by *Tetrahymena* causes scaliness, whitish lesions on the skin and opportunistic secondary infection by bacteria.

Hexamita Dujardin, 1838

Members of *Hexamita* possess oval body, bilateral symmetry, provided by four pairs of flagella, three anterior for locomotion and one posterior, besides two spherical nuclei located at the anterior end (ROTHENBACHER & BOHL, 1975; FOIN, 2005). They are opportunistic endoparasites of the intestinal tract of wild and farmed marine, freshwater and ornamental fishes in tropical and temperate waters (ROTHENBACHER & BOHL, 1975; EIRAS, 1994; FRANCIS-FLOYD & REED, 1994; FOIN, 2005). It can also be found in the swimbladder, liver, spleen, blood, kidney, and heart of hosts (FOIN, 2005). After analysis of 7,139 fish specimens between the years 1987 and 1990, Békési (1992) registered the presence of *Hexamita* in Brazil from the intestine of *Prochilodus brevis* (syn. *P. cearensis*). These parasites present a monoxenic life cycle; the pear-shaped trophozoites change to spherical before cellular division (WOO, 2006). They can be horizontally transmitted by the releasing of trophozoites and oocysts into the water from the fish feces that will be ingested by other hosts (LOM & DYKOVÁ, 1992; FOIN, 2005). The diagnosis can be made by analysing the feces of infected fish under high magnification (KLINGER & FRANCIS-FLOYD, 1998; FOIN, 2005).

Distribution of Parasites in Brazil

Several protozoan parasites of fishes have been reported in national territory, as a result of efforts of Brazilian researchers from different localities. Nevertheless, unpublished data of the present authors indicate that much more observations are yet to be reported as shown in Figure 11.

Attention on the Control Strategies

The use of chemicals in order to control protozoan parasites can be, sometimes, difficult to administer, costly, not completely efficient and even environmentally hazardous. Dozens of protocols used in many other countries for tropical and temperate fishes have been adapted to fish farms in Brazil with no scientific criteria. In fact, if applied erroneously, it may cause environmental degradation such as impairment of water quality (pH and dissolved oxygen alterations can lead to great fish mortality), besides the fact that several chemotherapeutics employed are corrosive, carcinogenic or even explosive. On this view, they must be manipulated with PPE's (Personal Protection Equipment) and special care. Nevertheless, in field practices it is usually observed low knowledge when employing these techniques. Prophylactic measurements or immunoprophylaxis adopted in the fish farms and ornamental industry are important tools to minimize the effects of parasitism and to stimulate the fish immune system (MARTINS et al., 2011).

In order to control outbreaks of diseases in fish farms it is fundamental to first characterize carefully the hygienic-sanitary state of the facility. This includes being aware of disease diagnosis

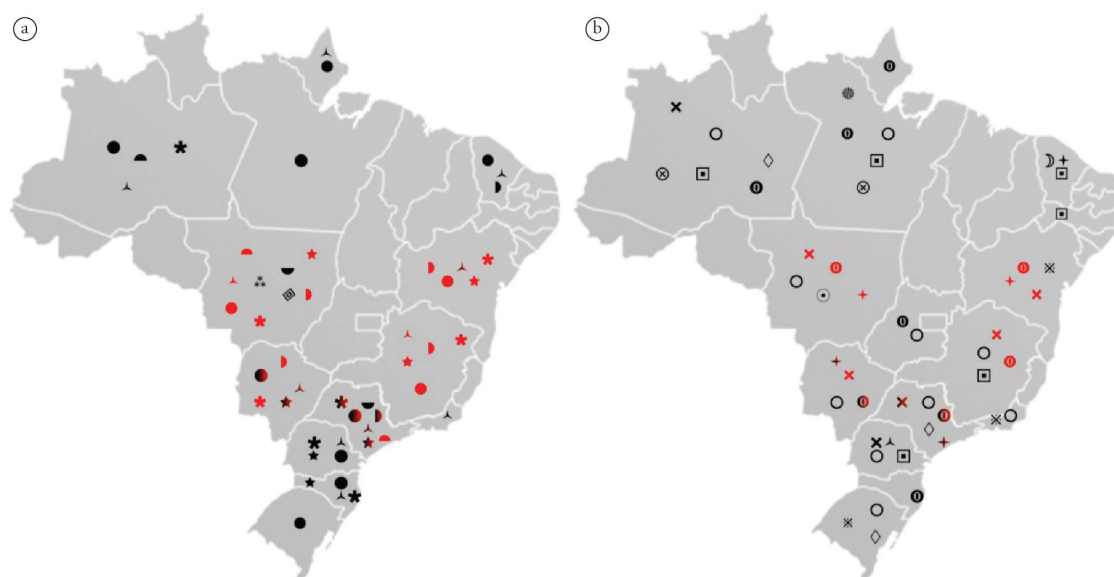


Figure 11. Geographical distribution of (a) ciliated protozoan and (b) other protozoan parasite groups of fishes from Brazil. Symbols indicate the register of the below-mentioned species: ⊙ *Entamoeba pimelodi*; ✱ *Amyloodinium*; + *Cryptobia*; ⤵ *Hexamita*; ✕ *Ichthyobodo*; ⊖ *Piscinoodinium*; ○ *Trypanosoma*; □ *Calyptospora*; ⊗ *Cyrtilia lignieresii*; ⌘ *Eimeria lepidosirensis*; ◇ *Haemogregarina*; ⤴ *Apiosoma*; ✱ *Balantidium*; ✱ *Chilodonella*; ★ *Epistylis*; ● *Ichthyophthirius multifiliis*; ◐ *Nyctotherus piscicola*; ⬠ *Rynchodinium paradoxum*; ◑ *Tetrahymena* and ▲ *Trichodinidae*. Colour black indicates previous reports; colour red indicates unpublished data from the present authors.

history, recognition of pathogen dispersal and/or transmission modes involved. The use of chemotherapeutics cannot be linked only when an outbreak of mortality occurs, in which treatment is employed as a rescue measure to reduce the economic losses. Such products must be applied strategically in each rearing phases in order to have the best fish response, with lowest environmental impact and safety to the operating person.

Disease control in hatcheries that produce fingerlings is a key point to reach the high sanitary quality and regular production (PÁDUA & CRUZ, 2014). In these farms, transmission of diseases from the broodstock to eggs, larvae and fingerlings deserves special attention. Efforts to control diseases must be concentrated on these units, which represent the beginning of the production cycle.

In tilapia hatcheries where the eggs are collected from the mouth, it may be necessary to disinfect them before storage in incubators. The use of Chloramine-T is an efficient measure for disinfecting eggs against viruses, bacteria, fungi and parasites found in this process. In order to proceed, the use of small capacity circulating systems (200 – 1,000 L) should be implemented so as to supply 2 to 6 incubators that will recirculate water with 30 mg/L of Chloramine-T, during 10 to 20 min of exposition. It is important to assure that water will circulate inside a mechanical filtration system with the aid of an acrylic filter mat. In this case, it is worth mentioning that the use of activated carbon elements or other substances with adsorption effects is not recommended, since they could kidnap the molecule of the drug used for disinfection.

Adequate management include not handling fish when abiotic factors such as water temperature and pH are exceeding limits generally considered safe for the fish species, avoid translocation of fingerlings from one place to another without proper care, always use good quality feeds, monitor regularly the quality of the water entering the farm, avoid water sharing among ponds

and disinfect fish handling equipment. After that, it is necessary to adopt other measures of biosecurity to ensure larvae remain free of parasites during subsequent rearing phases. For such, it is important to sectorize fish farm facilities, thus delimitating areas for equipments and for passage of employees. Other important measures to avoid disease dissemination include preventing entry of wild fish in the farm (this can be done using screens in the water supply canal), implementing sanitary barriers such as cleaning and disinfecting ponds, using a quarantine tank, depurating fish before transport and using *wetlands* in the water supply canal.

Wetlands macrophytes such as *Eichhornia* spp. (Figure 12) can be considered an efficient tool to contain dispersion of resistant forms of parasites, such as eggs and resistant cysts into the farm, as well as to provide a huge surface area for attached microbial growth. However, one must establish a routine for renewal of these aquatic plants so that the saturation capacity of retention of particles in its submerged roots does not occurs. To this end, it must be performed the removal of adult plants, or under the senescent stage, every 15 to 45 days, varying according to the season and the amount of suspended material in the water.

After storage of fingerlings in a farm destined to fish fattening, the use of sanitary measures may be expensive and sometimes operationally difficult. On the other hand, the implementation of sanitary measures in hatcheries is more economic and efficient in suppressing or eradicating the disease (PÁDUA & CRUZ, 2014). Similar to that found in industrial pig and poultry farming, young fish should be sent to growing units in a good health status and possibly vaccinated, minimizing thus parasite hazard to an acceptable level. It must be emphasized that it is complex to achieve fish completely negative for parasites and so prophylaxis and best management practices are the best ways of achieving adequate health status of farmed fish (BOYD et al., 2008).



Figure 12. Constructed wetland in freshwater fish farm for use as a sanitary barrier and water cleaning.

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