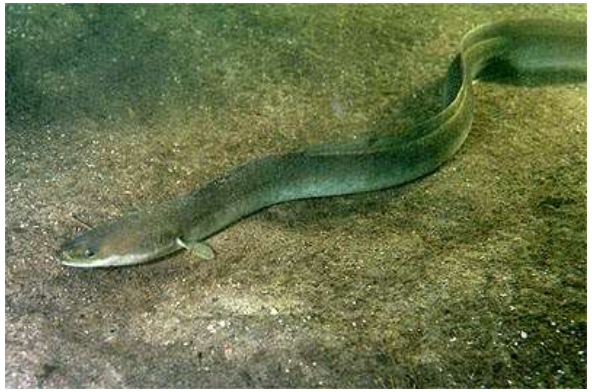
Hexamita and *Spironucleus*

Introduction:

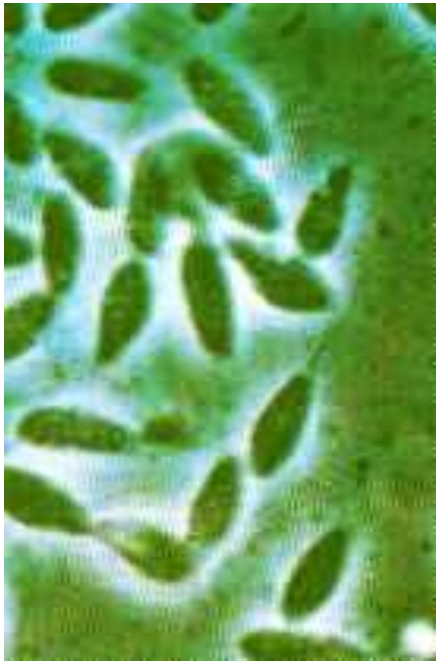
- reported in the digestive tract of fishes(sometimes in internal organs)
- direct life cycles
- they are difficult to distinguish under the light microscope
- known as hexamitosis, spironucleosis and possibly 'hole-in-the head' disease in salmonids, cyprinids and ornamental aquarium fishes
- Sporadic outbreaks of fatal systemic spironucleosis have occurred in cyprinids ,eels ,tropical aquarium fishes, Atlantic salmon and chinook salmon

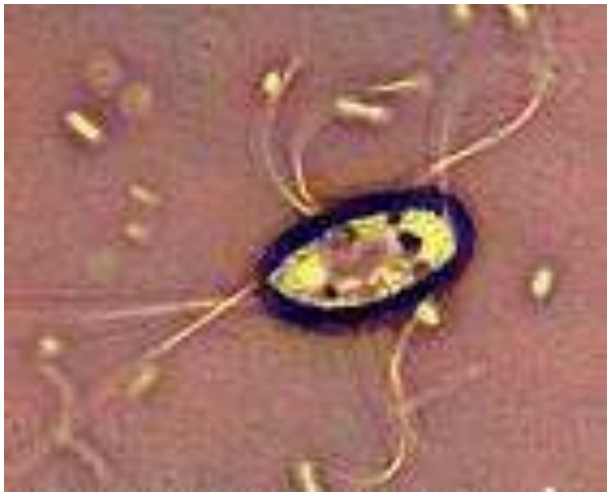
Geographical distribution and host range:

- very wide host range and geographical distribution
- These parasites are in wild, farmed and aquarium fishes in cold, temperate and warm waters.
- They have been reported in Acipenseridae, Anguillidae, Catostomidae, Centarchidae, Cyprinidae, Cyprinodontidae, Gadidae, Gasterosteidae, Mugilidae, Percichthyidae, Percidae, Salmonidae, Siganidae and Sparidae



- ***Hexamita salmonis*** has been reported in fishes in North America, Europe and Asia. It is usually found in freshwater fishes but it has also been reported from marine fishes.
  - The disease is most commonly found in fingerlings, although yearlings and smolts may also show clinical signs of the infection.
  - Outbreaks of the disease are usually sporadic in aquaculture facilities, and they have seldom been reported in wild fishes





- ***Spironucleus vortens*** is in angelfish Florida, in discus and in and in ide (*Leuciscus idus*).
  - causative agent in 'hole-in-the-head' disease in cichlids. The parasite is also known as 'discus parasite' as it is often found in discus fish

- *Spironucleus* are known to be pathogenic and cause disease in salmonids maintained in sea cages. ***Spironucleus barkhanus*** has been isolated from Atlantic salmon (*Salmo salar*), grayling (*Thymallus thymallus*) and Arctic charr (*Salvelinus alpinus*) in Norway

Morphology:

- Live trophozoites of *Hexamita* and *Spironucleus* vary from elongated to nearly spherical and the two genera are usually difficult to separate.
- The lengths of live organisms may reach 20 µm and the organisms have bilateral symmetry
- *Hexamita* has an elongated to spherical body and spherical nuclei, while a pyriform body and elongated nuclei are characteristic of *Spironucleus*

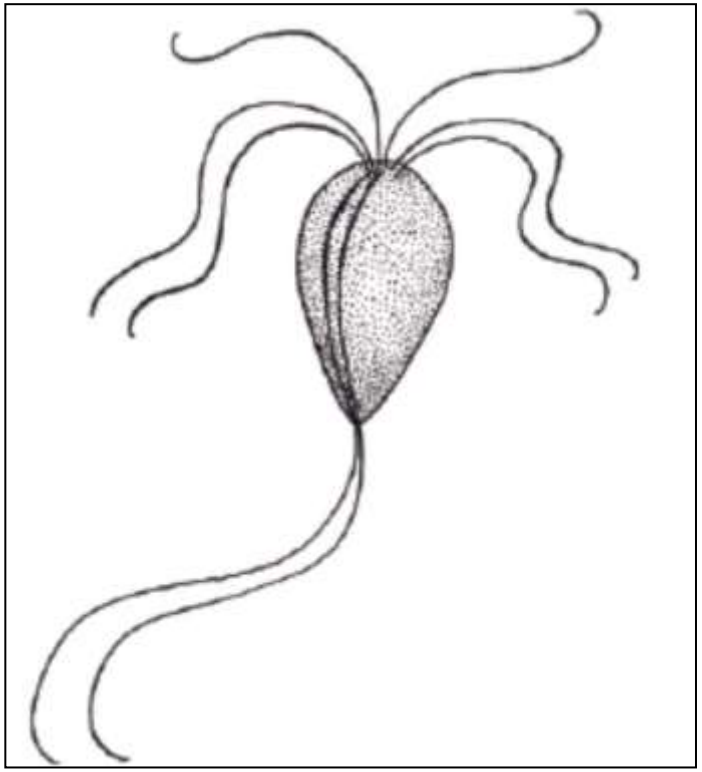
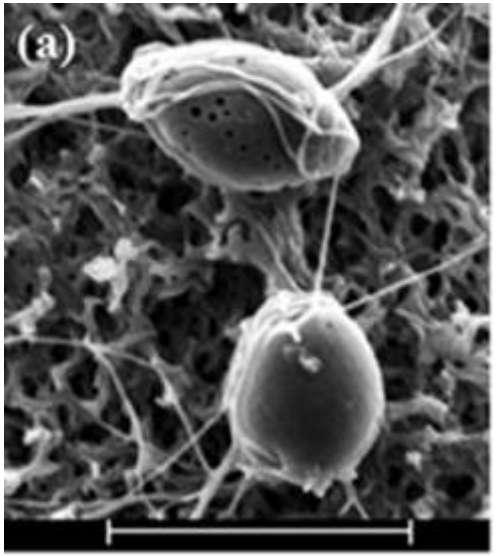
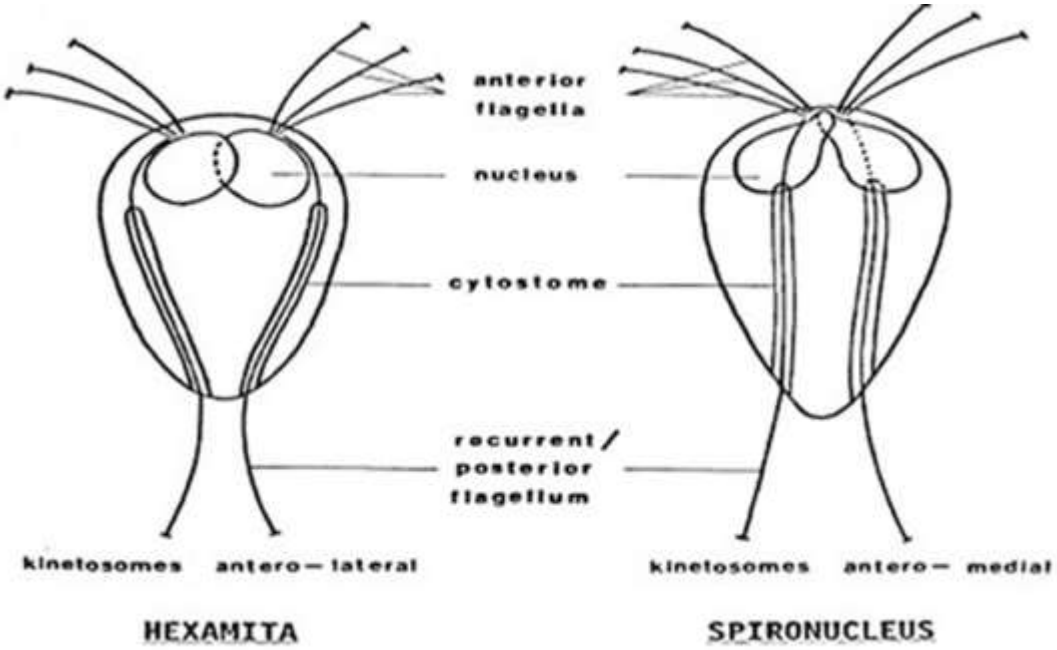


Diagram of a typical diplomonad flagellate with diagnostic features: size (from 5 to 20 µ m long, excluding the flagella); eight flagella (three pairs anteriorly, one pair posteriorly); pyriform to ellipsoidal to egg - shape to tapering body.

- the two recurrent flagella in their canals are located between the nuclei in *Spironucleus* while those in *Hexamita* are on the surface of the nuclei
- Diplomonad cysts are usually oval in shape, and each cyst usually contains two recently divided flagellates.



Scanning electron micrographs of *Spironucleus vortens*. Normal, untreated trophozoites are pyriform-shaped possessing 8 flagella



Clinical signs and pathology:

- *Intestinal infections:* Trophozoites are commonly found in the lumen of the intestine, while cysts are often not seen.
- the flagellate interfered with nutrition by competing for essential nutrients and/or by damaging the intestinal epithelium to affect digestion and absorption.

- Systemic hexamitid infections have been reported in cyprinids, eels, salmonids and tropical aquarium freshwater fishes , with trophozoites in the blood, gall bladder, heart, kidney, liver, spleen, eye, brain, muscles, mesentery and abdominal cavity. There are also reports of hexamitids in cranial skeletal tissues and in pustules on the skin of infected cichlids

- weakness, whirling, listlessness, abdominal distension, anaemia, faecal pseudocasts, pale stringy faeces, a red vent, exophthalmia and dark coloration

- Fish infected with *H. salmonis* may have anaemia, ascites, enteritis and intestinal contents that are yellowish and watery or jelly-like, with excessive amounts of mucus.

- Infected aquarium tropical fish may have yellow mucus in their intestines, enteritis and enlarged and inflamed gall bladder.

- The pathology associated with the infection varies from no effects to haemorrhages in the intestine , catarrhal enteritis and hepatocellular necrosis

This Tiger Oscar has succumbed to hole in the head disease

Oscar with hole in the head disease

Unilateral exophthalmia in Atlantic salmon experimentally infected with *Spironucleus barkhanus*

Diagnosis

- Wet mount of skin, feces, or viscera with parasites
- Histopathology of lesion with parasites

Wet mount of diplomonds

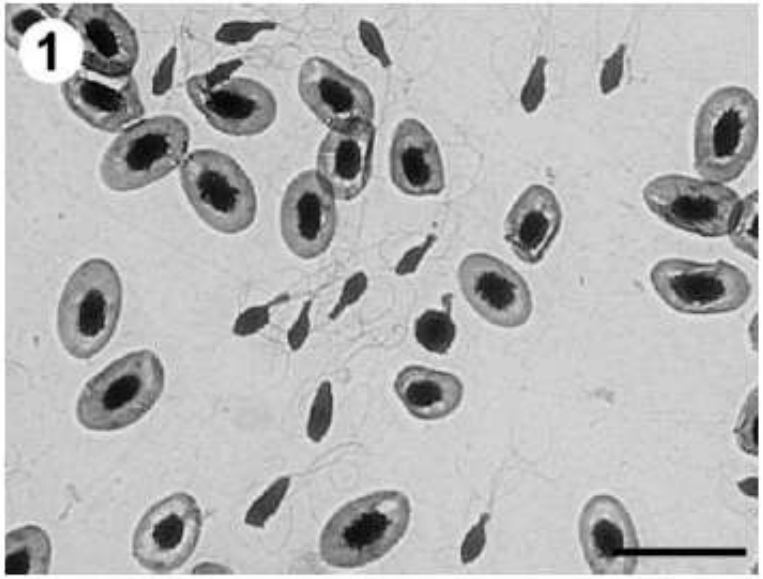
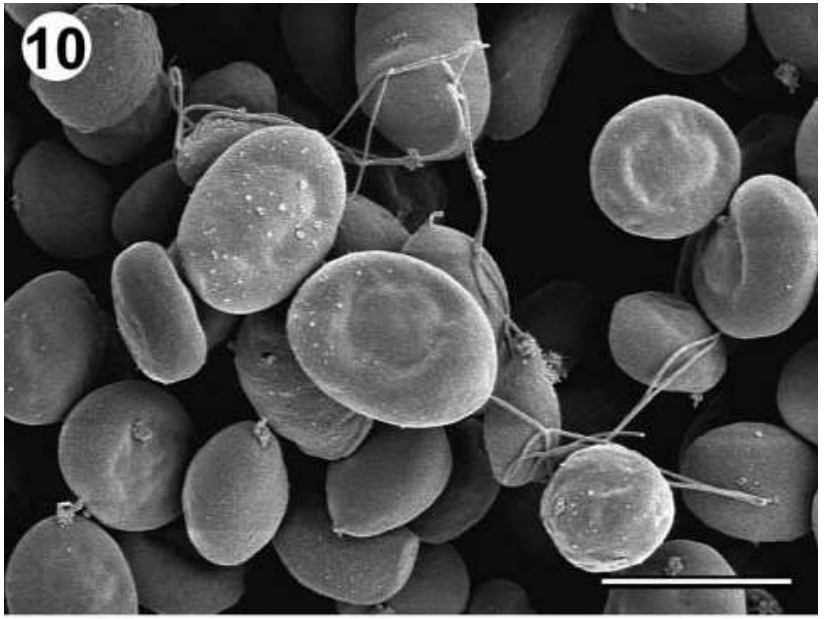
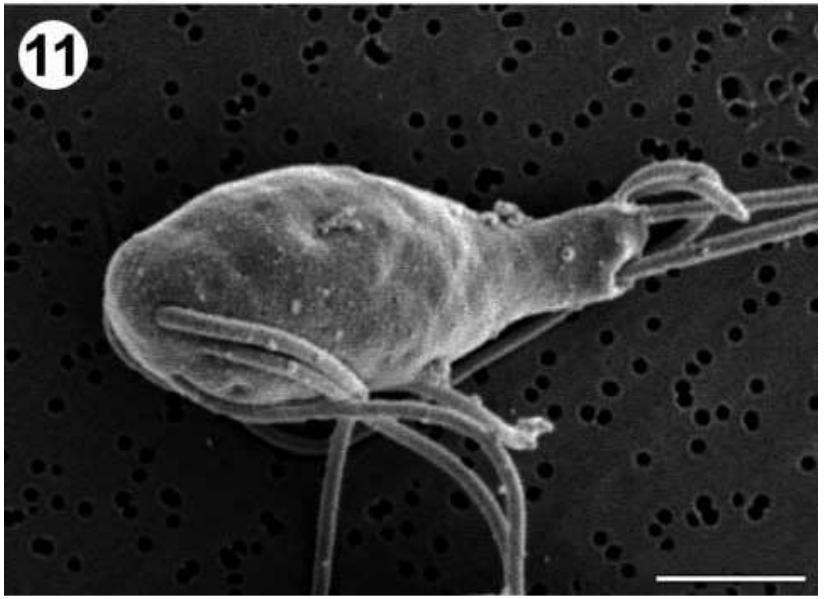


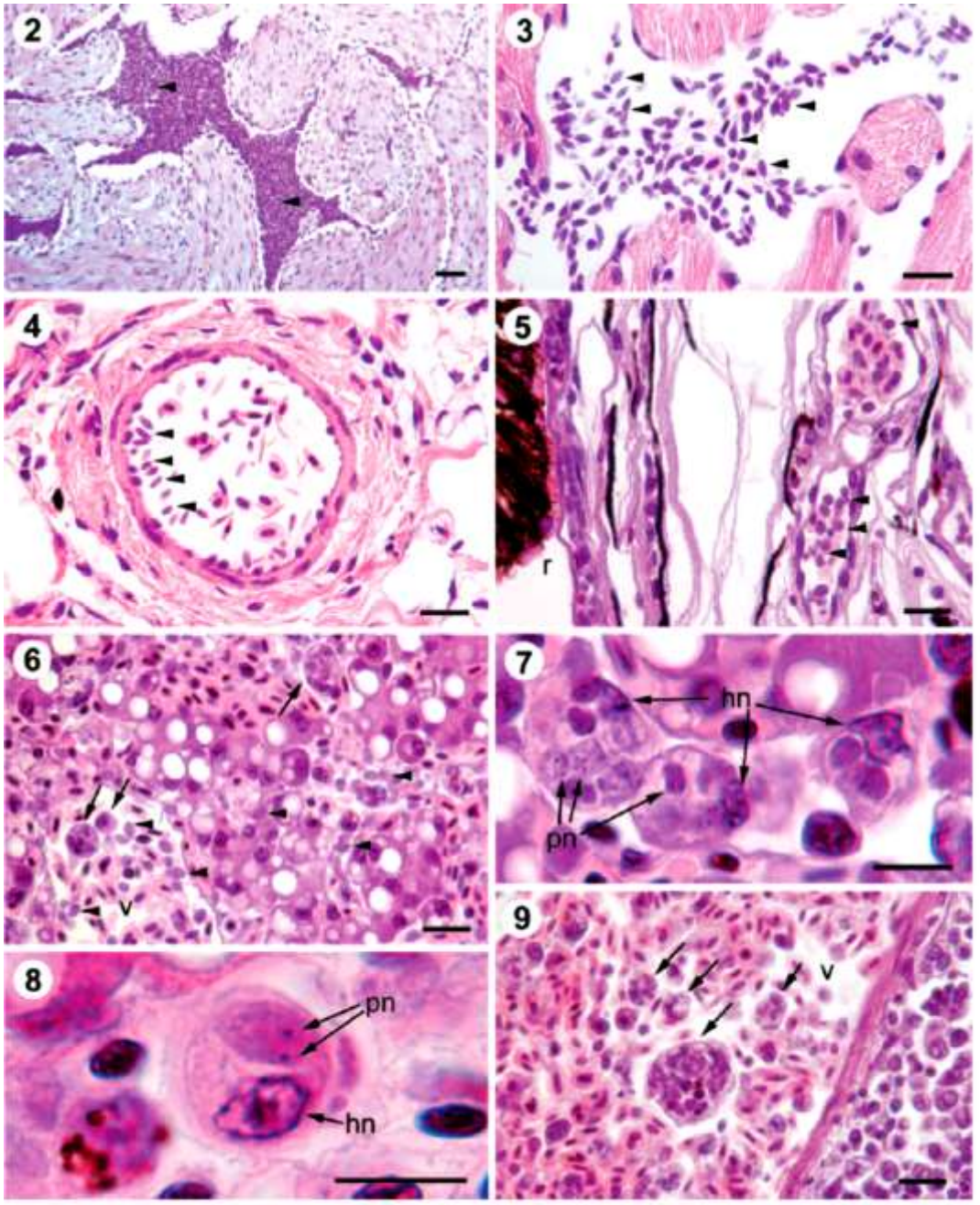
Fig. 1. *Salvelinus alpinus*. Air-dried and Diff Quick®-stained blood smear from Arctic char infected with *Spironucleus barkhanus*. Scale bar = 10 µm





Figs. 10 & 11. *Salvelinus alpinus.* Scanning electron micrographs of blood from Arctic char showing presence of trophozoites of *Spironucleus barkhanus*. Scale bar in 10 = 10 µm;in 11 = 2 µm

- Figs. 2 to 9. *Salvelinus alpinus*. Light micrographs of histological sections from Arctic char infected with *Spironucleus barkhanus* stained with haematoxylin and eosin
- Fig. 2. Numerous trophozoites of *S. barkhanus* (arrowheads) within lumen of bulbus arteriosus.
- Fig. 3. Trophozoites of *S. barkhanus* (arrowheads) within ventricular lumen.
- Fig. 4. Trophozoites of *S. barkhanus* (arrowheads)within lumen of coronary artery.
- Fig. 5. Trophozoites of *S. barkhanus* (arrowheads) within vessels of choroid plexus of the eye; r = retina.
- Fig. 6. Trophozoites of *S. barkhanus* (arrowheads) and host cells (arrows) with intrecellular stages of *S. barkhanus* in vessels and sinusoids of the liver.
- Fig. 7. Three host cells in liver sinusoid with varying numbers of intracellular stages of *S. barkhanus*; hn = host cell nucleus, pn = flagellate nucleus.
- Fig. 8. Single *S. barkhanus* inside host cell;
- Fig. 9. Host cells (arrows) with varying numbers of intracellular stages of *S. barkhanus*, within lumen of a vein (v) in the head-kidney. Scale bars in (2–6) and (9) 20 µm; scale bars in (7 & 8) = 10 µm



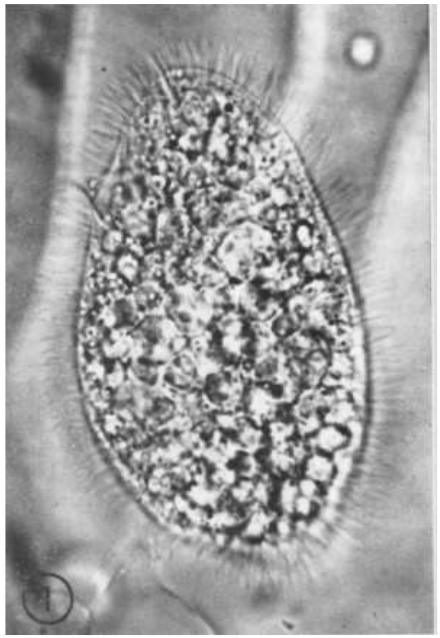
Treatment:

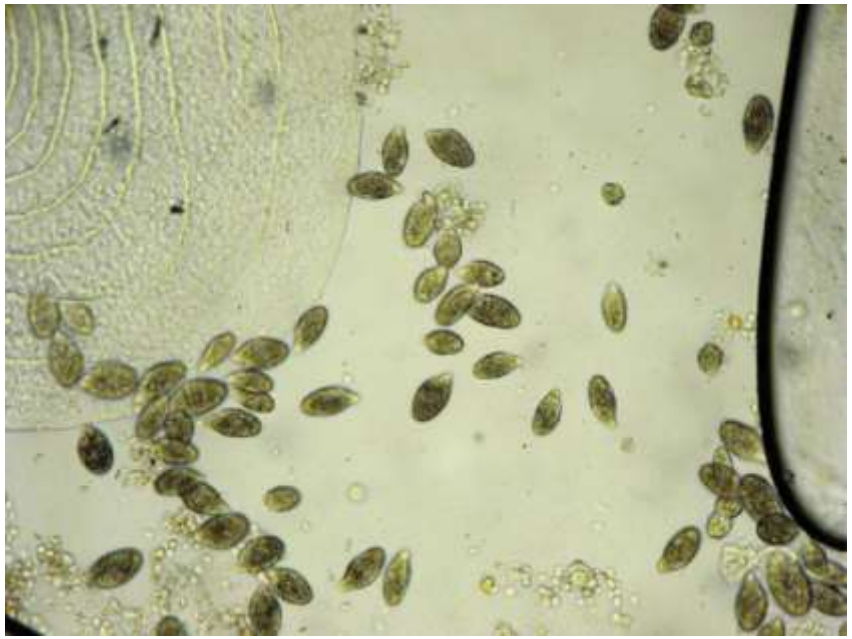
1. Metronidazole oral
2. Metronidazole prolonged immersion
3. Magnesium sulfate oral
4. Raise temperature to 35 ° C (95 ° F) for 7 days(for aquarium fish)

- Determining the infection intensity is important for both treatment and prognosis:
  - Occasional field with 1 – 5 organisms: no treatment needed
  - Average of 5 – 15 organisms in the field: no treatment needed unless no other cause of poor health is identified; watch closely for more serious infections
  - Average of 15 – 30 organisms in the field: treatment needed
  - Average of 30 – 100 organisms in the field: severe infection and therapy essential

Tetrahymenosis
(TET Disease, Guppy Disease)

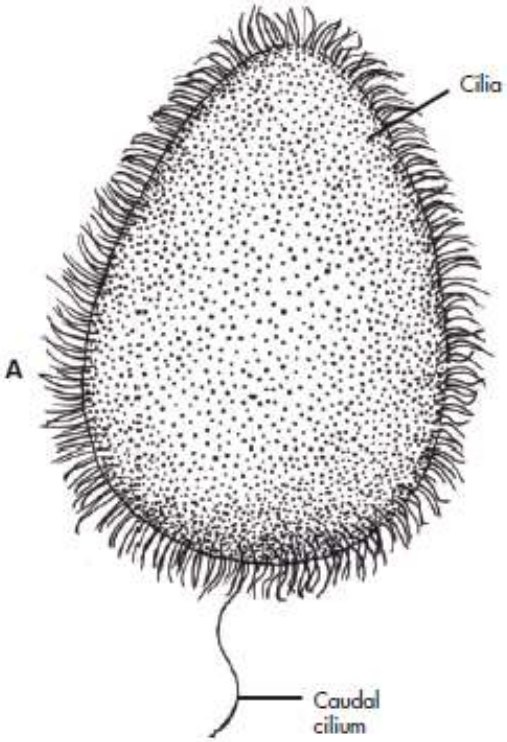
- The important pathogenic agent to ornamental freshwater fish where it may cause severe mortalities .
- This protozoan has a pear-shaped body covered by a row of cilia
- these ciliates do not form cysts and studies suggest that *Tetrahymena* penetrates the host epithelium (especially where there are wounds) reaching the blood and parasitizing the gills, kidney, eyes and brain



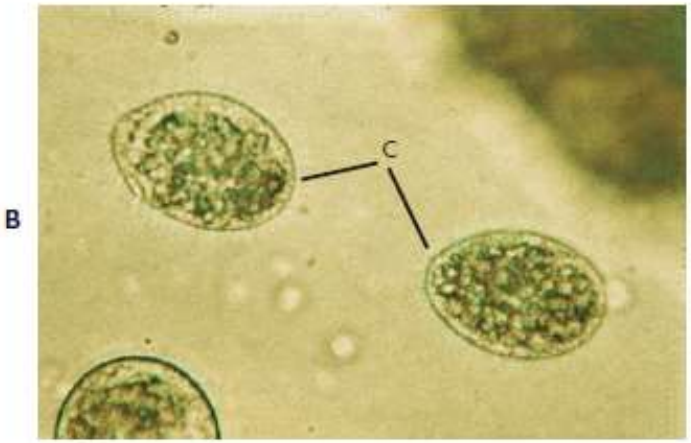


Tetrahymena sp. from the skin of guppy (*Poecilia* sp.)

Diagram with key characteristics: size (~ 30 – 60 × 50 – 100 µ m); pyriform or radially symmetrical, ovoid body; evenly distributed cilia; long caudal cilium (present only in some species [e.g., T. corlissi]).



B. Wet mount of Tetrahymena . Note long cilia covering body. Formalin - fixedspecimen. (B photograph courtesy of G. Hoffman.)



Epidemiology/Pathogenesis:

- Tetrahymenids are typically free - living ciliates, but some species can be highly lethal fish pathogens.
- Reproduction is typically by binary fission; some species (e.g., *T. corlissi*) can produce small reproductive cysts (2 – 8 tomites).
- The species most commonly causing disease is *Tetrahymena corlissi* , which can infest/infect fish and amphibians.
- Called guppy disease because of its predilection for guppies, the disorder also affects other livebearers, cichlids, and tetras

Clinical signs:

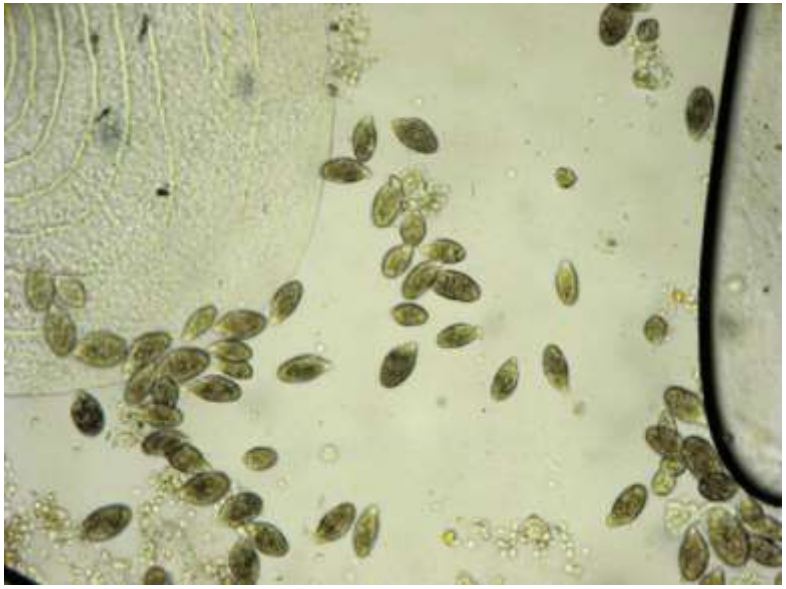
- Typical signs of protozoan ectoparasite; also, areas of muscle swelling
- Guppies can appear normal one day and be dead the next
- In tropical aquarium fishes the infection is manifested by whitish patches, due to masses of ciliophorans in copious amounts of mucus (Johnson, 1978). Fish scales are bristled as the outer skin is lost and the tissue swells.
- A mass of ciliophorans may form a rim around the eye orbit ('spectacled eye').
- *Tetrahymena pyriformis* - like ciliates can damage the skin and invade the internal organs of common carp, catfish and rainbow trout.
- *Tetrahymena* also can cause disease in some crustaceans

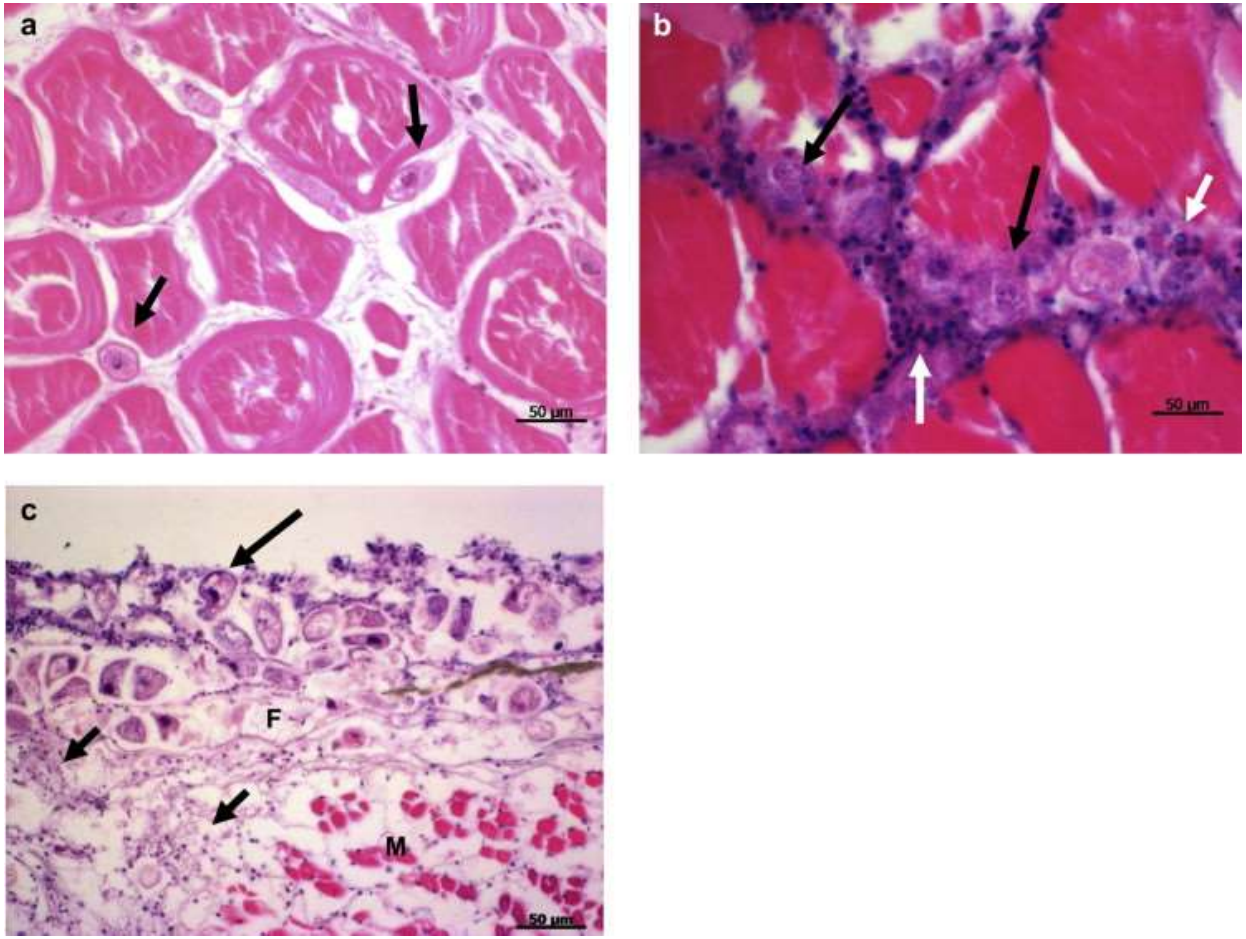
Diagnosis:

- The mucus production and epithelial damage caused by *Tetrahymena* may appear grossly similar to ich but are easily differentiated by identifying the parasite.
- *Tetrahymena* may be confused with free - living, nonpathogenic ciliates. Shape, size, movement (like a spiraling football), and presence of typical invasive lesions should be used for differentiation.
- Penetration of ciliates into muscle and deep tissues is highly diagnostic for *Tetrahymena* .

Method of Diagnosis:

- Wet mount of skin, gills, or internal organs with parasite
- Histopathology of skin, gills, or internal organs with parasite



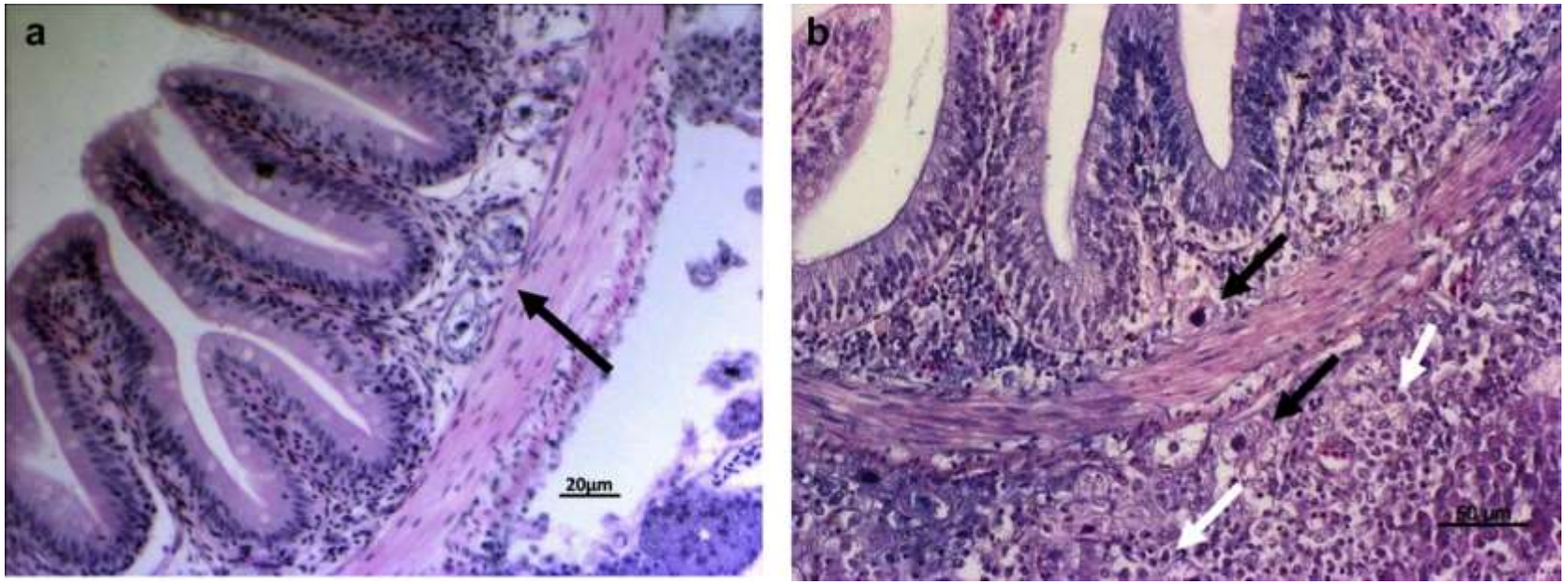


Histological analysis of muscle and associated skin from fish infected with *Tetrahymena*.

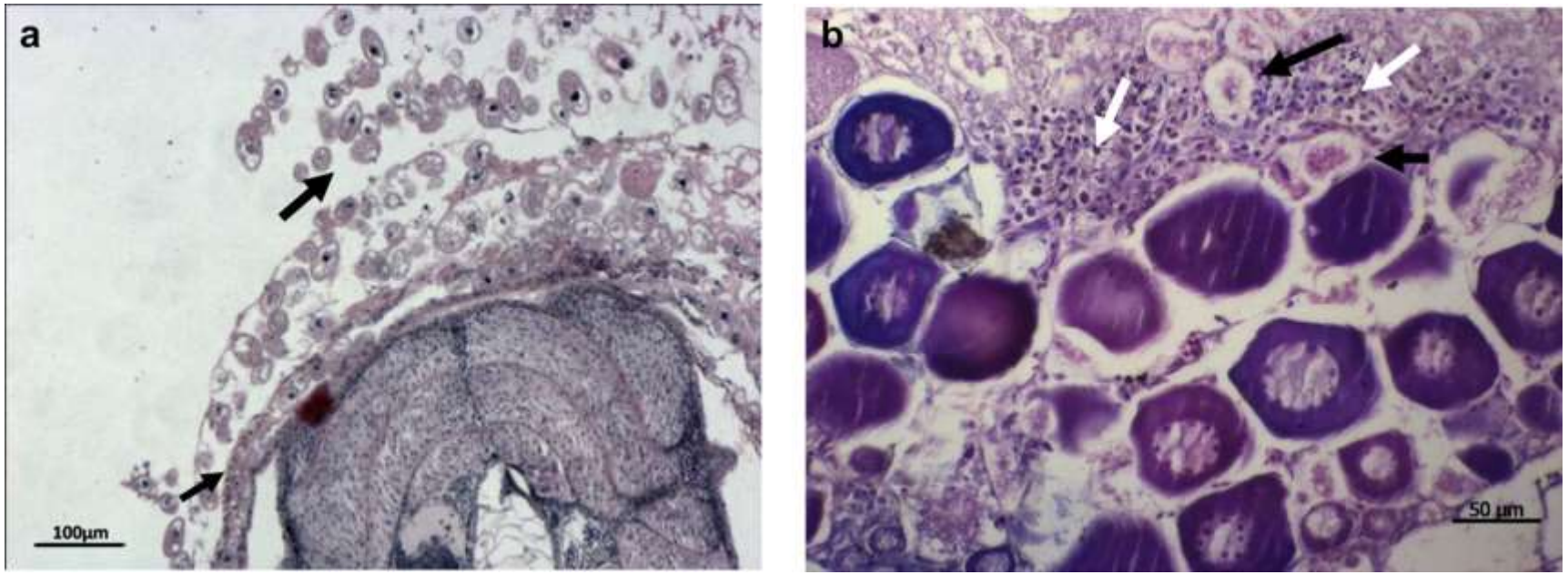
(a) Muscle tissue from a guppy showing *Tetrahymena* between muscle fibres (arrows) with no evident inflammatory response.

(b) (b) Muscle tissue from a koi carp with infiltrating leucocytes (primarily mononuclear cells; white arrows) around the parasite (black arrows).

(c) (c) Goldfish muscle and associated skin in which *Tetrahymena*is seen in the epithelium (long arrow), reaching subdermal fat (F) and the musculature (M). Leucocytes infiltrating the muscle and subdermis are indicated by short arrows. HE.



Histological analysis of intestine from fish infected with *Tetrahymena* (black arrows).
(a) Angelfish intestine showing *Tetrahymena* in the intestinal mucosa and no associated inflammation.
(b) Goldfish intestine showing *Tetrahymena* in the intestinal mucosa and submucosa and associated infiltrating leucocytes (white arrows). HE.



Histological analysis of the reproductive system from fish infected with *Tetrahymena* (black arrows). (a) *Tetrahymena* around the muscle tissue of a molly embryo.
(b) *Tetrahymena* adjacent to and invading the ovaries of a goldfish with associated infiltrating leucocytes (white arrows). HE.

Treatment:

- Only cases without systemic disease are treatable and may require several treatments.
- The environment should also be improved. Feeding a diet high in the essential fatty acid arachidonic acid aided the recovery of guppies from the
- Formalin bath

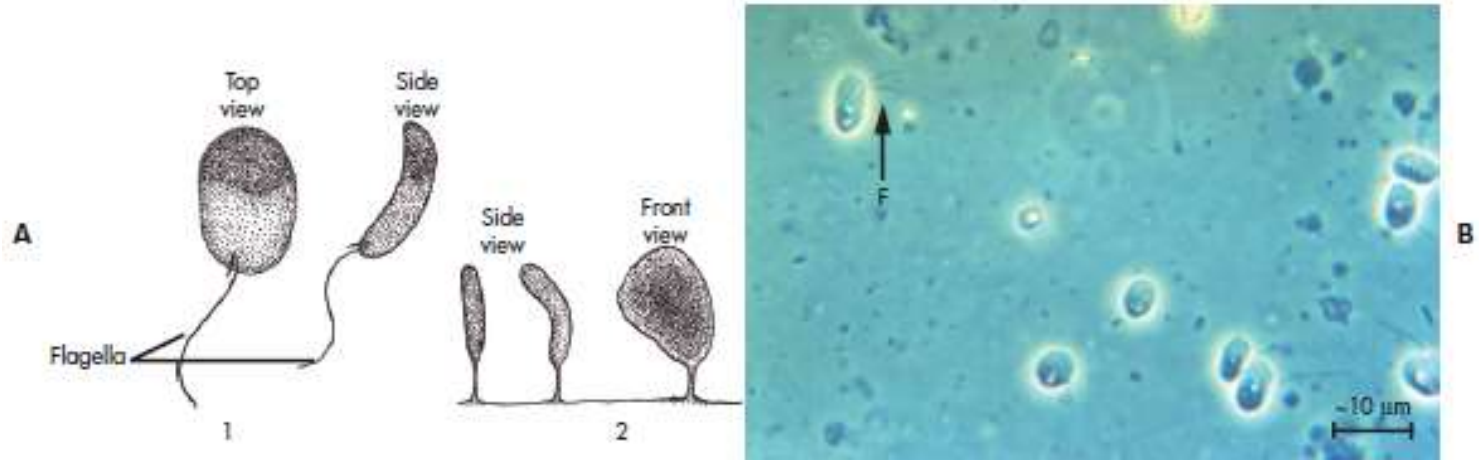
Ichthyobodosis (Costiosis)

Epidemiology:

- *Ichthyobodo necator* (*Costia necatrix*) is one of the smallest ectoparasites (about the size of a red blood cell).
- *Ichthyobodo* is especially dangerous to young fish and can attack healthy fry and even eggs.
- In older fish it is associated with some type of predisposing stress.
- *Ichthyobodo necator* causes disease over a wide temperature range (2 – 30 ° C).
- While classically a disease of freshwater fish, *Ichthyobodo* also occurs in purely marine fish.
- Recent morphological and molecular studies have resulted in the description of a new species, *Ichthyobodo hippoglossi* , which infests Atlantic halibut

Pathogenesis:

- *Ichthyobodo* exists in two forms :
- The detached, mobile form (A 1 and B) has two or, if predivisional, four flagella, all of which are difficult to see in actively moving parasites.
- While the parasite feeds on the fish, it is curled into a pyriform shape and is attached to and penetrates the epithelium
- The transition between forms occurs within a few minutes.



Ichthyobodo . Diagrams with key characteristics:
A.(1) Free - swimming stage: size (~ 5 – 8 × 10 – 15 µ m); slightly asymmetrical; oval body on top view; flattened, crescent shape on side view; single or paired flagella directed posterolaterally. (2) Attached stage: pyriform shape; flagella are not easily seen when attached.
B. Wet mount of the free - swimming stage of I. necator . F = flagellum.

- *Ichthyobodo* can cause considerable mortalities —sometimes with little obvious pathology (Fig. D), but other times with spongiosis and epithelial sloughing.
- Tissue irritation also leads to epithelial hyperplasia and increased mucus production, giving fish a bluish cast (slime).

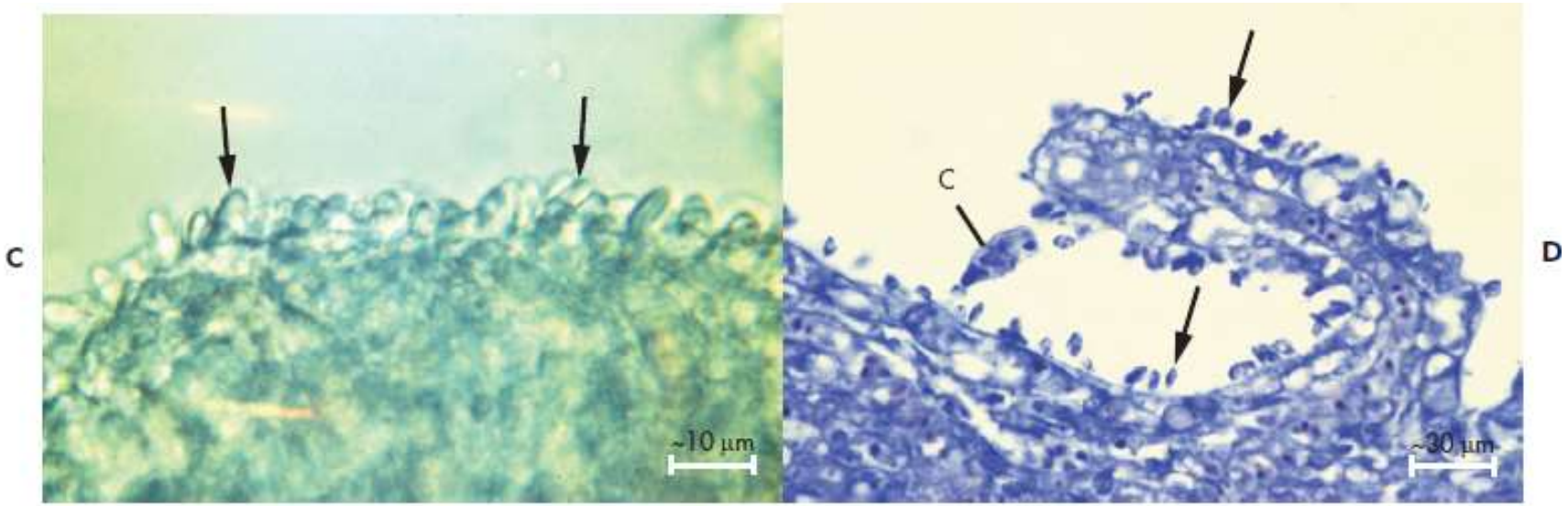
- **Clinical signs:** typical signs of protozoan ectoparasite; especially, drop in temperature; bluish or whitish film on body

Diagnosis

- Diagnosis of the genus *Ichthyobodo* is easily made from skin or gill biopsies
- The free - swimming form exhibits a characteristic flickering motion when it moves, which is caused by the change of refractility when it turns its crescent - shaped body.
- Attached parasites are more difficult to detect, but, in heavy infestations, they can be located by focusing up and down at high magnification on the edge of the gill epithelium, where they form palisades. They may also be seen slowly swaying while attached.

Method of Diagnosis:

1. Wet mount of skin or gills with parasite
2. Histopathology of skin or gills with parasite



C. Wet mount of many Ichthyobodo (arrows) attached to the gill epithelium.
D. Histological section of gill with a heavy I. necator infestation (arrows). Note the pyriform, dorsoventrally fl attened shape on side view. A larger, unrelated ciliate (C) is also present. Giemsa.

Treatment:

1. Formalin bath
2. Formalin prolonged immersion
3. Potassium permanganate prolonged immersion
4. Raise temperature > 30 ° C (86 ° F)
5. Salt bath (freshwater only)
6. Secnidazole oral
7. Triclabendazole
8. Metronidazole oral