

***Ichthyobodo* (= *Costia*)**
(protist: flagellate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Flagellates are protists that swim using one or more flagella (undulipodia); each arising from a small centriole (basal body, kinetosome) and having a microtubular axoneme core (2+9 configuration). Rather than forming a monophyletic group, flagellates are divided into several disparate groups: metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). Most kinetoplastids are parasitic in vertebrate or invertebrate hosts (some in plants) whereas the remainder are free-living aquatic organisms. All species are characterized by the possession of extranuclear DNA in the form of a kinetoplast, a unique structure formed by massed DNA (circles or lattice) within the single large mitochondrion near the flagellar basal body. The flagellates reproduce by longitudinal binary fission and parasitic species may have simple monoxenous (one-host) or more complicated heteroxenous (two-host) life cycles involving different developmental stages. Ichthyobodonids include members of the genus *Ichthyobodo* (syn. *Costia*) which have two heterodynamic flagella and are parasitic on fish.

Classification:

Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Excavata (with conspicuous ventral feeding groove)
Group: Discoba (diverse group supported robustly by molecular studies)
Phylum: Euglenozoa (flagella inserted in anterior pocket, some heterotrophs, some autotrophs (with chloroplasts))
Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)
Subclass: Prokinetoplastina (small group supported by molecular studies)
Family: Ichthyobodonidae (2 heterodynamic flagella arising from pocket that continues as a groove)
Genus: *Ichthyobodo* (= *Costia*) (parasitic on gills/skin of fish)
Species: *I. necator* (*C. necatrix*) (causes surface lesions on fish)

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Flagellated species have one or more flagella with an internal microtubular core (in a characteristic 2+9 configuration comprising 2 single central microtubules and 9 peripheral doublets) anchored to a submembranous protein structure (known variously as a centriole, basal body, kinetosome or blepharoplast). Many types of flagellated cells have been described and recent phylogenetic studies have classified them into several disparate groups: including the metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). While most flagellated protists are free-living organisms swimming and feeding in aquatic environments, representatives of several groups have developed symbiotic relationships with various hosts; some being endoparasitic in vertebrates (notably anaerobic metamonads in tubular organs, and heterotrophic euglenozoans occurring in blood or tissues), and some being parasitic in invertebrates (alveolates in crustacean tissues) (representatives tabulated below).

Higher taxonomy	Class or order	Family	Genera	Hosts (tissues)	Transmission*
Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: Metamonad (amitochondriate flagellates with karyomastigonts)					
Phylum: Fornicata (diplomonads)	Order: Diplomonadida (1-2 karyomastigonts)	Hexamitidae (2 karyomastigonts with binary axial symmetry)	<i>Giardia</i>	vertebrates (gut)	direct (f-o)
			<i>Hexamita</i> <i>Spironucleus</i>	vertebrates (tissues)	direct (f-o, w)
Phylum: Parabasalia (with parabasal body)	Order: Trichomonadida (3-5 anterior flagella plus recurrent flagellum)	Monocercomonadidae (costa absent, most without undulating membrane)	<i>Histomonas</i>	birds (gut, liver)	direct (f-o)
			<i>Dientamoeba</i>	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	<i>Trichomonas</i>	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	<i>Cochlosoma</i>	birds (gut)	direct (f-o)
Group: Discoba (diverse group supported robustly by molecular studies)					
Phylum: Euglenozoa (flagella inserted in anterior pocket, heterotrophs, autotrophs)	Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)	Ichthyobodonidae (flagellar pocket continues as groove)	<i>Ichthyobodo</i> (= <i>Costia</i>)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	<i>Cryptobia</i>	fish (gills, skin)	direct (w)
			<i>Trypanoplasma</i>	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	<i>Trypanosoma</i>	vertebrates (blood, tissues)	indirect (v-b)
		<i>Leishmania</i>	vertebrates (blood, tissues)	indirect (v-b)	
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups unified by molecular studies)					
Group: Alveolata (with cortical alveoli)					
Phylum: Dinoflagellata (with unique mesokaryotic nuclei)	Order: Blastodiniiales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	<i>Amyloodinium</i> <i>Crepidodinium</i> <i>Piscinoodinium</i>	fish (skin)	direct (w)
	Order: Syndiniiales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	<i>Haematodinium</i> <i>Ichthyodinium</i>	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	<i>Perkinsus</i>	gastropods, bivalves (tissues)	direct (w)

*f-o = faecal-oral transmission; v-b = vector-borne transmission, w = water-borne transmission; v = venereal transmission

Euglenozoans comprise a large group of excavates (with ventral feeding groove), most with 1-2 flagella inserted into an anterior pocket. Many species are free-living aquatic autotrophs possessing chloroplasts while others are free-living or symbiotic heterotrophs feeding on solutes, particles and even other organisms. Kinetoplastids are characterised by the possession of a kinetoplast (containing mitochondrial DNA separate from nuclear DNA), a flagellar pocket, basal bodies with three microtubular roots and paraxonemal (paraxial or paraflagellar) rods, and asexual multiplication by longitudinal binary fission. The unique kinetoplast is formed by massed DNA (circles or lattice) usually closely associated with the flagellar basal body (eukinetoplastic) although some species may be polykinetoplastic (with several kinetoplasts) or pankinetoplastic (irregular kDNA) and some mutants even dyskinetoplastic (without a kinetoplast). Two major kinetoplastid groups are recognized: bodonids with two flagella (most being free-living bacterivores in aquatic/terrestrial habitats); and trypanosomes with a single flagellum (most being parasites of animals or plants with monoxenous or dixenous life-cycles). Different kinetoplastid assemblages exhibit increasing morphological/ultrastructural complexity in their cellular organization thought to reflect evolutionary grades or clines. Amastigotes are simple non-flagellated cells, choano-, pro- and opistho-mastigotes are flagellated cells with elongate flagella, while epi- and trypano-mastigotes are flagellated cells with undulating membranes. Most kinetoplastids have amastigote and promastigote developmental stages but monoxenous parasites of insects (e.g. *Crithidia*, *Herpetomonas*) do not have more elaborate forms whereas dixenous parasites of plants or animals with invertebrate vectors (e.g. *Trypanosoma*, *Leishmania*) do have more morphologically complex forms such as epimastigotes and trypomastigotes.

Traditional classification	Molecular classification	Genera	No. spp.	Vertebrate hosts	Transmission (vectors)
F: Trypanosomatidae	SC: Metakinoplastina F: Trypanosomatidae	<i>Trypanosoma</i>	537	mammals, reptiles, frogs, birds, fish	indirect (arthropods, leeches)
		<i>Leishmania</i>	53	mammals, lizards	indirect (sand flies)
F: Bodonidae	SC: Metakinoplastina F: Parabodonidae	<i>Cryptobia</i> , <i>Trypanoplasma</i>	79	fish	direct or indirect (leeches)
	SC: Prokinetoplastina F: Ichthyobodonidae	<i>Ichthyobodo</i> (<i>Costia</i>)	5	fish	direct

Conventional taxonomic classification systems divide the kinetoplastids into 2 groups: the free-living bi-flagellated Bodonina; and the parasitic uni-flagellated Trypanosomatina. Over 600 species have been described on the basis of multiple phenotypic characters (host occurrence, geographic distribution, vectors, transmission cycles, morphology, development, pathogenicity, culture requirements, etc.). Modern molecular characterization studies, however, have shown that many traditional groups are polyphyletic and composed of numerous clades. Contemporary phylogenetic classifications recognize 2 main lineages: the Prokinetoplastina represented by 2 diverse genera (*Ichthyobodo* biflagellates ectoparasitic on freshwater and marine fishes, and *Perkinsella* (= *Perkinsiella*) aflagellates endosymbiotic (as parasomes or parasome-like organisms (PLOs)) in amoeba *Paramoeba* and *Neoparamoeba*); and the Metakinetoplastina containing 4 groups, including free-living aquatic eu-bodonids (with one genus *Bodo*), free-living neo-bodonids (with 10 genera, including *Rhynchomonas*), free-living or commensal/parasitic para-bodonids (with 5 genera, including *Cryptobia*, *Trypanoplasma*), and the parasitic trypanosomatids (containing some 39 genera, including *Trypanosoma* and *Leishmania*). Species belonging to the genus *Ichthyobodo* are common cosmopolitan ectoparasites of fishes; mostly on freshwater fish (esp. farmed and ornamental species) but they have also been observed on salmonids reared in estuaries as well as on strictly marine species. Ectoparasitic species were originally assigned to the genus *Bodo*, but were subsequently transferred to a new genus *Costia* which unfortunately was found to be preoccupied so they were transferred to another new genus *Ichthyobodo*. Initially, all infections were thought to be due to the species *Ichthyobodo necator* (syn. *Costia necatrix*), but recent studies suggest there are two species in freshwater fish (differing in size) and another in marine fish (different attachment disc and cytostome process). Molecular characterization studies performed on parasite isolates have identified multiple lineages, strains and substrains, and it has been suggested that there may be at least 9 putative ‘genotypic’ species.

Parasite species (and clade)	Mastigote length (µm)	Hosts	Location (disease)	Distribution
Family BODONIDAE (comprising free-living and parasitic genera)				
Genus <i>Ichthyobodo</i> (ectoparasites with free-swimming stage alternating with attached feeding stage)				
<i>I. necator</i> (syn. <i>Bodo necator</i> , <i>Costia necatrix</i>) (= lineage A, sp. I)	5-20	freshwater Salmoniformes: salmonid (brown trout, rainbow trout, brook trout, Atlantic salmon, chum salmon, pink salmon, sockeye salmon, masu salmon); Acipenseriformes: acipenserid (sterlet sturgeon); Anguilliformes: anguillid (European eel); Cypriniformes: cyprinid (common carp, goldfish, tench, barbs); Cyprinodontiformes: poeciliid (green swordtail, platyfish, guppy, molly); Cichliformes: cichlid (banded tilapia, blackchin tilapia, large African mouthbreeder, Mozambique tilapia, dwarf cichlid, southern mouthbrooder); Perciformes: percid (silver perch, European perch, pike-perch, spotted wolffish); Scorpaeniformes: gasterosteid (three-spined stickleback); Moroniformes: moronid (sunshine bass); Siluriformes: ictalurid (channel catfish); marine Clupeiformes: clupeid (scaly mackerel); Gadiformes: gadid (Atlantic cod); Mugiliformes: mugilid (mullet); Pleuronectiformes: paralichthyid (Japanese flounder), pleuronectid (turbot, European plaice, winter flounder)	skin, fins, gills (epidermal degenerative changes)	worldwide
<i>I. hippoglossi</i>	9-14	marine Pleuronectiformes: pleuronectid (Atlantic halibut)	skin, gills	Europe

<i>I. nitschei</i> (syn. <i>Tetramitus</i>) (= lineage B2.1?, spp. VII, VIII)		freshwater Cypriniformes: cyprinid (goldfish); Siluriformes: ictalurid (channel catfish); marine Pleuronectiformes: paralichthyid (Japanese flounder)	skin	Eurasia
<i>I. pyriformis</i> (syn. <i>Costia</i>) [may be small form of <i>I. necator</i>]	9-14	freshwater Salmoniformes: salmonid (rainbow trout, brook trout, golden trout, char)	skin	North America
<i>I. salmonis</i> (= lineage B1, sp. II)	9-13	euryhaline Salmoniformes: salmonid (Atlantic salmon)	gills, skin	Norway
Un-named isolates				
<i>Ichthyobodo</i> spp.	8-14	marine Salmoniformes: salmonid (Atlantic salmon, chinook salmon, chum salmon); Tetraodontiformes: tetraodontid (tiger puffer); Pleuronectiformes: paralichthyid (olive flounder, summer flounder), pleuronectid (European plaice, winter flounder, common dab, Atlantic halibut), scophthalmid (turbot); Gadiformes: gadid (Atlantic cod, haddock); Gobiiformes: gobiid (two-spotted goby); Centrarchiformes: oplegnathid (spotted knifejaw); Clupeiformes: dorosomatid (Bali sardinella); Labriformes: labrid (corkwing wrasse, ballan wrasse, cuckoo wrasse, goldsinny wrasse, rainbow wrasse, rock cook); Moroniformes: ephippid (Atlantic spadefish), moronid (European seabass); Mugiliformes: mugilid (flathead grey mullet, Lebranche mullet); Scorpaeniformes: gasterosteid (three-spined stickleback), scorpaenid (black rockfish); Spariformes: sparid (gilt-head bream)	skin, fins, gills	worldwide
<i>Ichthyobodo</i> sp. (= lineage B1, sp. III)	9-10	euryhaline Salmoniformes: salmonid (Japanese masu salmon, chum salmon)	fins, skin	Asia
<i>Ichthyobodo</i> sp. (= lineage B3, sp. IV)		marine Gadiformes: gadid (Atlantic cod)	gills, skin	Europe
<i>Ichthyobodo</i> sp. (= lineage B2.2, sp. V)		freshwater Moroniformes: moronid (hybrid striped bass)	skin, gills	North America
<i>Ichthyobodo</i> sp. (= lineage B2.2, sp. VI)		freshwater Cichliformes: cichlid (dwarf cichlid)	skin	North America (ex. Brazil)
<i>Ichthyobodo</i> sp. (= sp. IX)		freshwater Salmoniformes: salmonid (rainbow trout)	skin	North America

Parasite morphology: *Ichthyobodo* spp. form 2 morphologically different developmental stages: free-swimming trophozoites and attached ectoparasitic trophonts. Both forms contain a long tubular mitochondrion with numerous nucleoids so there are many small kinetoplasts scattered throughout the cells. The free-living forms are highly motile, swimming with a rapid-spinning but non-directional manner. They range in size from 8-20 x 9-16 µm and are oval-spherical in shape (sometimes comma-shaped) with a small anterior cytostomal protrusion (nose- or lip-like). Most cells are biflagellated with 2 heterodynamic (unequal) flagella arising from a flagellar pocket under the cytostomal protrusion that opens antero-ventrally into a longitudinal groove extending along the cell. One flagellum is short while the other is long and recurrent (trailing) but not adherent to the cell body (it does not form an undulating membrane). Early reports of cells with 4 flagella were considered to be predivisional forms, but recent studies have described *I. salmonis* cells with 4 flagella (comprising 2 pairs of unequal length). The cell is uninucleate with a single central rounded nucleus surrounded by several large pale vacuoles. A pale bent rod-like structure (axostyle) runs along the cell margin from the anterior protrusion to the posterior body. The cytoplasm contains numerous kinetoplasts varying in number within and between species: e.g. 25-113 for *I. necator* and 23-68 for *I. salmonis*. The attached forms (trophozoites) are non-motile ectoparasites anchored to host cells by small attachment discs at their anterior pointed ends through which a cytostome canal supported by microtubules penetrates into the cytoplasm. The attachment disc often differs between freshwater and marine isolates (being smooth in seawater and ridged in freshwater), but it is not known whether these are intra- or inter-specific variations or related to habitat. The cells range in size from 10-17 x 5-11 µm and are pyriform in shape with the attached pointed end containing the axostyle which extends posteriorly along the cell margin lateral to the flagellar pocket. The cells contain inconspicuous (non-emergent) flagella located in the longitudinal groove which is readily seen and sometimes forms a Y-shape with the flagellar pocket. Trophozoites appear to be elastic and often elongate stretching the cytostome to the midbody. The cell membrane comprises 2 membranous layers with a middle fibrillar layer, and the nuclear membrane also with 2 layers and single nucleolus. The cytoplasm contains numerous

kinetoplasts varying in number: e.g. 52-87 for *I. necator* and 21-46 for *I. salmonis*. While multiple species may exist according to genotypic studies, the morphotypic differentiation of species is difficult due to the pleomorphy of both free and attached stages. Recent studies have shown that marine *I. salmonis* cells are generally thinner than freshwater *I. necator* cells, their attachment region is smooth rather than jagged, and they possess 2 lateral spine-like projections.

Site of infection: Free-living stages swim around in the water column and may browse over substrates and sediments. The attached stages are ectoparasitic on fish and may be found on the gills (usually on the tips of secondary gill lamellae) and skin (often on the cuff of skin sheltered by the operculum, the pectoral and pelvic fins, and the area adjacent to the dorsal fin – trophozoites not normally found anterior to operculum). Infections have occasionally been reported in association with fish roe, and there are rare reports of flagellated cells feeding on detached decaying cells and scales in tanks and ponds (one worker even suggesting they were saprophagous stages). *Ichthyobodo* spp. are common ectoparasites on fish; frequently found on freshwater fish (farmed and ornamental species) but also on salmonids reared in estuaries as well as on strictly marine species. Their taxonomic classification is complicated as phenotypic studies (parasite morphology, host occurrence) suggest the existence of several species, while genotypic studies (gene sequences) have identified multiple lineages, strains and substrains, so far suggesting that 9 species may exist (most un-named). To date, infections have been detected in over 50 species of fish, including 19 species of freshwater fish belonging to 7 families in 7 orders, and 31 species of marine fish belonging to 16 families in 12 orders.

Pathogenesis: *Ichthyobodo* spp. feed on epithelial tissues by attaching to cells, penetrating them with a cytostomal canal supported by tubular fibrils and ingesting cell contents, and possibly even secreting digestive enzymes or toxic substances. Infections may cause clinical disease in fish (known as ichthyobodiosis, formerly costiasis) characterized by skin irritation, excess mucus production, flashing and rubbing behaviours and respiratory difficulties. Skin infections may lead to epithelial hyperplasia, Malpighian cell hyperplasia, goblet cell depletion, mucus secretion, cell necrosis, oedema, spongiosis, cloudy greyish discolourations, vacuolization and sloughing of the epidermis and damaged fins. Heavily infected fish often scrape themselves against substrates and rub against immersed objects or the sides of tanks. Gill infections may lead to epithelial hyperplasia, necrosis, mucus secretion, oedema, clubbing of filaments and lamellae, blood vessel collapse, melanomacrophage infiltrates and breathing difficulties. Heavily infected fish often swim near the surface, have difficulty maintaining upright position and swim erratically with occasional ‘flashing’ behaviour (rolling over exposing pale bellies). Cumulatively, infected fish become lethargic, listless, anorexic, and even emaciated before dying (most deaths due to osmoregulatory problems with haemodilution). Fish may become heavily infected within 1-2 weeks of infection and mortalities peak around 4-8 weeks. Disease is usually more severe in young fish, in malnourished fish, and in stressed fish (due to crowding, capture, translocation, competition with older fish or sudden changes in water quality, especially warmer water temperatures). The parasites are able to survive a wide range of environmental conditions, including temperature, salinity and pH ranges. They may cause significant problems in the culture of young salmonids and cyprinids, especially in hatcheries where fish occur in high numbers, and outbreaks are frequently associated with aquaria, culture ponds and fish in cage cultures.

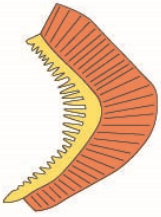
Developmental cycle and mode of transmission: The parasites have simple direct life-cycles involving free-swimming stages that actively locate fish hosts and attach to their external surfaces to feed. Under favourable conditions, free-living stages divide by longitudinal binary fission (predivision stages are usually larger and may have up to 4 flagella). Studies have shown that these motile stages multiply rapidly when water temperatures are between 10-25°C and that cells die at temperatures above 30°C. It has been suggested that cells may encyst under adverse environmental conditions to facilitate survival, and a few studies have reported inert non-flagellated stages when water temperatures dropped below 8°C, but typical environmentally-resistant membrane-bound cysts have not been observed. When in contact with fish hosts, free-swimming stages use their flagella for site selection and then attach using small flat ventral discs through which cytostomal connections are made to the host cell cytoplasm. Feeding trophozoites detach when replete or disturbed and flagella emerge or develop from their flagellar pockets and longitudinal grooves to restore motility. Infections are therefore water-borne with contamination by free-swimming (and dividing) stages arising from the surfaces of infected fish. The potential sources of infection depend greatly on the host specificities of the *Ichthyobodo* spp. which have yet to be determined. Some of the putative species have only been found in a few related hosts (stenoxenous), while others have been found in a broader range of hosts (euryxenous), albeit limited to sympatric species within specific aquatic habitats (freshwater, euryhaline or marine). Nonetheless, aquaculture facilities may become contaminated by the ingress of feral fishes, and some workers think that amphibian larvae (salamanders, frogs, toads) may also be potential sources of infection. Outbreaks are common in recirculating aquaculture systems, particularly hatcheries where fish are reared in small crowded conditions. Fish also appear to be more susceptible to clinical disease when stressed (and thus immuno-compromised) due to capture, handling, crowding, poor diet, and sudden changes in environment and water quality. Little is known about the immunology of infections although it is thought that recovered fish develop some degree of protective immunity against subsequent disease.

Differential diagnosis: Infections may be indicated by clinical signs of skin irritation, greyish cloudy lesions and behavioural changes, but must be confirmed by the microscopic detection of trophozoites in clinical samples (skin scrapings, mucus, gill snips). Specimens may be detected in wet mounts, fixed smears or histological sections, particularly after staining with iron haematoxylin, carmine or periodic acid-Schiff’s reagent, although trophozoites are fragile and may rupture during processing. Molecular biological

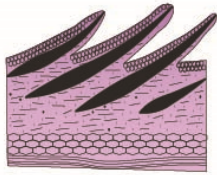
techniques have been used to detect and characterize isolates following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (small subunit (18S) ribosomal DNA and internal transcribed spacer regions). However, further studies are required to determine whether the different lineages or strains detected represent discrete species or species-complexes.

Treatment and control: Clinical infections in cultured and aquarium fish have been successfully treated using chemical bath treatments (formalin, acetic acid, copper sulphate, malachite green, pyridylmercuric acetate) as well as foodstuffs medicated with parasiticidal drugs (metronidazole, secnidazole, triclabendazole), although over 30 other drugs proved ineffective. Various control measures have also been used to decontaminate holding facilities by treating water with chemicals (methylene blue, quinine hydrochloride, malachite green, potassium permanganate, aureomycin, globucid, lysol) or draining, drying and disinfecting ponds or tanks with quicklime or chloride of lime before restocking. Several studies also eliminated parasites from tanks by raising the water temperature to 32°C for several days. Care should also be taken to avoid stressful and crowded conditions to reduce the risk of severe infections.

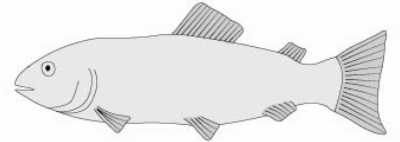
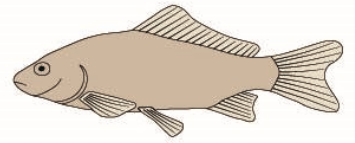
Ichthyobodo (syn. *Costia*)



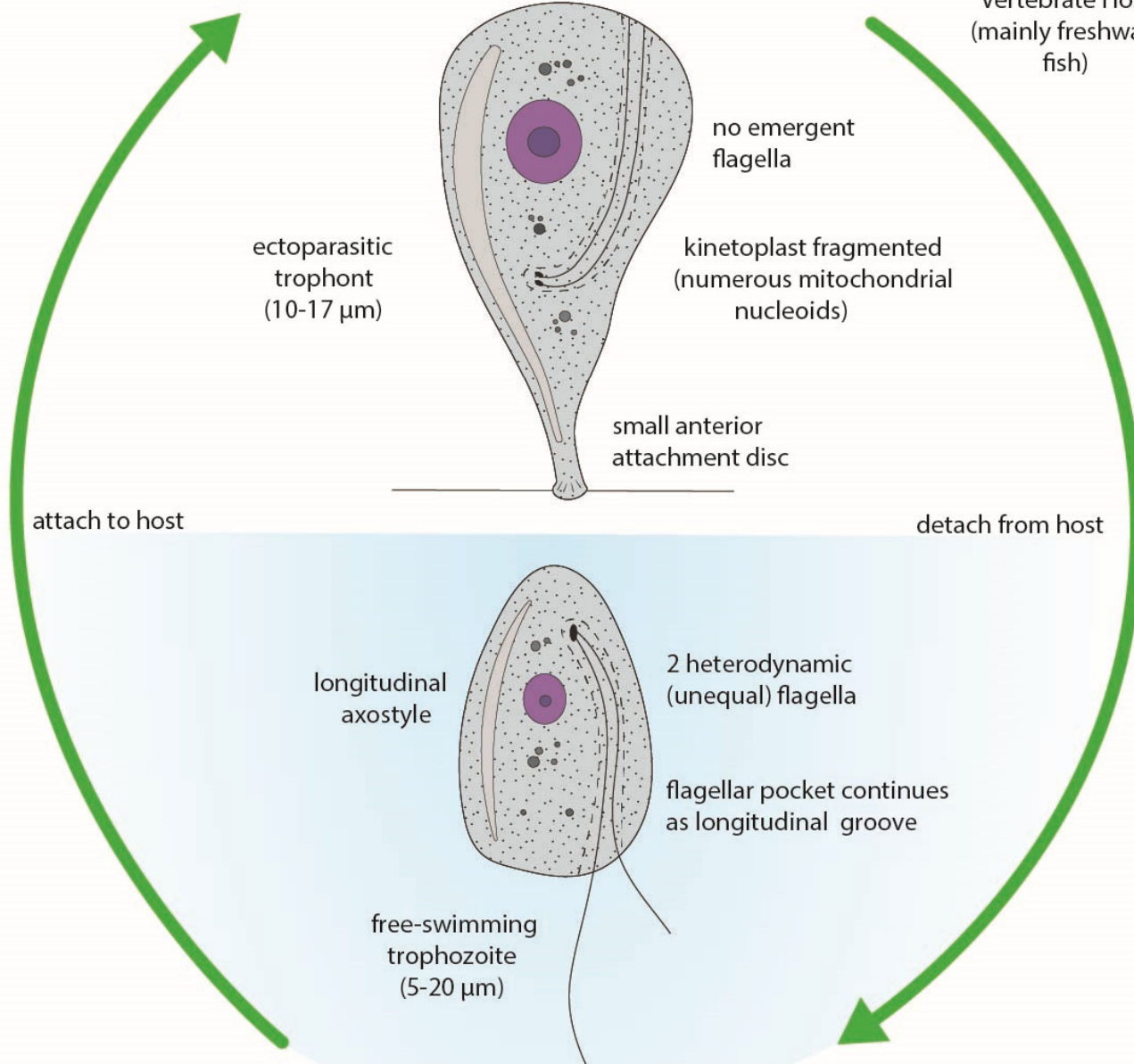
gills, skin
(lesions, respiratory disorders, emaciation)



multiply by longitudinal binary fission



Vertebrate Hosts
(mainly freshwater fish)



ectoparasitic trophont
(10-17 μm)

no emergent flagella

kinetoplast fragmented
(numerous mitochondrial nucleoids)

small anterior attachment disc

attach to host

detach from host

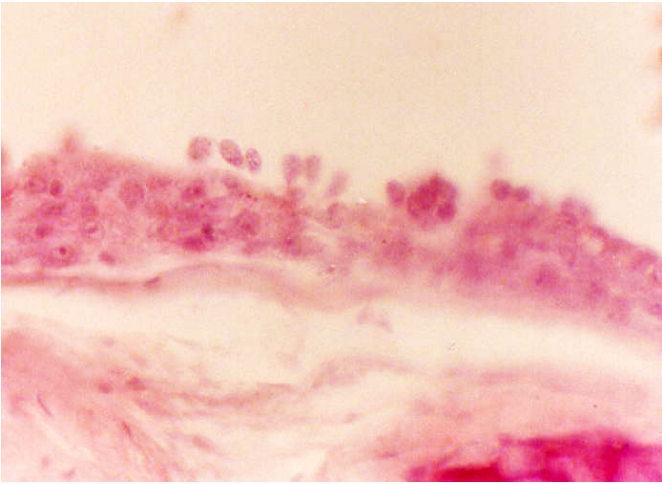
longitudinal axostyle

2 heterodynamic (unequal) flagella

flagellar pocket continues as longitudinal groove

free-swimming trophozoite
(5-20 μm)

direct transmission via trophozoites actively seeking hosts in water column



Ichthyobodo trophonts



Ichthyobodo trophont