

Cryptobia
(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. Parabodonids include members of the genus *Cryptobia* which are parasitic for fish, being epizotic on the gills and skin or endozoic in the gut.

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: Metakinetoplastina (large polyphyletic group supported by molecular studies)
Order: Parabodonida (biflagellated, recurrent flagellum attached or free)
Family: Parabodonidae (Cryptobiidae, Trypanoplasmatidae) (epizotic or endozoic in invertebrates and/or fish)
Genus: *Cryptobia* (parasitic on gills/skin of fish, direct cycles)
Species: various species cause infections in 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: Blastodiniales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	<i>Amyloodinium</i> <i>Crepidoodinium</i> <i>Piscinoodinium</i>	fish (skin)	direct (w)
	Order: Syndiniales (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: Metakinetoplastina 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: Metakinetoplastina 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

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*). The genus *Cryptobia* was originally proposed for biflagellated organisms in the reproductive systems of snails, but was later expanded to include similar organisms in other invertebrates as well as some aquatic vertebrates. The genus *Trypanoplasma* was originally proposed for biflagellated organisms in the blood of freshwater fishes and the crops of leeches. Some workers consider *Trypanoplasma* to be a junior synonym of *Cryptobia*, while others regard them as separate subgenera. Recent molecular phylogenetic studies have suggested that they should continue to be considered as separate genera, with epizoid/endozoic species (thought to undergo direct transmission) being retained within the genus *Cryptobia*, and haemozoic species (thought to undergo indirect transmission via leech vectors) forming the genus *Trypanoplasma* (note that many species have been shuffled between these genera). The genus *Cryptobia* is an enigmatic diverse assemblage of species described from a wide variety of hosts (including marine and freshwater fish, terrestrial and aquatic snails, platyhelminths, chaetognaths, leeches, reef geckos, and mole crickets) as well as several free-living aquatic representatives. [Note that molecular characterization studies have led to the transfer of several *Cryptobia* spp. originally described as free-living bacterivorous zooflagellates from anoxic marine sediments to special excavate taxa (including *Carpediomonas* and *Jakoba*)]. Most *Cryptobia* infections in fish appear to be nonpathogenic, but several species have been associated with diseases of the gills, guts and sometimes internal organs.

<i>Cryptobia</i> species	Mastigote length (µm)	Hosts	Location (disease)	Distribution
Non-haematozoic (ectoparasitic on gills or body surface, or endoparasitic in intestines), direct transmission				
<i>C. agitans</i>	5-12	freshwater Perciformes: centrarchid (bluegill, white crappie, black crappie); Cypriniformes: cyprinid (bighead carp, silver carp, grass carp, goldfish)	gills (cryptobiosis)	Eurasia, Africa, North America
<i>C. agitata</i>	5-8	freshwater Cypriniformes: cyprinid (bighead carp, silver carp)	gills, body	China
<i>C. antedonae</i>		marine Crinoidea: antedonid (rosy feather star)	coelomic fluid	Europe
<i>C. bialata</i>		brackish water (free-living)		Australia
<i>C. branchialis</i> (syn. <i>C. agitata</i> , <i>concava</i> , <i>rebae</i> , <i>indica</i>)	12-23	freshwater/estuarine Cypriniformes: cyprinid (carp, grass carp, bighead carp, silver carp, goldfish, olive barb, golden shiner); Cichliformes: cichlid (Nile tilapia); Gonorynchiformes: channid (milkfish); Perciformes: centrarchid (white crappie, black crappie), percoid (fantail darter); Siluriformes: clariid (African sharp-tooth catfish), ictalurid (channel catfish, brown bullhead); Spariformes: sparid (sea bream)	gills, body (cryptobiosis)	Europe, Asia, Africa, North America
<i>C. carinariae</i>		marine Gastropoda: carinariid (<i>Carinaria mediterranea</i>)	spermatheca	Europe
<i>C. carassii</i>		freshwater Cypriniformes: cyprinid (goldfish)	body	Europe

<i>C. catastomi</i>		freshwater Cypriniformes: catostomid (white sucker)	body	North America
<i>C. congeri</i>		marine Anguilliformes: congrid (European conger)	stomach	Atlantic
<i>C. coryphaenoideana</i>		marine Gadiformes: macrourid (Pacific grenadier)	stomach	North America
<i>C. dahli</i>	10-20	marine Scorpaeniformes: cyclopterid (lumpfish)	gut, bladder (nonpathogenic)	Europe
<i>C. dendrocoeli</i>		freshwater Platyhelminthes: dendrocoelid (<i>Dendrocoelum lacteum</i>), planariid (<i>Planaria albissima</i>)	gut	Europe
<i>C. desertorum</i>		terrestrial Gastropoda: helioid (<i>Helix desertorum</i>)	seminal vesicle	Africa
<i>C. eilatica</i>		marine Spariformes: sparid (seabream)	gills	Red Sea
<i>C. geccorum</i>	15-23	terrestrial Sauria: sphaerodactylid (reef gecko)	gut	North America
<i>C. gryllotalpae</i>	10-26	terrestrial Orthoptera: gryllotalpid (European mole cricket)	intestines	Europe
<i>C. helioid</i>		terrestrial Gastropoda: polygyrid (<i>Helix albolabris</i> , <i>tridentata</i>), helioid (<i>Helix pomatia</i>), discoid (<i>Helix alternata</i>)	seminal vesicles	Europe
<i>C. helicognae</i>		terrestrial Gastropoda: helioid (<i>Helix pomatia</i>)	seminal vesicle	Europe
<i>C. indistincta</i>		freshwater Cypriniformes: cyprinid (carp)	body	Eurasia
<i>C. innominata</i>		terrestrial Gastropoda: polygyriid (<i>Triodopsis tridentata</i>)	spermatheca	Europe
<i>C. intestinalis</i>	8-19	marine Spariformes: sparid (bogue)	oesophagus, stomach	Mediterranean, Adriatic
<i>C. isidora</i>		freshwater Gastropoda: planorbid (<i>Isidora tropica</i>)	seminal receptacle	Africa
<i>C. iubilans</i>	19	freshwater Cichliformes: cichlid (Texas cichlid, firemouth cichlid, convict cichlid, Jack Dempsey, blue discus); Siluriformes: clariid (vundu, African sharptooth catfish)	gut, internal organs (weight loss, death)	Mexico, Europe, Africa, North America
<i>C. liberia</i>		brackish water (free-living)		Australia
<i>C. limnorum</i>		freshwater Gastropoda: lymnaeid (<i>Limnaea stagnalis</i> , <i>palustris</i>)	seminal vesicle	Europe
<i>C. makeevi</i>		euryhaline Salmoniformes: salmonid (pink salmon, chum salmon)	body, gills	Asia
<i>C. rupestre</i>		terrestrial Gastropoda: helioid (<i>Helix singula</i>)	seminal vesicle	Europe
<i>C. sagittae</i>	60-100	marine Sagittoidea: sagittid arrowworm (<i>Sagitta bipunctata</i>)	midgut	Atlantic
<i>C. stilbia</i>		marine Argentiniformes: bathylagid (smoothtongue)	stomach	Mexico
<i>C. trematomi</i>		marine Perciformes: nototheniid (emerald rockcod)	stomach, intestines	Africa
<i>C. trocheurum</i>		terrestrial Gastropoda: helioid (<i>Helix</i> spp.)	seminal vesicle	Europe
<i>C. udonellae</i>	12-15	marine Platyhelminthes: udonellid (<i>Udonella murmanica</i>)	excretory channels	Russia
<i>C. vaginalis</i>		freshwater Clitellata (leeches): haemopid (<i>Haemopsis sanguisuga</i>), hirundinid (<i>Hirudo medicinalis</i>)	vagina	Europe
<i>C. ventriculi</i>		marine Scorpaeniformes: cyclopterid (lumpfish)	body	Atlantic

Parasite morphology: *Cryptobia* spp. only form one developmental stage: elongate biflagellated trophozoites. These cells have oval-elongate tapering (broader anteriorly) bodies (sometimes ribbon-like) ranging in size from 5-23 x 2-6 μm depending on the species (e.g. *C. branchialis* 7-23 x 2-6 μm , *C. iubilans* 5-12 x 3-6 μm). Trophozoites contain a single central ovoid nucleus and a smaller oval-elongate kinetoplast (containing mitochondrial DNA) located nearby. Adjacent flagellar basal bodies (microtubular complexes) give rise to 2 heterodynamic (unequal) flagella emerging anteriorly from a flagellar pocket, with the shorter anterior flagellum being free, while the longer flagellum is recurrent (trailing) and attached to the cell body for most of its length before extending freely from the posterior end. The recurrent flagellum does not form a permanent undulating membrane, but it draws out the pellicle when it beats giving the appearance of a narrow undulating membrane (in contrast, the recurrent flagellum of

Trypanoplasma spp. form prominent undulating membranes). Trophozoites also possess a large anterior contractile vacuole for fluid regulation within the cell.

Site of infection: Trophozoites are usually detected in association with aquatic hosts, many being ectozoic on the skin or gills and several being endozoic in the intestines, although some occur in terrestrial invertebrates and a few are free-living in brackish water. [Note that organisms that are endozoic in the blood (= haemozoic) have been transferred to the genus *Trypanoplasma* (and are considered separately in next section)]. A total of 34 parasite species have been described from a wide variety of hosts, including 26 species of freshwater fish (from 10 families), 7 species of marine fish (from 6 families), 11 species of terrestrial and aquatic snails (from 6 families), 3 platyhelminth species, 2 leech species, one arrowworm species, one crinoid species, one gecko species and one cricket species.

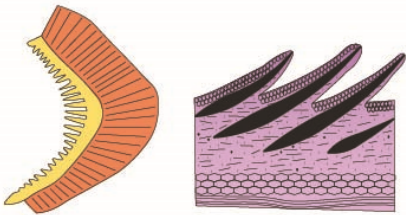
Pathogenesis: Infections by most *Cryptobia* spp. remain asymptomatic or subclinical, but a few species may cause a mild clinical disease (known as cryptobiosis) involving the gills, skin or alimentary tracts of fish. Trophozoites attach to host epithelia by the tip or free portion of the recurrent flagellum expanding to form a ridged ledge. The parasites feed on solutes and small suspended organic materials in the water column or associated with host mucosal membranes. Infections by *C. branchialis* and *C. agitans* on the skin or gills of fish may cause skin abnormalities, discolourations or deformities, excessive mucus production, scale loss, chronic inflammation, gill congestion, hyperaemia, anaemia, respiratory difficulties, anorexia, weight loss and eventually death (mostly in cultured carp, goldfish and catfish). Infected fish are more prone to secondary bacterial infections leading to pale or red skin patches and fin rot. Infections by *C. iubilans* in the stomach and/or intestines of fish can cause multifocal granulomatous inflammation resulting in lethargy, anorexia, stunted growth, emaciation and death (mostly in cichlids). Infections may sometimes spread to visceral organs (liver, spleen, kidney) not by gut perforation, but more likely by intracellular uptake by macrophage-like cells. Infections appear to be more prevalent and severe in young fish, small fish and stressed fish (inadequate diet, poor water quality, crowded conditions and concomitant infections).

Developmental cycle and mode of transmission: Trophozoites multiply asexually by longitudinal binary fission and those of parasitic species have been found to survive free-swimming in aquaria for up to 2 days. Ectozoic species, such as *C. branchialis*, are transmitted directly between fish by the transfer of trophozoites through close body contact or via flagellates in the water column. Infections are particularly prevalent in small aquaria or tanks sharing reticulated water as well as crowded cultures where fish cohabit for protracted periods. Endozoic species, such as *C. iubilans*, are transmitted directly between fish via the faecal contamination of water by trophozoites which subsequently invade new hosts. Experimental studies have also shown that some infections in cichlids may be transmitted by cannibalism when infected tissues are consumed. This led to the suggestion that piscivory or scavenging may be involved in transmission cycles, and it was even proposed that *C. dahli* from lumpfish may be transmitted via prey animals (pelagic crustaceans, annelids, jellyfish) acting as secondary hosts, although this remains to be proven.

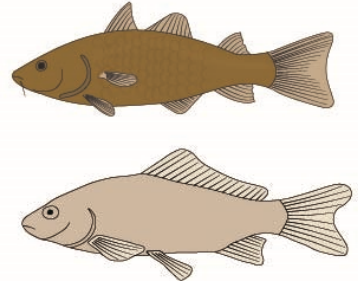
Differential diagnosis: A preliminary diagnosis may be afforded based on clinical signs and history, but infections need to be confirmed by the direct microscopic detection of trophozoites in clinical samples (skin/mucus scrapings, excised gills, gut content, mucosal scrapings, faeces or organ impression smears). Wet mounts may be examined by high-contrast microscopy looking for motile trophozoites, or fixed smears or histological sections may be examined after staining with Giemsa, iron haematoxylin, carmine or periodic acid-Schiff's reagent. Molecular biological techniques have been used to characterize several parasite isolates following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (small subunit (18S) ribosomal DNA).

Treatment and control: Ectozoic infections in fish responded reasonably well to bath treatments in various chemicals, including solutions of formalin, malachite green, chloride of lime, copper sulphate, and iron sulphate. However, similar treatments did not work against endozoic infections. Several studies have found that treatment of fish with some nitroimidazoles (dimetridazole) or thiazoles (nitrothiazole) did reduce but not eliminate endozoic parasite burdens. Various preventive measures have been recommended to reduce parasite transmission between fish, including screening new stock, isolating infected individuals for treatment or culling, promptly removing carcasses from tanks, disinfecting holding facilities, using treated water and keeping fish healthy in stress-free conditions (by providing proper nutrition and good water quality, avoiding crowding and over-handling, and treating any concomitant infections).

Cryptobia

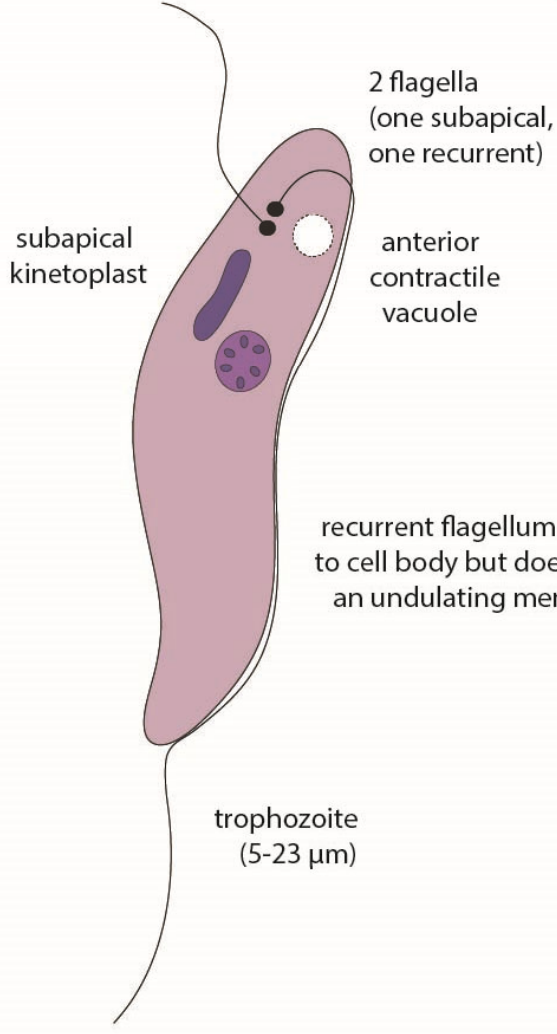


gills, skin
(discolourations,
congestion)
(some endozoic
in gut)



Vertebrate Hosts
(marine and
freshwater fish)

divide by longitudinal
binary fission



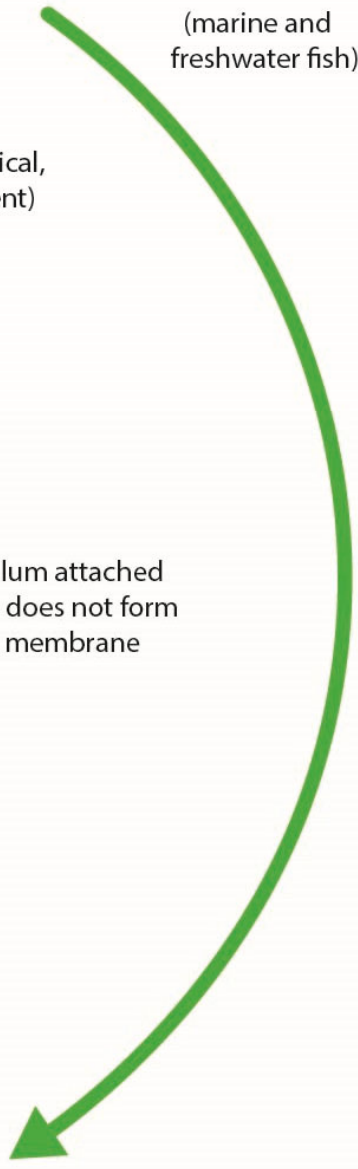
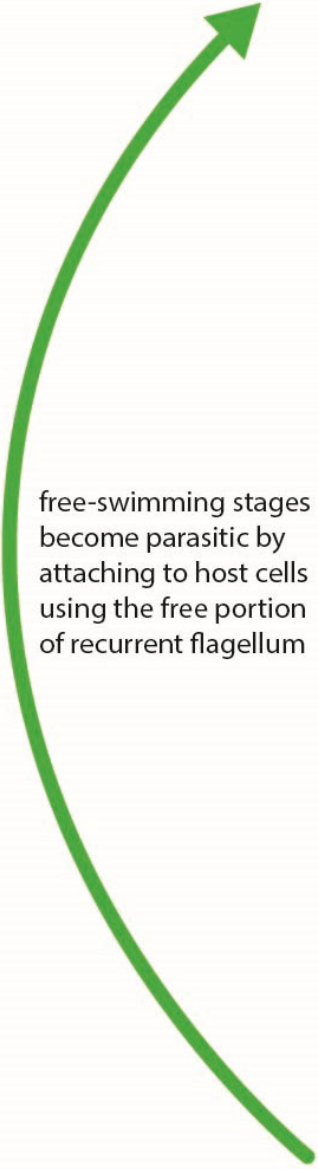
free-swimming stages
become parasitic by
attaching to host cells
using the free portion
of recurrent flagellum

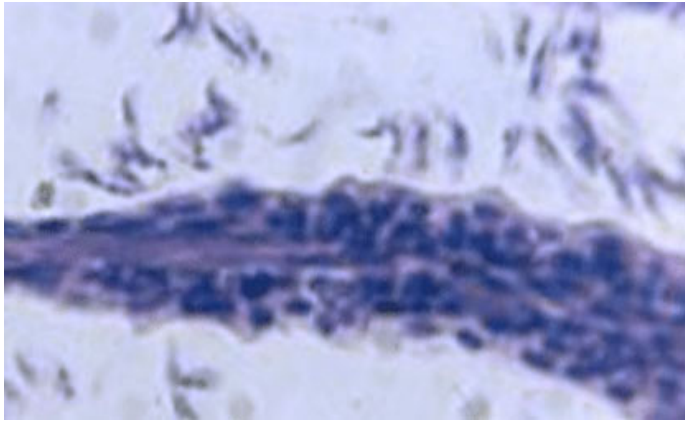
recurrent flagellum attached
to cell body but does not form
an undulating membrane

trophozoite
(5-23 μm)

no cyst formation

direct transmission via trophozoites
actively seeking hosts in water column





Cryptobia trophozoites on fish gill



Cryptobia trophozoite