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Some diseases in aquatic fish with spotlight on Pathology, morphology and pathogenicity- A review

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Review Article

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Abstract

The Phylum Protozoa include many organisms divided into ecto and endo parasites in fish world which is responsible for diseases. Thus, they may result in social and economic effects in several places. Also, secondary bacterial infections can occur, leading to great losses and mortality.

So, this assessment provides the most vital protozoan parasites determined in farmed fish, with recognition on its analysis, distribution, transmission routes, host-parasite relation and histopathology which is useful for investigators in figuring out the definitive diagnosis of fish pathogens.

Keywords: *Parasites of fish, morphology, pathology, pathogenicity of diseases.*

Introduction

The Phylum Protozoan include many organisms divided into endo and ectoparasites in fish **Lom & Dykovā (1992)**. They are causing diseases in aquaculture leading to harm and growth reduction of the fish host **Moraes & Martins (2004)**, infection of secondary bacteria and mortality, which affect global aquaculture manufacturing **XU *et al.* (2012).** So, protozoan diseases are resulting in massive losses of the economic fishing industry **Bondad-Reantaso *et al.* (2005).** In this way, the importance of disease impact must be highlighted and numbers of curative measures and prophylactic have suggested, to clarify effective techniques for early diagnosis and rapid management practices in fish farms to get a continuous production **Pādua & Cruz (2014).**

This evaluate offers the most vital protozoan parasites located in farmed fish, with recognition on its diagnosis, distribution, transmission routes, host-parasite relation and histopathology which helpful for researchers in determining the proper diagnosis of fish pathogens.

Ciliophora:

Unicellular protozoans have mobile cilia covering outer surface. They consist of Cytostome, macronucleous and micronucleous. They reproduce via binary fission and conjugation. The principle representatives are Apiosoma, Chilodonella, Ichthyophthirius multifiliis, Balantidium, Epistylis, Rhynchodinium paradoxum, Nytotherus, Trichodinidae and Tetrahymena **Li *et al.* (2008).**

Apiosoma:

Involves ciliated sessile peritrichid protozoans

in mature phase, with a conical body form supplied with contractile and nutritive vacuoles, infundibulum (oral cavity), scopula (where the parasite attaches to host surface), peristomial disc, macronucleous and micronucleous **El – Tantawy *et al.* (2013).**
As other Sessile Peritrichids (like Heteropolaria Foissner and Schubert, 1977 and Epistylis) they attach to host but not enter the epithelium, So feeding via hanging material filtration in the water. This phenomenon is named epibiosis, as the host is basibiont and the ciliate is epibiont **Pādua *et al.* (2012b).** They present in fish ponds; rarely affect marine or ornamental fish.

Life cycle:
Reproduction is through conjugation and binary fission **Lom& Dykovā (1992).** Also, they can extend free swimming migratory stage which detaches from the colonization to look for new hosts in water (telotroch). Bad water quality and excessive fish stocking factors increasing infection.

Transmission:
It's far transmitted by way of the free swimming infective telotrochs.

Diagnosis:
The microscopiclly examination is primary technique for diagnosis of Apiosoma of fresh-mounted of skin fish , fins and gills (Fig.1a,b). The parasite has a firm and elongated (40-70 µm) body shape, it's vase shape and identified even in low parasite density infections. The following staining technique as silver nitrate impregnation (Fig.1c), protargol ang Giemsa (Fig.1d),Heidenhain, Ehrlich or Harris heamatoxylin and neutral red used **Li *et al.* (2008).** The identification is through length and width of body, macronucleus, micronucleus and peristomial disc; scopula; body shape; position of contractile vacuole and peduncle width **Li *et al.* (2008); El –Tantawy *et al.* (2013)**.

Pathogenesis and clinical signs:
As the parasite attaches to the host by scopula and not invade epithelial cells, the pathological alterations are discrete or even less evident. When heavily infested on the fish gills, these parasites cause reduced breathing. So, fish suffering from respiratory distress with gasping for air **Durborow (2003)**. Clinical symptoms are not characteristic however they are related to respiratory distress and hyperventilation as darkness of skin. **Li *et al.* (2008)** observed lack of equilibrium, gasping the air from surface and anorexia **Durborow (2003).**

Histopathology:
The most common pathologies on the gills were hyperplasia, lamellar apposition and vacuolisation. Abundant necrotic cells as an indication of a serious complication **Yandi *et al.* (2017).**

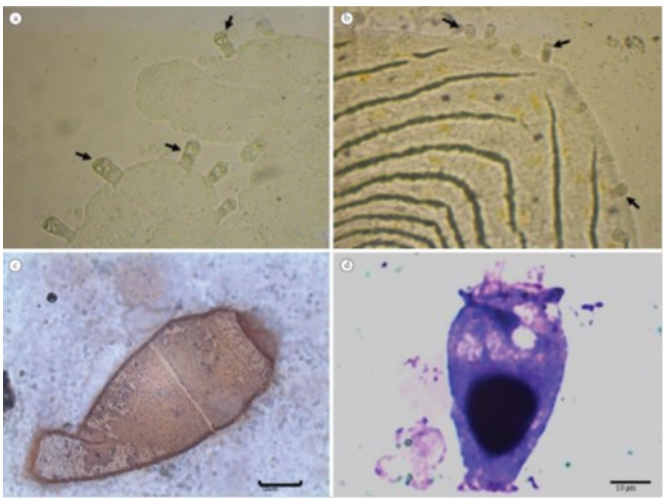

Figure (1). Apiosoma attached on the epithelium (a) and scale (b) of Nile tilapia larva Oreochromis niloticus in fresh-mounted slide. Silver nitrate impregnated specimen to take a look at the cilia (c) and Giemsa stained (d) displaying the nuclear apparatus.

Treatment: heavily infested ponds formalin or copper sulphate or potassium permanganate is effective.

Chilodonella:
Leaf-shaped ciliated protozoans, oval, dorsoventrally flattened, somewhat asymmetric (Fig.2a-c) and mobiles. Macro and micronucleus well appeared (Fig. 2c), two longitudinal rows of ciliary kineties on the ventral surface located (Fig. 2b,d). Its species are free-residing but some parasitize skin, gills and fins of each marine, estuary and fresh water fish **Pādua *et al.* (2013a)**. The hosts are damaged by two species: Chilodonella hexasticha, particularly in tropical fishes and Chilodonella piscicola specifically in temperate and subtropical waters.

Life cycle:
It has monoxenic life cycle with division on the host transversally (Fig.2d), sexually occur by conjugation **Pādua *et al.* (2013a).**

Transmission:
Occurs mainly by contact directly with infested and uninfected one. High stock density and poor water quality are factors affecting infection. All utensils should be taken into consideration as a vital aspect for dissemination of the parasite in fish farming.

Diagnosis:
Microscopically, scraps of skin, fins and gills can be examined. In mounted slides, the parasite moves rapidly to a single direction. Silver nitrate impregnation method with Heamatoxylin or Giemsa are important **Pādua *et al.* (2013a).**

Symptoms:
Lazy fish, gasping, clamped fins, scrubbing, and opened gills.

Pathogenesis and clinical signs:
Chilodonella, in comparison to other ciliates of similar life cycle as trichodinids, it causes severe lesions. Due to its abrasive action on the epithelium, Pathological changes occur. Filaments of gill have highly sensitivity to the parasites. In acute stage, excessive mucus production with gill congestion maybe located. In significantly infested fish, inflammatory infiltrate, desquamation, necrosis and epithelial proliferation are visible **Pādua *et al.* (2013a).** As defense system is severely affected, secondary bacterial infections are accelerated (Fig.1c). Clinical signs consist of breathing trouble, lack of equilibrium and appetite. Whitish lesions on the gills, darkened skin, scaleness, ulcers and haemorrhagic regions are found in secondary bacterial infection.

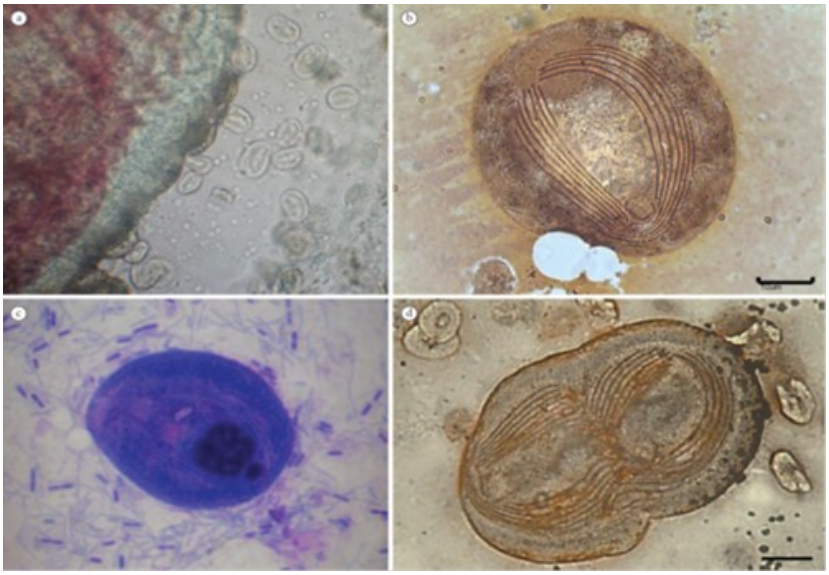

Figure (2). Chilodonella hexasticha discovered in mounted slides from Oreochromis niloticus gills (a), ciliary kineties in staining of silver nitrate (b), Giemsa stain of nuclear apparatus and bacteria (c), silver nitrate for transversal fission (d). Bar: 10 μm (b,d).

Histopathology:
Attach on fish skin and gills. After infection, fish secretes excessive mucus, with acute to subacute dermatitis and hyperplasia **Noga (2000). Bruno *et al.* (2006)** reported that gill lesions in Chilodonellosis include hyperplasia, damaged gill function and necrosis. The primary cause of fish mortality is respiratory failure which occurred due to gill hyperplasia.

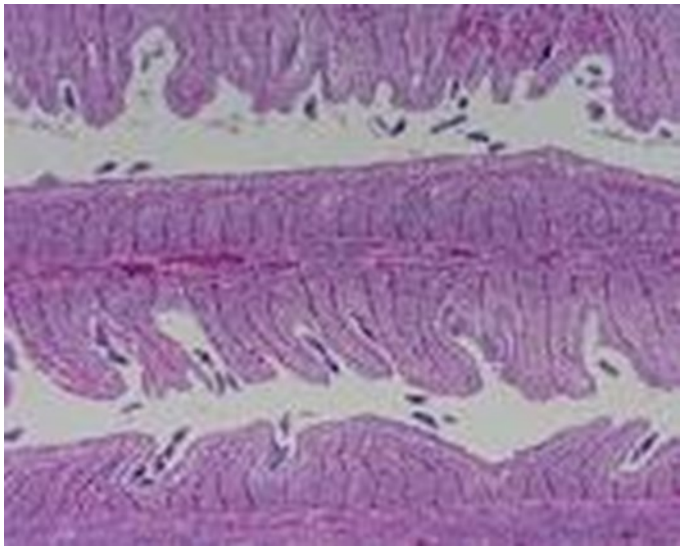

Figure (3). Massive Chilodonella sp. are present in the gill epithelium showing an extensive gill filament epithelial hyperplasia, with complete fusion of lamellae with occasional pseudocyst formation. There is a mononuclear cell infiltrate in the filament connective tissue and in the epithelium at the base of the lamellae.

Treatment:
Prevention is usually better, filter with activated carbon is recommended also periodical test of water quality.

Epistylis
Epistylis have main characters as ciliates with bell shaped frame, and a protracted peduncle (non contractile). Within the apex, zooid ovoid - form cell is placed have contractile vacuoles, cilia and nucleus **Lom & Dykovā (1992)**. As Apiosoma, this parasite attached by aid the fish and feed on debris which suspends in water **Pādua *et al.* (2012a)**. This ciliate might lead to host damage when invasion by secondary bacterial infection

Life cycle:
Asexual reproduction occurs through binary fission of the zooids (Fig. 4b). Additionally, they may reproduce via conjugation on its sexual reproduction **Ishikawa *et al.* (2012).**

Transmission:
Infective telotrochs are transmitted to new hosts for attachment to broaden new colonies. As a routine for fish farming, they upload manure to the ponds to enhance the primary productivity of the water leading to improved phytoplankton and zooplancton production for fish feeding **Visse (2007).**

Diagnosis:
Whitish to yellowish blubby growth colonies of epistyliasis at the fish (gills, fins, surface, head, mouth and operculum) through naked eye may be confused with fungal infection. So, it needs to be observed by fresh-mounted slides and determined with the microscope (Fig.4a). Contraction actions of the zooids provide the final diagnosis. In early infestation, the colonies by the naked eye might not be detected **Pādua *et al.* (2013b)**. Diameters of zooid and peduncle, colony shape, arrangement of buccal ciliary and nucleus form are essential **Li *et al.*. (2012).**

Differential diagnosis:
Formation of branched colonies is used to differentiate Epistylis from different sessile peritrichids, Apiosoma and Ambiphrya. Alternatively, different ciliates which include Carchesium and Zoothamnium additionally branch colonies were developed like Epistylis. So, they may be differentiated by Epistylis move-

ment of peduncle contraction.

Pathogenesis and clinical signs:

Its pathogenicity is widely related to the existence of bacteria at the peduncle (Fig.4b, arrow heads). It leads to irritation and epithelium inflammation at the point of attachment. Afew authors assured that Aeromonas hydrophila is liable for the parasite colonies and secreting lytic enzymes which degrade the near tissue. So, haemorrhagic changes related to the redsore sickness. In Fish highly infested with Epistylis sp. , skin ulcer and fin erosion were observed associated with enzyme of bacteria activity may be seen (Fig.4c,d) **Pādua *et al.* (2012a).** In large amount, it influences water flow over gills leading to asphyxia. Commonly, the fish death takes place after secondary bacterial systemic infection.

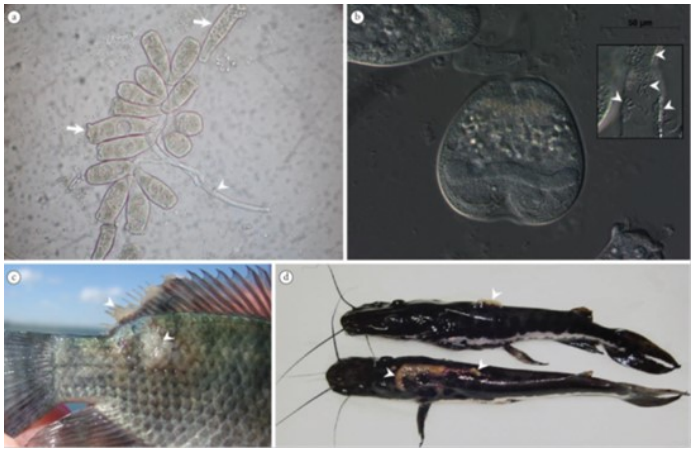

Figure (4). Epistylis sp. (a)fresh slides beneath light microscope . Zooid binary fission (b) micro-organism (arrow heads) attached at peduncle of the ciliate. Oreochromis niloticus (c)with erosion of fin and lack of scale (arrowheads) related to colonization of Epistylis , and hybrid surubin pseudoplatystoma sp. displaying overall dorsal fin erosion and Epistylis sp. colonies on the head and dorsal fin (arrow heads).

Histopathology:

Histopathology displayed necrosis and degeneration of epithelium near the colonies, excessive desquamation and mucus production with inflammatory infiltrates **Pādua *et al.* (2012a).** Additionally, hemorrhagic lesions and necrotic dermatitis with ulceration are host response for the infection (Fig.5) **Purivirojkul & Khidprasert (2009).**

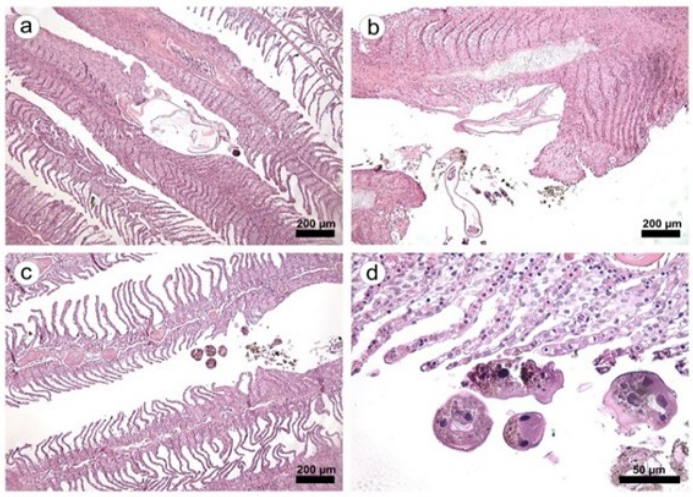

Figure (5). Lernaea cyprinacea attached to a branchial filament, leading to evident epithelial hypertrophy and hyperplasia, 200 µm. b) Crustacean parasite and Epistylis spp. causing lamellar damage, with regions of hemorrhage, necrosis and massive inflammatory infiltrates, 200 µm. c) Epistylis spp. near regions of epithelial hyperplasia and scaling, 200 µm. d) Epistylis spp. parasitizing branchial filament, 50 µm.

Treatment: salt is highly effective (salt bathes).

***Ichthyophthirius multifiliis* (ICh):**

It is important parasite infected the fish; it is the causative agent of ichthyophthiriasis or white spot disease which has an effect on gills, eyes, fins, and skin of fish of farm. ICh is not always host precise, so;any fresh water fish may additionally transmit it **Eiras (2013a).** During a survey for parasitology of ornamental fish from North Brazil, **Tavares-Dias *et al.* (2009)** located I.multifiliis in the gills of Paracheirodon axelrodi, Hyphessobrycon copelandi and Dianema urostriatum. Numerous species of fish are affected by ICh as silver catfish (Rhamdia quelen) **Martins *et al.* (2013).**

Life cycle:

It is a compulsory pathogen, which can not survive without presence of fish. Its life cycle is monoxenic, includes one fish to be finished. As Piscinoodinium and Amyloodinium, the life cycle has three stages as:

I. Theront: its infected stage and mobile form measuring 30 to 50 µm; it must find their host, if not will be die.

II. Trophont: its adult one also mobile stage in cell epithelium can attain 800 to 1000 µm in diameter.

III. Tomont: its free form, provided by way of a cyst to protect it. They divide asexually and originate 500 to 1000 tomites (daughter cell) which become infected one of theronts and invasion a new host.

Transmission:

It's transmitted and takes place directly from the theronts or by co-habitation with infested fish. Utensils and water transport in fish farm act as a source of infection for ICh **Xu *et al.* (2007).** The discharge of theronts into the water is associated with temperature. At water temperatures over 24°C, the life cycle is completed rapidly. However, the life cycle may be inhibited when temperatures are under 10°C or above 28°C **Ishikawa *et al.* (2012).**

Diagnosis:

Diagnosis of Ichthyophthiriasis depends totally on the detection of trophonts inside skin of host macroscopically, and analysis of skin, fins and gill scrops microscopically (Fig. 6a) **Pādua *et al.* (2012b).**

Under microscope, the cellular pear-shaped theronts and mature trophonts covered by a layer of external cilia with a horse-shoe shaped nucleus were evident (Fig. 6b).

Differential diagnosis:

We need to differentiate among I. multifiliis and dinoflagellate Piscinoodinium Pillulare. Both of them, have the same color and shape, but this dinoflagellate has no cilia around the body. In addition to, the rounded nucleus in place of horse shoe shaped nucleus.

Pathogenesis and clinical signs:

Pathological changes related to ICh are associated with invasion of theront at the epithelium. This process enhances host inflammatory response associated by excessive proliferation of goblet cells **Pādua *et al.* (2014).**

The primary symptom was existence of small spots with white colour on surface of fish involving gills, skin, eyes and buccal cavity. It is prevalent to find fish difficult respiration and flashing behavior **Martins *et al.* (2000); Ishikawa *et al.* (2012).**

Histopathology:

I. multifilis is large in size contains a horseshoe -shaped macronucleus. It causes acute to subacute dermatitis with hyperplasia**.** The epithelial erosions and ulcers are damaging and they may lead to secondary infections **Purivirojkul & Khidprasert (2009).** Infection by Ichthyophthirius associated with epithelial hyperplasia especially in gills **Bruno *et al.* (2006)**. Histopathological picture reveals also the paracitosis with inflammatory infiltrates and proliferation of mucous cells (Fig.6c,d). In severe cases, secondary lamella of gills is fused, degeneration and necrosis of epithelial cells forming numerous ulcers after emission of mature trophonts **Pādua *et al.* (2014)**.

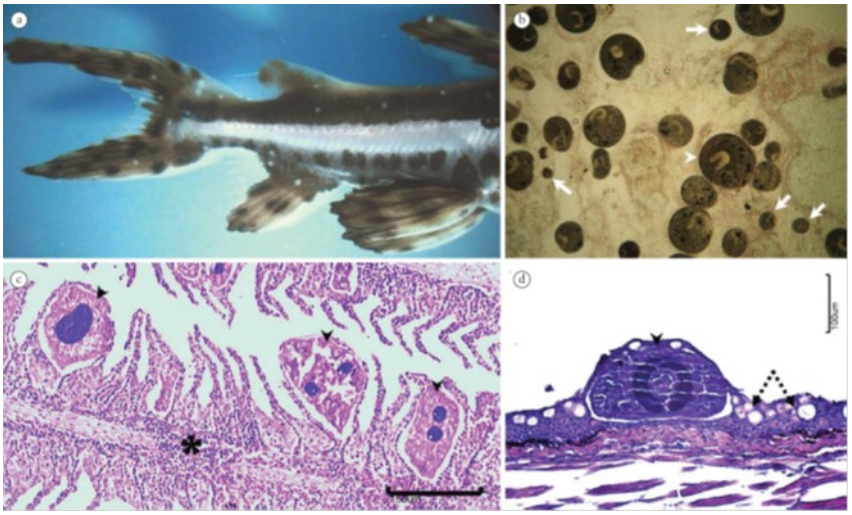

Figure (6). Hybrid surubim pseudoplatystoma sp. describe skin white spots at fin and skin (a). I.multifiliis in freshmounted from skin , mature trphont and horse shoe shaped macronucleus (b- arrowhead) and numerous immature trophonts in developed levels (b continued arrow). Histology of lamella gills (c), with inflammatory infiltrate (c – asterisk), and the parasites present in fish skin (d- arrow head). Cell mucous Proliferation subsequent to attachement parasites (d- dotted arrows). Bar: a 150 µm (c) and 100 µm (d).

Trichodinidae :

They are mobile ciliated parasites, having general characters as: a body covered with membrane is slender in shape surrounded by adoral ciliary spiral, macronucleus is horse shoe shaped and present denticulate ring around adhesive disc and denticle is present (Fig.7c) **Basson & Vanas (2006)**. Paratrichodina, Trichodinella both genera are parasitizing in fresh and marine water fish **Pādua *et al.* (2011a)**. They are discovered in zooplanctonic microcrustacean **Silva *et al.* (2009)**, gastropod mollusc **Pinto *et al.* (2006)**, bivalve mollusc from mangrove **Sabry *et al.* (2013)**, ornamental fishes **Martins *et al.* (2012)**, wild fishes **Bittencourt *et al.* (2014)**, in addition to farmed fish **Valladão *et al.* (2013)** and amphibians **Fernandes *et al.* (2011).**

Gills, body surface and buccal cavity are at risk of Trichodinids infestation, small number of them have become endoparasites of their hosts **Lom & Dykovā (1992).**

Proliferation within the environment related to low water quality, total number of bacteria and environmental elements of the fish species **Palm & Dobberstein (1999).** Their reproduction in fish farms suppressed with high water temperature **Basson & Vanas (2006); Martins *et al.* (2010) and Yemmen *et al.* (2011).**

Life cycle:

Life cycle of Trichodinids are monoxenic, reproduction particularly by conjugation and binary fission may be occur (Fig.7d). In a short time, rapid reproduction with 100% prevalence can take place **Martins *et al.* (2010).**

Transmission:

They can transmit horizontal through direct contact, contaminated water and infected fish farming utensils.

Diagnosis:

By microscopical examination of fins, gills and skin of scraps of diseased one (Fig.7a,b). In fresh- mounted slides, it's moved in circle rapidly. Silver nitrate impregnation (Fig.7c) and Giemsa or haematoxylin are commonly used for specific diagnosis **Lom (1958); Vanas & Basson (1989).**

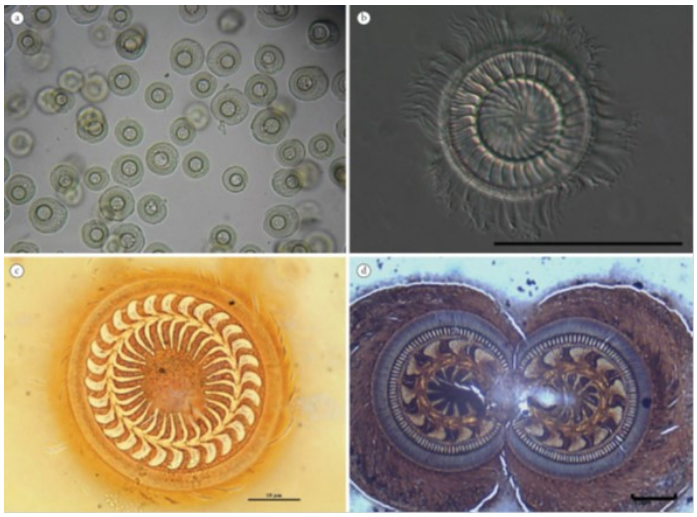

Figure (7). Trichodinids in fresh-mounted slide from skin (a), Trichodina centrostrigeata in differential interference contrast microscope (b), adhesive disc of Trichodina magna in silver nitrate impregnation (c) and an adhesive disc of a trichodinid in binary fission in silver nitrate impregnation (d). Bar: 50 µm (b) and 10 µm (c,d).

Pathogenesis and clinical signs:

Strong attachment of Trichodina to the epithelium, leads to occurrence rim of the border membrane (bites) into epithelial cells which causing damage to the epithelial cells of fish **Basson and Vanas (2006).**

Acute phase of the sickness specifically, fingerlings and larvae resulting in ulcers, in gills secondary lamella subepithelial oedema displace it, hyperplasia and mononuclear inflammatory infiltrates **Valladão *et al.* (2014)**. In chronic phase, epithelium mucus cell is increased and occur hyperplasia with partial or overall fusion of secondary lamellae in gill filament, gill necrosis and inflammatory infiltrate **Valladão *et al.* (2013).**

Clinical symptoms are not precise. Skin darkness, whitish regions inside the gills, flashing at surface and hypoxia can present **Pādua *et al.* (2011a).** Irregular swimming on surface and lethargy also observed in diseased one **Valladão *et al.* (2013).**

Histopathology:

Trichodina sp. is found in infected skin and gills. They inhabit the surface of fish, causing damage. Occasionally, some species may infect the urinary bladder, oviducts or gastrointestinal tract **Noga (2000).** It causes subacute dermatitis with hyperplasia **Purivirojkul & Khidprasert (2009). Bruno *et al.* (2006)** found that infection by Trichodina associated with epithelial hyperplasia especially in gills (Fig.8).

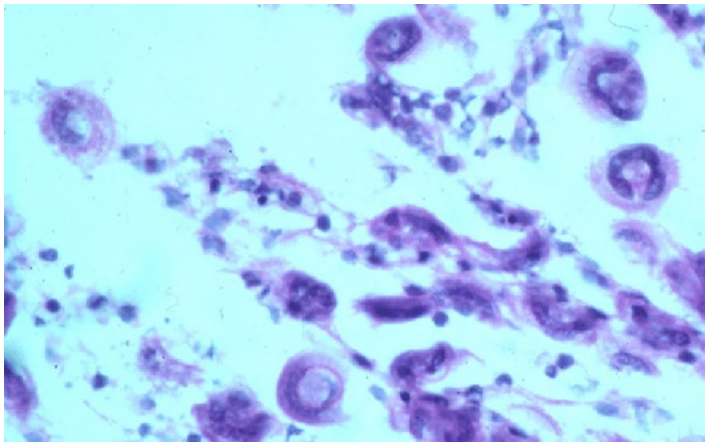

Figure (8). Histopathology of Trichodina sp. in gill tissue of fish. Note multiple parasites in this H&E stained tissue section.

Treatment: potassium permanganate 1.5 gm/220 gallon . Not used with salty water.

Flagellate

As *trypanosome cryptobia, Ichthyobodo, Amyloodinium ocellatum and Piscinoodinium pillulare*. They have one or more flagella to motion. Mostly ectoparasites and another can parasitize in internal organs. Their reproduction can occur by binary fission longitudinally as kinetoplastids or through a 3-phased lifestyles cycle as dinoflagellates **Eiras (1994).**

Amyloodinium ocellatum

Its consists of dinoflagellates varied shape relying on the stage of life. It causes velvet disease. It influences marine, brackish farmed fish, may additionally induce essential mortalities and economic losses **Levy *et al.* (2007); Pereira *et al.* (2011); Moreira *et al.* (2013).**

Life cycle:

The parasite have effect on gills and skin in farmed and wild marine fishes, its monoxenic parasite, as having low host specificity **Reed & Francis–Floyd (1994); Abreu *et al.* (2005); Woo (2007).**

No intermediate hosts in their life cycle, they are showing these 3 stages: The dinospore, infective form with transverse and longitudinal **Guerra–Santos *et al.* (2012); Woo & Ardelli (2014).** The trophont, a pear or rounded shaped, with golden to brownish colour, exhibits chloroplasts and is able to grow on the host when attached to the fish surface or gills by its rhizocysts. And tomont, the reproductive stage with successive divisions developed on aquaria or pond (Fig.9). Binary fission occurs by divided tomonts several times and frees swimming production **Francis–Floyd & Floyd (2011); Woo & Ardelli (2014).**

Transmission:

Transmit by infective dinospores through direct contact in the water. Parasite can transmit effortlessly to wild fish **Roberts-Thomson *et al.* (2006).**

Diagnosis:

By microscopical examination of body surface and gills scraping **Montgomerybrock *et al.* (2001); Abreu *et al.* (2005); Guerra–Santos *et al.* (2012).** In addition to, histological sections of parasitized tissue to clarify the parasite **Guerra–Santos *et al.* (2012)**. Serological analysis with specific antibodies has little information **Cecchini *et al.* (2001).** Giemsa, heamatoxylin and eosin and may- grunwald are the most famous staining methods **Guerra –Santos (2011)**. As well as iodine to reveal amyloid granules. In dinospores, flagella, diameters of cellular body, distribution pattern of epitheca and hypotheca plates, a peduncle, rhizocysts, pores and nodules are the principle characteristics for parasite identity **Landsberg *et al.* (1994)**. As well as measurements of trophont **Abreu *et al.* (2005).**

Pathogenesis and clinical signs:

During attachment of ryzoids with epithelial cells make mechanical action, tissue changes took place **Abreu *et al.* (2005).** The principle symptoms are erratic swimming, lethargy, anorexia and flashing onto the substrate and emaciation in addation skin pigmentation and emaciation **Francis–Floyd & Floyd (2011); Guerra–Santos *et al.* (2012).**

Visible small white spots are seen on fins and skin. Infected fish scratch their skin against items or ponds wall.

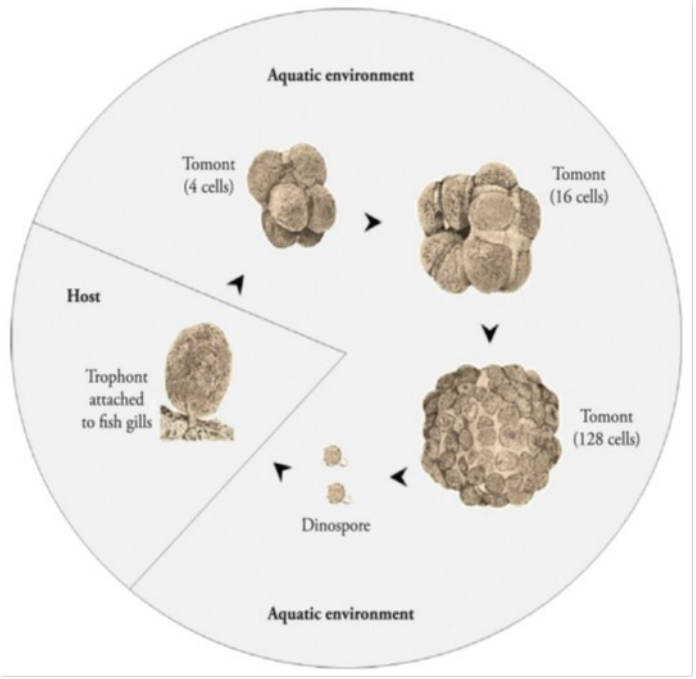

Figure (9). Life-cycle of *Amyloodinium ocellatum*. Modified from **Pereira *et al* (2011).**

Histopathology:
Amyloodinium sp. and Piscinoodinium sp. cause necrotic dermatitis to their host. The gills are the primary site of infection. Heavy infestations can cause gill epithelial hyperplasia to severe hyperplasia of the entire gill filament, inflammation, hemorrhage and necrosis. In addition, filament degeneration and necrosis may also are reported in case of Piscinoodinium **Noga (2000).** In acute phase, excessive production of mucous and congestion of gills are observed **Guerra–Santos *et al.* (2012).** Vacuolar degeneration, hyperplasia and secondary lamellae fusion also detected **Cruzlacierda *et al.* (2004); Saraiva *et al.* (2011).**
Treatment: using chloroquine or phosphate also increasing pond temperature or adding salt.

Piscinoodinium pillulare:
It considers the causative agent of piscinoodiniasis or velvet disease, has the same characters of A. ocellatum in morphology and development but normally found in freshwater fish causing Rust disease **Noga & Levy (2006).** It is not host-specific **Martins *et al.* (2001).** 3 forms of dinoflagellate from fish scraps can be observed microscopically: banana and pear shaped as well as mature rounded brownish parasite **Martins *et al.* (2001); Foin (2005).** Different developmental stages may be found in excessive infestations **Tavares–Dias *et al.* (2009)**.

Life cycle:
Similar to A. ocellatum (3-phased cycle). Immobile trophonts attach to fish by way of rhizocysts **Klinger & Francis–Floyd (1998); Foin (2005).** On substrate, tomonts go through sequent divisions to produce dinospores which supplied with flagella and free-swimming.

Transmission:
Contact with infested fish from infected water and utensils and by transporting live fish among fish farms, because water carries different stages of parasite **Foin (2005)**.

Diagnosis:
Skin and gill scrapes is used for diagnosis. Presence of trophonts attached on gill filaments in stained slides with diluted Giemsa gives clear diagnosis (Fig.10) or via detection of amyloid granules by iodine staining **Martins *et al.* (2001).**

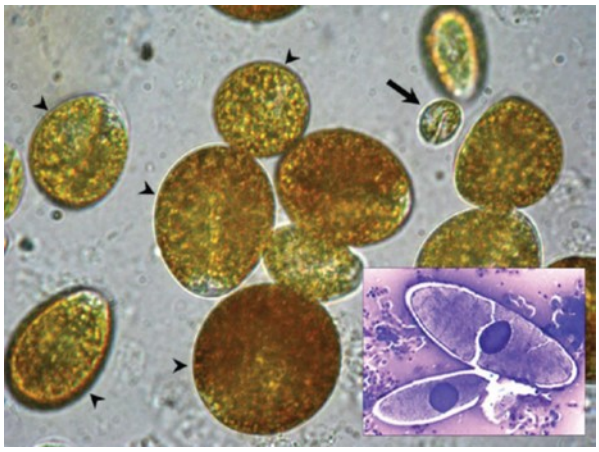

Figure (10). *Piscinoodinium pillulare from gill of tambaqui Colossoma macropomum.* Trophonts with pear shape (round arrowheads) with one dinospore (arrow). Two parasites stained with Giemsa showing oval to rounded nucleus without micronucleus.

Pathogenesis and clinical signs:
Although no clinical signs appeared in fish in low parasitic infestations, irritant action of the parasite has occurred in severe infestation (chronic form) leading to "flashing" on the substrate **Sant'Ana *et al.* (2012)**.

Pathological picture:
Trophant observed attached to skin, gills or fins as sac- like form. Macroscopical finding of gills in highly infestation with P. pillulare may show brownish colour (Fig.11a,b). All of interlamellar space among secondary lamellae was filled with trophonts of P. pillulare that attached to the primary lamellae (Fig.11c), in which the rhizocysts attached to epithelium (Fig.11d).
Proliferative changes together with fusion of secondary lamellae, infiltration of inflammatory cells, haemorhage, epithelium degeneration and necrosis, subepithelial oedema and ulcers had been determined. Excessive mucous production on skin and gill were observed **Martins *et al.* (2001)**.

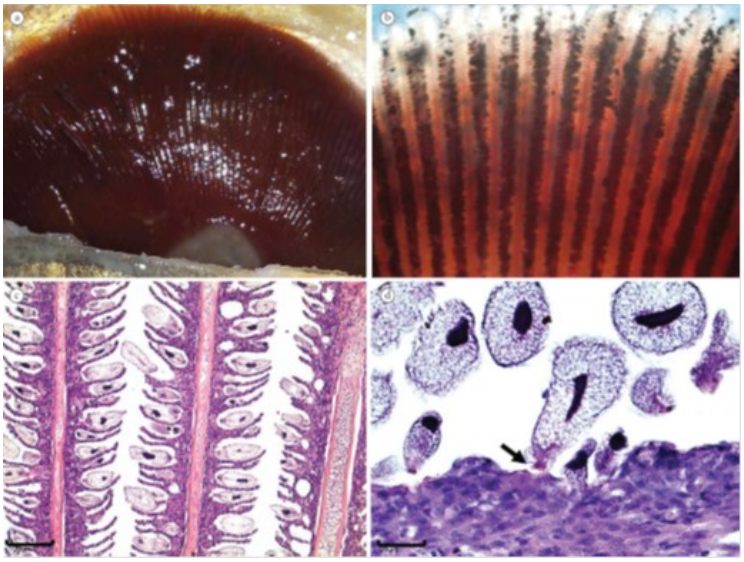

Figure (11). Pathological alterations in fish with piscinoodiniasis. Brownish gill of tambaqui Colossoma macropomum (a), masses of parasites attached to gill filaments located in stereomicroscope (b), as well as from histological section (c), rhizocysts penetrating host's skin (d- arrow). Bar: 100 µm (c) and 25 µm (d).

Trypanosoma

A haemoflagellate parasite, exists in marine and fresh water, causing risk in aquaculture. Trypanosoma species have elongated, cylinder-shaped body with more or less thin extremities, loose flagellum, with undulating membrane beside the nucleus (Fig.12) and volutin granules disposed in the center of the body **Hussein *et al.* (2010); Pādua *et al.* (2011b).**

Life cycle:

Those protozoans with a heteroxenic life cycle concerning an annelid Hirudinea as an intermediate host and vertebrates as definitive hosts. Asexual reproduction through cell division qualified in infested knifefish Gymnotus aff. Inaequilabiatus **Pādua *et al.* (2011b).** Amastigote, epimastigote and tripomastigote were located in stomach of leech Batracobdella gemmata, vector of trypanosomiasis to the loricariid catfish Hypostomus punctatus **D'agosto & Serra–Freire (1993).**

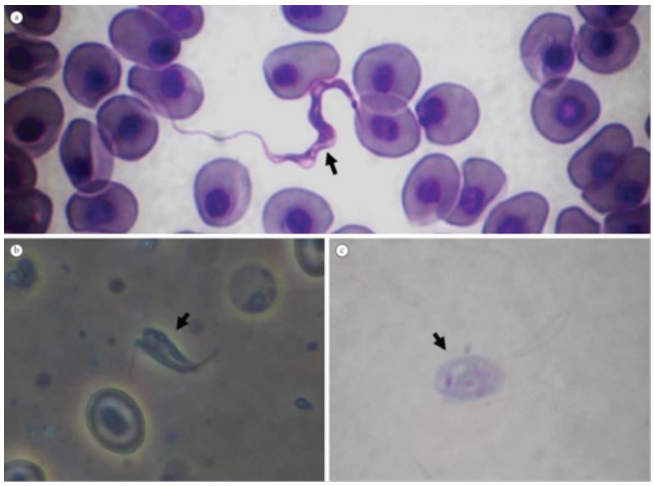

Figure (12). *Trypanosoma sp. (a) blood of tuvira Gymnotus aff. Inaequilabiatus; Cryptobia sp. (b) scrapes of gill of hybrid tambacu (C. macropomum x P. mesopotamicus) and Ichthyobodo sp. (c) Giemsa stained skin of Nile tilapia*

Transmission:

It is blood parasite, transmission takes place as the leech invade fishes **Bruno *et al.* (2006).** In loricariids, they attached to non covered areas by plates or to gill filaments **D'agosto & Serra –Freire (1993).**

Diagnosis:

Stained blood smear with Giemsa alone or mix of Grünwald- Giemsa or May Grünwald-Giemsa-Wright considered simple and less sensitive methods for morphological characterization of haemoflagellates. Blood mounted slide with centrifugation of blood is specific for analysis **Woo (1969)**. Stained sections of liver, kidney and spleen with Ehrlich haematoxylin and eosin may enhance analysis **Hussein *et al.* (2010).** Identification of the Parasite ought to be done via morphological and morphometric characteristics besides molecular phylogenetic techniques **Maslov *et al.* (2001); Hussein *et al.* (2010); Ferreira & Avenant–Oldewage (2013).**

Pathogenesis and clinical signs:

Due to high parasitic infestation in cultured fish, we can observe: splenomegaly, nephromegaly, liver colour alterations and pale gills.

Histopathology:

All organs with trypanosomiasis presented different levels of alteration, accompanied by large numbers of the parasite in small and large vessels (Fig.13). The main findings were mast cell infiltrates in gills and intestine, multifocal aggregates of melanomacrophages in liver, pancreas, spleen and kidney **De Jesus *et al.* (2018).**

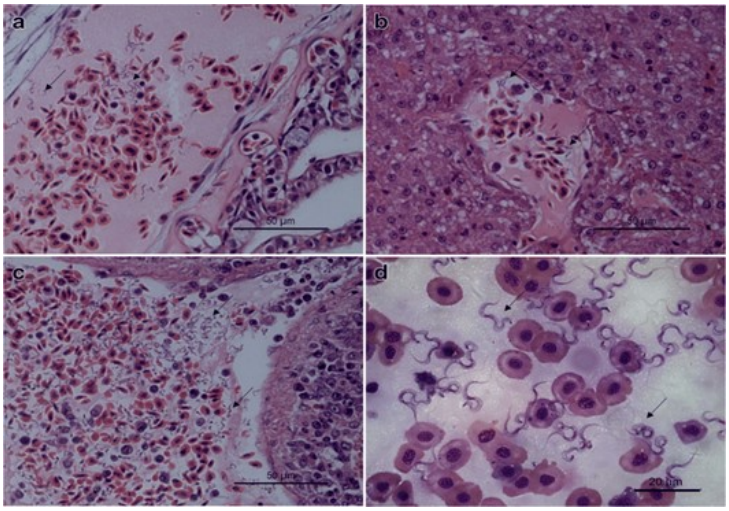

Figure (13). Trypanosoma micropteri sp. trypomastigotes in gill (a), liver (b), head kidney (c), and blood (d). a–c Histological sections stained with H&E. d Wright–Giemsa stained peripheral blood smear.

Cryptobia:

Biflagellates have general characters as: body is triangular shape, at anterior end there are a kinetoplast and a nucleus as well as two flagella; free flagellum and attached one to body which is responsible for the movement (Fig.12b) **Kuperman *et al.* (2002); Bruno *et al.* (2006).** Most of them are ectocommensals **Ranzani–Paiva *et al.* (2005).** Cryptobia branchialis Nie has been recorded in exclusive continents **Kuperman *et al.* (2002)**.

Life cycle:

It has monoxenic life cycle and its reproduction through longitudinal fission. Parasite can invade fish skin and gills of fresh and marinewater **Kuperman *et al.* (2002).**

Transmission:

Blood inhabitant, transmitted horizontally through direct contact or by fishery utensils and contaminated water which can be a source of parasitism in aquaculture **Bruno *et al.* (2006)**.

Diagnosis:

Skin and gills mounted smears examined microscopically to identify Cryptobiosis. Silver albumose (protargol) impregnation stained infected smears can provide a correct diagnosis **Kozloff (2004)**. **Bruno *et al.* (2006)** observed kinetoplast by Giemsa or Feulen stain.

Differential diagnosis:

Fresh-mounted microscope slides from gills must be made carefully to differentiate between *Cryptobia* spp. and *Ichthyobodo* spp. due to their similarity. Both of them have two flagella. Flagellum *of Cryptobia* spp. presents rapid movement but flagellum *of Ichthyobodo* shows circular movement.

Pathogenesis and clinical signs:

Young tilapia with high parasitic infestation showed excessive production of gill mucous and edema of gill filaments **Kuperman *et al.* (2002).**

Histopathology:

Cryptobia spp. can invade the gastric epithelium (Fig.14), causing gastric dilation, submucosal granuloma, gastric perforation, peritonitis and full thickness muscle necrosis **Bustos *et al.* (2011).**

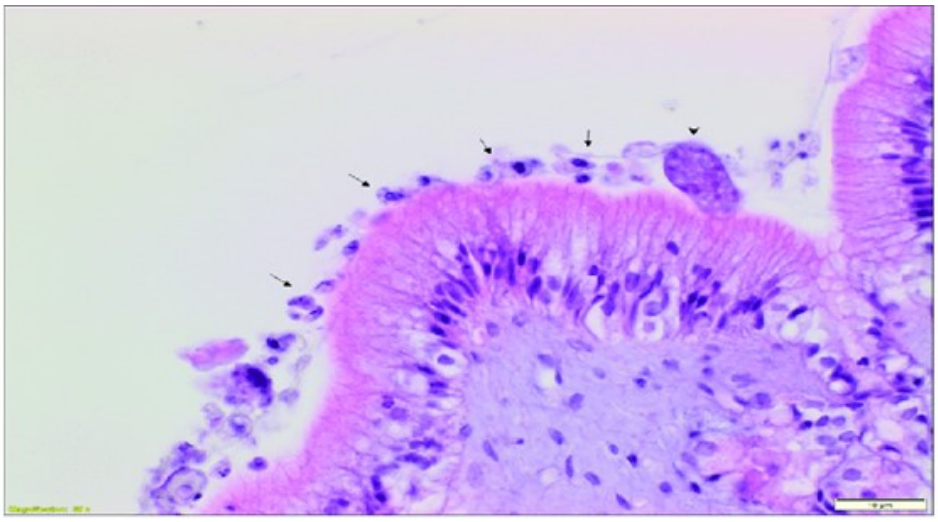

Figure (14). Cryptobia sp. (arrows) and Cycloptericola sp. (arrowhead) on the mucosal surface of stomach from lumpfish. H&E stain. Scalebar 10 µm.

Ichthyobodo:
Small biflagellated, Causing ichthyobodiasis, the kinetoplastids are present in fins, gills and skin **Lom & Dykovā (1992); Todal *et al.* (2004).** Ichthyobodo necator in some Brazilian fish was recorded **Eiras *et al.* (2012).** Like Cryptobia, body is pear shape (Fig. 12c), circling or zigzag movement was noted if it attached to host **Lom & Dykovā (1992).**

Life cycle:
It has monoxenic life cycle, reproduction is asexual through longitudinal fission of parasitic cell can occur **Lom & Dykovā (1992).**

Transmission:
Horizontal transmission occurs by direct contact, contaminated water and equipments.

Diagnosis:
By haematoxylin, Feulgen or Giemsa stained mounted smears of gills and skin **Lom & Dykovā (1992); Todal *et al.* (2004); Isaksen (2013).** Distribution and kinetoplast morphology are useful for identification **Moreira *et al.* (2004)**. Histopathological evaluation taken into consideration as an efficient tool for diagnosis of ichthyobodiasis **Urawa *et al.* (1991); Bruno *et al.* (2006).** Molecular methods of DNA extraction is sensitive, but not used as a routine diagnosis **Isaksen *et al.* (2012).**

Differential diagnosis:
Cryptobia is pyriform with two flagella which are unequal and posterior-lateral extension, but Ichthyobodo is elongated with one posterior and one free posterior flagellum.

Pathogenesis and clinical signs:
No significant mortality observed. In larvae and younger fishes, low intensity infections have been found. Non specific clinical symptoms including gill and skin color changes and breathing problem **Urawa *et al.* (1991); Lom & Dykovā (1992).**

Histopathology:
Parasitic attachment leads to epithelial cells destruction **Lom & Dykovā (1992)**, epithelium proliferation and degeneration with decreased the production of mucous were observed **Urawa *et al.* (1991)**.
Ichthyobodo necatrix induces severe erosion and ulcerative dermatitis following epithelial hyperplasia and increased mucus production (Fig.15) **Noga (2000).**

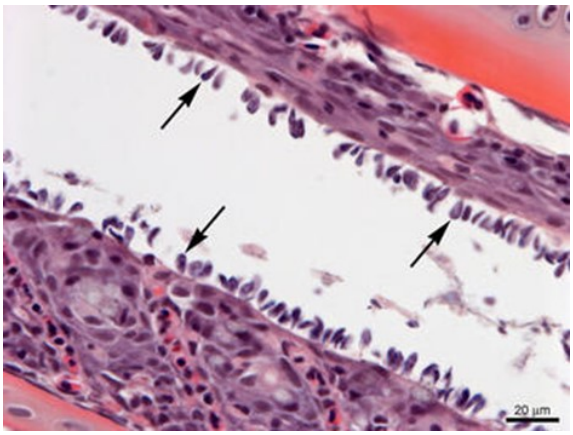

Figure (15). Gills from Lost River suckers with a heavy infestation of Ichthyobodo sp. (arrows).

Tetrahymena:

It is an essential pathogen in freshwater fish causing high death rate **Bruno *et al.* (2006).** Its body is a pear shap has a row of cilia covering it , as well as macronucleus and micronucleus (Fig.16). When there are wounds, these ciliates penetrate the host epithelium reaching the blood and parasitizing the gills, kidney, eyes and brain **Eiras (2013a).** Tetrahymena causes whitish lesions on the skin due to cutaneous infestation and secondary bacterial infection can occur **Bruno *et al.* (2006).**

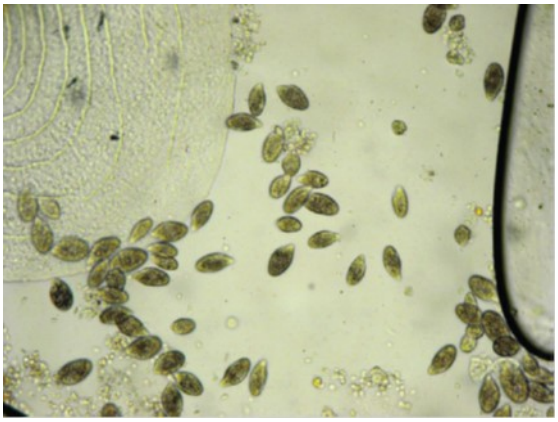

Figure (16). *Skin of guppy showed Tetrahymena sp. (Poecilia sp.)* 40x magnifications

Histopathology:

Tetrahymena pyriformis are capable of disseminating infections with dermal ulceration. It may invade various internal organs as kidney or brain (Figure 17) **Noga (2000).**

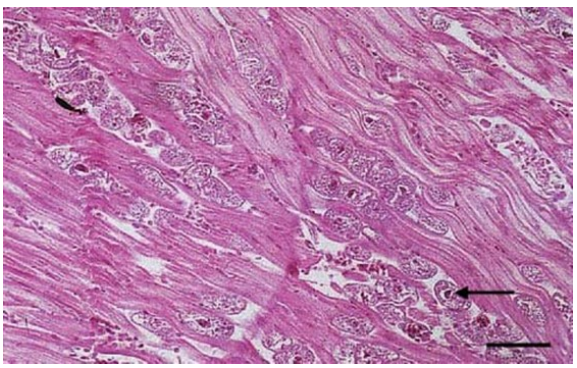

Figure (17). Tetrahymena corlissi between muscle fibres of the guppy Poecilia reticulata. Some of the parasites show the characteristic pyriform shape, whereas others are distorted by crowding into tissue. Oval macronucleus is visible in some organisms (arrow). H&E. Scale bar = 50 µm**.**

Hexamita:

Hexamita members have the following characters: body is oval shape, bilateral similarity, locomotion is through three anterior pairs of flagella and one posterior pair, on anterior end there are two rounded nuclei **Foin (2005).** Also, intestinal tract endoparasites **Eiras (1994); Francis–Floyd & Reed (1994); Foin (2005).** It may be located in blood, swimbladder, liver, kidney, heart and spleen **Foin (2005)**. **Békési (1992)** recorded existence of Hexamita in the intestine of Prochilodus brevis in Brazil.

Life cycle:

They are monoxenic lifecycle. Before cellular division, the pear-shaped trophozoites change into spherical **Woo (2006).**

Transmission:

Horizontally transmitted by discharging trophozoites and oocysts from the fish feces into the water. So, other hosts will ingest them. **Lom & Dykovā (1992); Foin (2005).**

Diagnosis:

Analysis of the feces of infected fish under high magnification **Klinger & Francis–Floyd (1998); Foin (2005).**

Histopathology:

They are associated with a full-thickness gastritis and may penetrate blood vessels leading to dissemination. Hexamita spp. are reported in the kidney of many fish causing renal tubular necrosis (Fig.18) **Purivirojkul & Khidprasert (2009).**

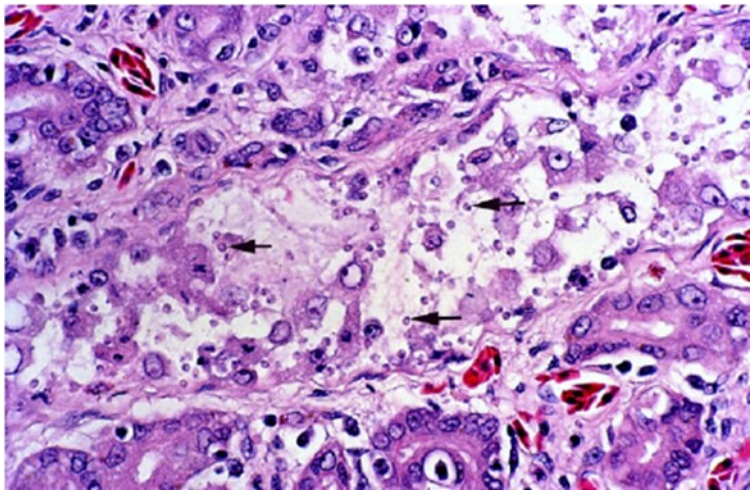

Figure (18). Spironucleus (Hexamita) infection of the renal tubules of a tortoise. Note tubular necrosis and microorganisms (arrows).

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