

Ichthyophthirius

(protist: ciliate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). One protistan supergroup known as SAR comprises the Stramenopiles (with heterokont flagella), Alveolata (with cortical alveoli) and Rhizaria (with fine pseudopodia). Three major alveolate groups are recognized: ciliates, apicomplexans and dinoflagellates. Ciliated protozoa are unique eukaryotes as they exhibit nuclear dualism (vegetative macronucleus and reproductive micronucleus), the process of conjugation (exchange of micronuclei between pairs), have membrane-bound sacs (subpellicular alveoli) supporting the plasma membrane, and move using cilia (2+9 undulipodia with compound subpellicular infraciliature). Most ciliates are free-living in aquatic and terrestrial habitats, but some are symbiotic in vertebrate and invertebrate hosts. Ten major monophyletic lineages are recognized on the basis of their infraciliature, i.e. the ultrastructural organization of their kinetids (comprising basal bodies (= kinetosomes) and associated microtubular ribbons and fibrils). Members of the subphylum Intramacronucleata are united by the presence of microtubules inside the macronuclear envelope during division; including the oligohymenophoreans ('few membrane-bearer') with an adoral zone of buccal (oral) ciliature. Hymenostomes have a paroral membrane located on the right side of the buccal cavity and three membranelles on the left side, while their bodies are mostly covered by uniform somatic ciliature. Ophryoglenines have a buccal cavity opening at the bottom of a deep depression and they possess a lens-like (watchglass) organelle. Ichthyophthiriids are monoxenous (one-host) ectoparasites of fishes. Trophonts infect epithelial tissues, often causing visible lesions evident as white spots (disease known as freshwater whitespot, ichthyophthiriosis or simply 'ich'). When replete, they leave the host and form encysted stages (tomonts) in the external environment. These cysts produce hundreds of infective stages (tomites) which are released as swimmers (theronts) which actively seek new hosts.

Classification:

Domain: Eukaryota (membrane-bound nucleus)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria)
Group: Alveolata (with cortical alveoli)
Phylum: Ciliophora (with cilia, nuclear dualism, pellicular alveoli, reproductive conjugation)
Subphylum: Intramacronucleata (microtubules occur inside macronuclear envelope during division)
Class: Oligohymenophorea (distinct oral ciliature, comprising right paroral membrane and 3 left membranelles)
Subclass: Hymenostomatida (right paroral dikinetid plus 1-3 left polykinetids)
Order: Hymenostomatida (preoral suture, somatic monokinetids)
Suborder: Ophryoglenina (with organelle of Lieberkuhn (watchglass organelle))
Family: Ichthyophthiriidae (monoxenous ectoparasites, form encysted tomonts which release swimmers/theronts)
Genus: *Ichthyophthirius* (ectoparasitic on skin/gills of fish)
Species: *I. multifiliis* (causes whitespot disease ('ich') in freshwater fishes)

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Cells with different modes of locomotion do not form separate monophyletic assemblages as previously thought, but rather are distributed across several disparate supergroups (as evidenced by recent molecular phylogenetic analyses). One protistan supergroup known as SAR comprises the Stramenopiles (with heterokont flagella), Alveolata (with cortical alveoli) and Rhizaria (with fine pseudopodia). Three diverse alveolate groups are recognized: Ciliophora (with cilia), Dinoflagellata (with flagella) and Apicomplexa (with gliding motion, some also with flagellated microgametes). Ciliated protozoa are unique amongst the unicellular eukaryotes because they are the only group to exhibit nuclear dualism. Individual cells possess two different types of nuclei; vegetative macronuclei and reproductive micronuclei. Asexual reproduction occurs by transverse binary fission across rows of cilia (homothetogenic fission) whereas some species exhibit sexual reproduction by the phenomenon of conjugation (temporary fusion of two conjugates which exchange micronuclei). As their common name implies, ciliates are also characterized by the possession of simple cilia, or compound ciliary organelles, in at least one stage of their life cycles (compound subpellicular infraciliature is universally present even when cilia are absent). Cilia are elongate hair-like extensions of the cell membrane with an internal microtubular core (universal 2+9 configuration = 2 single central microtubules surrounded by 9 peripheral doublets). They are organelles of motility used for locomotion and/or feeding. Cilia (singular, cilium) are similar in ultrastructure to flagella (singular, flagellum), and they are collectively often called undulipodia (singular, undulipodium) because both use cross-linked proteins (dynein-walking mechanism) to undulate about their basal kinetosome (unlike the rotary motion unique to flagella in bacteria). Ciliates, together with dinoflagellates and apicomplexans, possess subpellicular alveoli which are membrane-bound sacs beneath the plasma membrane. Alveoli are thought to serve many varied functions: ranging from support (helping maintain body shape, act as fulcrum for undulipodia); metabolism (storage); osmoregulation (mucocysts); excretion (extrusomes); protection (toxicysts, trichocysts); and even hunting (haptocysts).

Most ciliate species are free-living in aquatic or terrestrial habitats but many are commensals in vertebrate or invertebrate hosts and some are parasitic. Early classification systems recognized three main classes of ciliates mainly on the basis of their patterns of somatic (body) and buccal (oral) ciliation. The ‘lower holotrichs’ have simple body and oral ciliature; most are free-living species but some are highly specialized symbionts aiding cellulose digestion in herbivores. The ‘higher holotrichs’ have simple body ciliature but more specialized oral ciliature forming membranelles; most occur as free-living organisms but some live as commensals or parasites in a range of animals. The ‘spirotrichs’ have reduced body ciliation but well-developed oral ciliature forming an adoral zone of membranelles; most are bacterivores living in aquatic and terrestrial habitats. More recently, ten major monophyletic lineages have been recognized on the basis of their infraciliature; i.e. the ultrastructural organization of their kinetids (comprising basal bodies (= kinetosomes) and associated microtubular ribbons and fibrils). These lineages (ranked as classes) have been well supported by modern molecular biological studies using several gene sequences.

Class	Etymology	Defining characters	Lifestyles*	Genera covered
Subphylum: Postciliodesmatophora [somatic dikinetids with postciliodesmata (overlapping microtubular ribbons)]				
Karyorelictea	‘primitive-nucleus’	macronuclei not dividing but replaced by division of micronuclei	free-living (aquatic benthic/planktonic)	
Heterotrichea	‘different-hair’	compound ciliary organelles around mouth, macronuclei divided by external microtubules	free-living (aquatic planktonic/benthic)	
Subphylum: Intramacronucleata [macronuclei divided by internal microtubules]				
Spirotrichea	‘coiled-hair’	conspicuous right and left oral ciliature, left polykinetids leading into oral cavity	free-living (aquatic, terrestrial)	
Litostomatea	‘simple-mouths’	cytostome with noncurved tubular cytopharyngeal apparatus (rhabdos)	free-living (often predatory), symbiotic	<i>Balantidium</i>
Phyllopharyngea	‘leaf-throated’	mouth with radial microtubular ribbons (phyllae), some with sticky feeding tentacles	free-living (aquatic), epizoic, symbiotic	<i>Chilodonella</i>
Colpodea	‘breast-shaped’	reniform bodies, somatic cilia with transverso-desmata (overlapping ribbons)	terrestrial, some aquatic (bacterivores)	
Nassophorea	‘pot-bearer’	oral nematodesmata well-developed (basket-like nasse or cyrtos supporting cytopharynx)	free-living (aquatic, terrestrial)	
Prostomatea	‘before-mouth’	simple apical mouths, some with oral microtubular band, some with oral brush	free-living (often predatory)	
Plagiopylea	‘misshapen-marker’	with twisted oral tubes, most with hydrogenosomes	free-living (anoxic habitats)	
Oligohymenophorea	‘few membrane-bearer’	typically with ventral groove containing mouth and compound ciliary organelles (usually adoral zone of three membranelles)	free-living, epizoic, symbiotic (microphagous)	<i>Uronema</i> , <i>Ichthyophthirus</i> , <i>Tetrahymena</i> , <i>Trichodina</i> , <i>Vorticella</i>

*Symbiosis *sensu lato* ranges from commensalism, mutualism and parasitism (depending on the benefit/detriment to the host)

The class Oligohymenophorea contains ciliates whose somatic (body) kinetosomes (basal bodies) are associated with unique ultrastructural elements (collectively referred to as infraciliature) comprising anteriorly-directed overlapping kinetodesmal fibrils, divergent postciliary microtubular ribbons and radial transverse ribbons. Members are considered to be ‘higher’ holotrichs as there is clear distinction between the oral and somatic ciliature. They possess distinctive oral kineties consisting of a right paroral kinety (membrane) and typically 3 left oral polykinetids (membranelles) located in a buccal or infundibular cavity with an inconspicuous cytopharyngeal apparatus. They are widely distributed as free-living or symbiotic forms, although a few are parasitic mainly in fish. The class contains 6 subclasses (Apostomatia, Astomatia, Hymenostomatia, Peniculia, Peritrichia, Scuticociliatia) differentiated predominantly on the basis of their patterns of somatic and oral ciliation. The hymenostomes have a well-defined buccal cavity and conspicuous oral ciliature evident as a paroral membrane of dikinetids on the right side and 1-3 left polykinetids (membranelles) on the left side, while their bodies are mostly covered by somatic ciliature. The single order Hymenostomatida contains 2 suborders: Ophryoglenina (cells with dense somatic ciliature, preoral sutures, buccal cavity opening at the bottom of a deep depression, presence of a unique lens-like organelle of Lieberkuhn (watchglass organelle), and teloparakinetel stomatogenesis); and Tetrahymenina (cells with uniform somatic ciliature, inconspicuous oral cavity (except in species that undergo macrostome transformation), absence of organelle of Lieberkuhn, and monoparakinetel stomatogenesis). Ophryoglenines also exhibit division whilst free-swimming or by palintomy within cysts. The ciliates may also develop into histophagous forms which feed on other aquatic organisms (invertebrates and vertebrates). Two families are recognized: Ichthyophthiriidae (monoxenous (one-host) ectoparasites of fishes); and Ophryoglenidae (monoxenous parasites on the tissues of molluscs and other invertebrates). Both families form feeding trophonts which drop off their hosts to form encysted tomites that divide internally into tomites which are then released as swarmers (theronts). The genus *Ichthyophthirus* (syn. *Chromatophagus*, *Ichthyophthirioides*) is monotypic with the single species *I. multifiliis* causing skin lesions (freshwater white spot disease) on ornamental, farmed and wild freshwater

fishes. It is thought to have originated in Asia on carp but now has a global distribution on a wide range of fish species. It has extremely low host specificity (euryxenous) and can infect virtually any freshwater fish species. It is an important pathogen in temperate and tropical regions and causes significant economic losses in many aquaculture industries, including the ornamental fish trade, and epizootics in wild fish populations resulting in mass mortalities. There is some conjecture about the existence of different parasite races, which may have different temperature tolerances, being adapted to hosts with specific temperature preferences, or they may be geographic races varying in virulence in introduced and/or endemic fish species. Several reports have been made of 'ich'-like parasites (*Ichthyophthiriodes browni* and *Neoichthyophthirius scholtfeldti*) from tropical freshwater fish, and a similar parasite, *Cryptocaryon irritans* (originally classified as *I. marinus*) found on marine fish and associated with marine white spot disease. Despite many similarities in ciliate biology, molecular phylogenetic studies have found that *Cryptocaryon* is more closely related to protostome ciliates than to the hymenostomes like *Ichthyophthirius*.

<i>Ichthyophthirius</i> species	Hosts	Location (Clinical signs)	Distribution
<i>I. multifiliis</i> (syn. <i>I. cryptostomus</i> , <i>Chromatophagus parasiticus</i>) [type species]	freshwater fish (Anguilliformes: anguillid (short-finned eel, African longfin eel); Atheriniformes: melanotaeniid (eastern rainbowfish, Lake Eacham rainbowfish); Characiformes: acestrorhynchid (red-tailed freshwater barracuda, big-eyed cachorro), characid (banded tetra, black tetra, feather tetra, cardinal tetra), erythrinid (wolf fish, gold wolf fish), gasteropelecid (black-winged hatchetfish, marbled hatchetfish), lebiasinid (diptail pencilfish, one-lined pencilfish), serrasalmid (tambaqui, pirapitinga, small-scaled pacu), triportheid (dusky narrow hatchetfish, curtus hatchetfish); Cichliformes: cichlid (oscar, blue tilapia, Nile tilapia, Mossambique tilapia, saddle cichlid, convict cichlid, royal acara cichlid, dwarf cichlid, flag cichlid, perunsukkula-ahven, angelfish, blue discus, red discus); Clupeiformes: dorosomatid (threadfin shad, American gizzard shad); Cypriniformes: cyprinid (goldfish, common carp, grass carp, mrigal carp, silver carp, bighead carp, common barbel, shabout, common chub, roundtail chub, barbel chub, Papaloapan chub, tench, redtail barb, straightfin barb, smallmouth yellowfish, Caucasian scraper, Levantine scraper, bhakura, zebrafish, rohu, Levantine barbel, common snowtrout); Cyprinodontiformes: cyprinodontid (Soffia toothcarp, leukakilli), fundulid (blackstripe topminnow), goodeid (splitfin, relict splitfin), poeciliid (twospot livebearer, porthole livebearer, sailfin molly, short-finned molly, guppy, green swordtail, moonfish); Esociformes: esocid (northern pike); Galaxiiformes: galaxiid (common galaxias, climbing galaxias); Gobiiformes: eleotrid (giant bully), gobiid (round goby, monkey goby, bighead goby); Moroniformes: moronid (white bass, striped bass); Mugiliformes: mugilid (greenback mullet, flathead grey mullet); Osmeriformes: retropinnid (Australian smelt); Osteoglossiformes: osteoglossid (arapaima, silver arowana); Perciformes: chaetodontid (butterflyfish), latid (barramundi), percichthyid (Murray cod, trout cod, golden perch, Macquarie perch, river blackfish), percid (yellow perch), terapontid (silver perch); Salmoniformes: salmonid (rainbow trout, brown trout, brook trout); Scorpaeniformes: gasterosteid (three-spined stickleback); Siluriformes: clariid (African sharptooth catfish, vundu), claroteid (golden Nile catfish), heptapterid (South American catfish), ictalurid (channel catfish), loricariid (common pleco, tiger pleco, starlight bristlenose pleco), pangasiid (Pangas catfish), pimelodid (sailfin pim, spotted sorubim, barred sorubim), plotosid (eel-tailed catfish), sisorid (south sisorid catfish); Synbranchiformes: mastacembelid (Euphrates spiny eel); Anura: limnodynastid (striped marsh frog, tadpoles)	skin, fins, gills, eyes ('ich' = whitespot disease, death)	worldwide (esp. aquaria, culture ponds)

Parasite morphology: The parasite *I. multifiliis* forms three developmental stages: trophonts, tomonts containing tomites, and theronts. Trophonts on fish are variable in size (from 30 µm up to 1-1.5 mm in diameter) and possess a horseshoe-shaped macronucleus encircling a single micronucleus (although up to four micronuclei have been observed). Depending on their size, the trophonts have may up to 2,000 meridional (longitudinal) rows of somatic (body) cilia (kineties) interrupted only by a subapical vestibulum (mouth) with up to 15 buccal (oral) kineties lining the cylindrical prebuccal space. The trophonts possess many peripheral mucocysts (crystalline and secretory), several contractile vacuoles (water regulation organelles) and a unique anterior discoid body known as Lieberkuhn's (or watchglass) organelle (complex photo-receptive structure absent in the marine species *Cryptocaryon irritans*). As trophonts feed and grow, Lieberkuhn's organelle disappears and the cell fills with vacuoles (food, lipid, glycogen, protein). When replete, trophonts leave the host and form free tomonts which become encysted on available substrates. The tomonts begin internal division and repeatedly divide to form 250-2,000 smaller tomites. The tomites are mouthless but have active somatic cilia which keeps them spinning within the cyst. The tomites eventually break through the cyst wall to become free-swimming cylindrical theronts (swarmers) that actively seek hosts. Theronts range in size from 25-70 x 15-22 µm and are covered with 36-48 meridional kineties converging anteriorly around the pre- and post-oral sutures. The pre-oral suture forms an elevated pellicular crest raised apically into a pointed elevation (perforatorium) used to penetrate host tissues. Theronts possess an ellipsoidal macronucleus with an adjacent spherical micronucleus, one contractile vacuole with several excretory pores, a single anterior Lieberkuhn's organelle and a long caudal cilium.

Site of infection: Trophonts may infect the epidermis, gill filaments, cornea, mouth and tongue of their fish hosts. They generally occur in surface epithelia adjacent to the basal lamina, but invasions into subepidermal tissues may occur in advanced stages of disease.

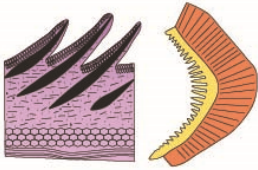
Pathogenesis: *I. multifiliis* causes freshwater white spot disease known as ichthyophthiriosis (or 'ich') in freshwater fishes. Theronts use the elevated pointed ridge (perforatorium) to penetrate host tissues and they discharge mucocysts to form a sticky envelope glued to the host's epithelium. Within minutes, the parasites penetrate deeper into epithelial or epidermal tissues where they feed (exhibiting intense phagocytic activity) and grow (increasing their volume up to 3,000 times). The trophonts cause significant tissue damage as a result of histolysis and trauma and usually form grey-white pustules in the skin of their hosts. Infected fish produce excess mucus to combat the irritation but many epidermal cells are destroyed and are sloughed. In some tropical fishes, pustules are not formed and the ciliates remain free on the surface or burrow into the epithelium. In other fishes, the parasites may form nonpurulent 'pustules' characterized by epithelial hyperplasia. Irrespective of lesion type, skin damage often predisposes to secondary bacterial infections. Heavy infections of the gill filaments may also interfere with gas exchange and may prove fatal. Lesions containing engorging trophonts appear as visible white spots up to 1-1.5 mm in diameter. Affected fish are anorexic and lethargic and may exhibit respiratory difficulties (gulping air, increased opercular flaring, crowding inlets) and 'flashing' (rapid turning to expose belly, often involving contact with substrates). Fish surviving infection exhibit some protective immunity against subsequent infections, with many disease outbreaks (epizootics) appearing cyclic (seasonal) due to the waxing and waning of protective immunity in fish populations and age-cohorts. While parasite densities are generally low in wild fish populations, heavy infections may develop in closed systems with reticulated water supplies (aquaria, tanks, ponds) leading to mass mortalities and significant economic losses. Disease risk factors include host susceptibility (some host species are more resistant than others), physiological condition (suboptimal due to poor nutrition, over-crowding, spawning, chemical pollutants, and other stressors), immunological status (varies with age and previous exposure) and environmental conditions (particularly poor water quality and warming waters over spring).

Developmental cycle and mode of transmission: Parasites are not transmitted directly from fish to fish, but rather via contamination of the water by dividing stages which release free-swimming stages that actively seek new hosts. Engorged trophonts of *I. multifiliis* are liberated from ruptured pustules on fish into the water column where they settle on convenient substrates or on the bottom. Within hours, they form a gelatinous cyst (tomont) which undergoes a series of 10-12 divisions (repeated binary fission) without intervening growth thus producing numerous equally-sized tomites (250-2,000 per cyst). [*C. irritans* produces fewer tomites per cyst (< 200) and they are smaller cells cleaved from the larger tomont]. Tomonts complete division within 24 hours at 15-25°C but up to 6 days at 5°C. Tomites are then released to become free-swimming theronts which actively search for new hosts. Theronts generally survive for up to 24 hours but their infectivity decreases after 12 hours. When theronts infect suitable hosts, they transform to trophonts which usually feed for around 7 days at 20°C, although they can persist for several weeks or even months at lower temperatures. The number, size and duration of the life-cycle stages for either species depends upon prevailing environmental conditions, particularly temperature (no development occurs below 2°C or above 30°C). The whole life-cycle may be completed in as little as 3-8 days at 23-24°C, but it progressively takes longer at lower temperatures (up to 3 months at 4-5°C).

Differential diagnosis: Infections are diagnosed by the detection of characteristic pustules on the external surfaces of fish and then confirmed microscopically by observing trophonts in fresh wet tissue preparations. Pustules are generally white to grey in colour and variable in size and shape. In heavy infections, the skin may become thickened, ulcerative and/or necrotic with flaking scales. Trophonts appear heavily ciliated and contain a distinctive large horseshoe-shaped macronucleus. Various molecular techniques have been utilized to characterize ciliate isolates; including immunoblot analyses of immobilization antigens, and polymerase chain reaction (PCR) amplification of partial DNA sequences (18S small subunit (SSU) ribosomal DNA); but such tests are restricted in use to molecular epidemiology or immunology research rather than diagnostics.

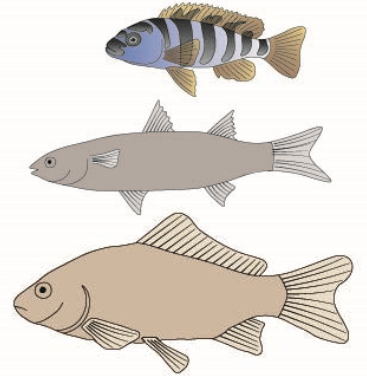
Treatment and control: Seasonal variations observed in the prevalence of *Ichthyophthirius* infections have been attributed to seasonal differences in water quality and the abundance of susceptible hosts. The prevalence and severity of infections is often exacerbated by poor water quality and/or overstocking which stresses fish and affects their susceptibility. While ciliates do not multiply on their hosts, their numbers amplify greatly in water sources through tomtom/tomite division and theront release. Many outbreaks have been associated with the introduction of new stock and closed culture systems are particularly at risk of sustained contamination. Quarantining new stock, destocking and resting ponds and periodic flushing of tanks and ponds with clean fresh water have all been shown to reduce contamination levels. Water treatment strategies used to prevent infections by reducing theront production and survival have included raising water temperature (usually possible only in aquaria or small tanks) or adding salt (to increase salinity), quicklime (to increase pH) or toxic chemicals (chlorine, chloramine, formalin, potassium permanganate, copper sulphate, silver nitrate, sodium percarbonate, methylene blue, ronidazole, bronopol). There is currently no acceptable curative drug for inclusion in fish food, although preliminary studies with antiprotozoals (quinine, toltrazuril, salinomycin, secnidazole, amprolium, metronidazole) and immune enhancers (ascorbic acid) have shown promise. Diseased fish infected with trophonts have been treated by bathing in dilute concentrations of toxic chemicals (formalin, malachite green, methylene blue, copper sulphate, hydrogen peroxide, peracetic acid). However, most of these chemicals are not appropriate for fish destined for human consumption and others are contraindicated for aquaria fish due to toxicity issues. Repeat treatments are often required, the frequency depending on their efficacy as well as the multiplicative potential of the parasites (kinetics and dynamics dependent on water temperature). Previous infections have been shown to impart some protective immunity to fish against subsequent re-infection and experimental studies have been relatively successful in protecting fish vaccinated with various ciliate extracts, especially those containing immobilization (i-) antigens. However, the logistics and mechanics of vaccine production and delivery in aquatic systems remain problematic.

Ichthyophthirius



skin, gills
(irritation, cystic lesions
'whitespot/ich')

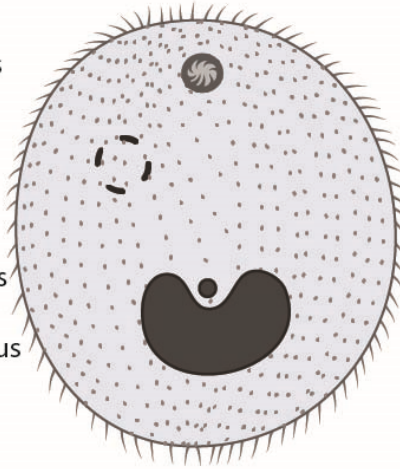
may reproduce asexually
(by transverse binary fission)
or sexually (by conjugation)



Vertebrate Hosts
(freshwater fish)

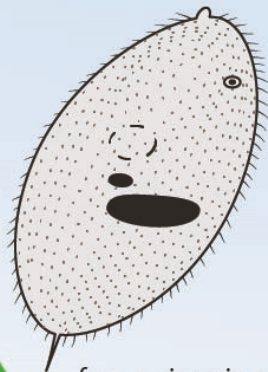
obligate monoxenous ectoparasites

watchglass
organelle
contractile
vacuole
micronucleus
macronucleus



subapical
vestibulum
meridional
kineties
mucocysts

trophonts
(30-1500 μm)



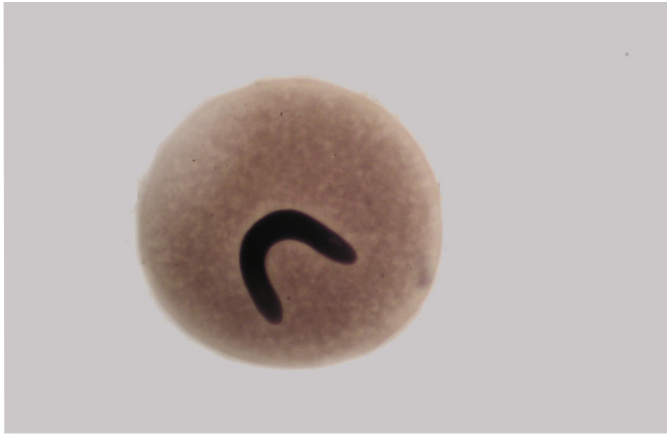
free-swimming
theronts
(25-70 μm)

36-48
meridional
kineties

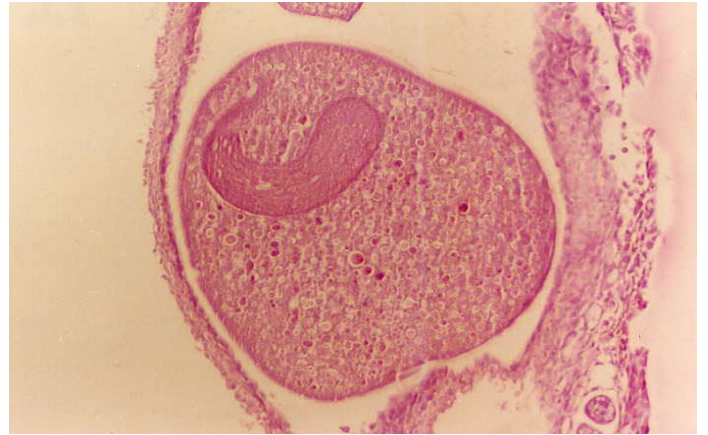


reproductive cysts
(tomonts) with
tomites

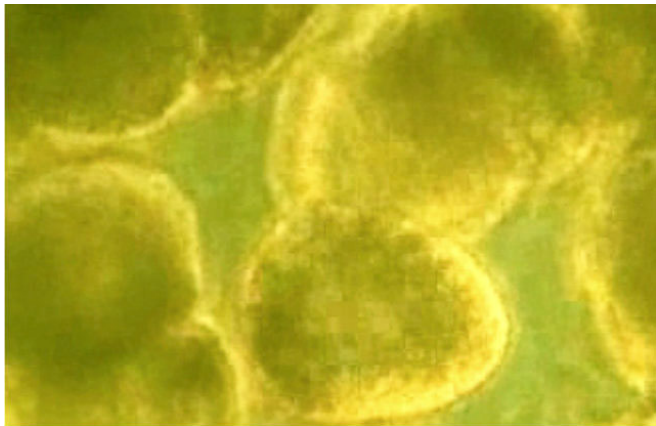
transmission via water contaminated by
dividing stages which release host-seeking 'swarmers'



Ichthyophthirius trophont from fish skin



Ichthyophthirius trophont on fish gill



Ichthyophthirius tomites from aquarium



Ichthyophthirius white-spot lesions on fish