

Chilodonella
(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 phyllopharyngids ('leaf throated') with cytopharyngeal phyllae. Chlamyodontid ciliates have dorsoventrally flattened bodies, with only the ventral surface being ciliated. In chilodonellids, the ventral ciliature is reduced to two longitudinal belts close to the body margins. Many *Chilodonella* spp. are free-living aquatic organisms, but some are ectoparasitic on the gills and skin of freshwater fishes, sometimes causing respiratory difficulties and mortalities.

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: Phyllopharyngea (cytopharynx with leaf-like phyllae)
Subclass: Phyllopharyngia (cyrtos, ventral cilia, free-living/symbiotic)
Order: Chlamyodontida (body dorsoventrally flattened, ventral cilia thigmotactic)
Family: Chilodonellidae (reniform body dorsoventrally flattened, two fields of dorsal ciliary rows)
Genus: *Chilodonella* (parasitic in fish on gills/skin)
Species: various species cause hypoxic gill diseases 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. 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)	
Oligo-hymenophorea	‘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 Phyllopharyngea contains ciliates with an infraciliature consisting of unique ultrastructural elements supporting their kinetosomes (lateral kinetodesmal fibril, reduced transverse microtubular ribbon accompanied by a left-directed transverse fibre, and convergent postciliary ribbon extending posteriorly to accompany those of adjacent kineties). While the class has been named after the possession of prominent cytopharyngeal phyllae (radial microtubular ribbons), some representatives (Suctoria) actually lack mouths and phyllae and possess unique sticky feeding tentacles. Depending on the classification system used, the class contains 2-4 subclasses, nominally identified as Chonotrichia, Phyllopharyngia, Rhynchodia, and Suctoria. The subclass Phyllopharyngia (also known as Cyrtophoria) is characterized by ciliates with mouths whose oral ciliature consists of one preoral and 2 circumoral kineties, with phyllae and rod-shaped nematodesmata forming a distinct cyrtos (cytopharyngeal basket). Two orders are recognized: Dysteriida containing 17 genera of free-living ciliates with laterally compressed bodies; and Chlamyodontida containing 17 genera of free-living or symbiotic ciliates with dorsoventrally-flattened bodies. Chlamyodontids include free-living (aquatic, terrestrial) and symbiotic (epizoic, parasitic) species, but they are essentially free-swimming ciliates that may attach to substrates by thigmotactic ventral cilia. They are classified into 6 families (Chilodonellidae, Chitonellidae, Chlamyodontidae, Gastronomidae, Kryoprodontidae, Lynchellidae) on the basis of their structural features. Members of the family Chilodonellidae have reniform dorsoventrally-flattened bodies with somatic cilia arranged into 2 longitudinal fields on the ventral aspect only. Five genera are recognized (*Chilodonatella*, *Chilodonella* (syn. *Chilodon*), *Phascolodon*, *Pseudochilodonopsis*, and *Thigmogaster*) with members of the genus *Chilodonella* characteristically having an arched right ventral ciliary belt and long phyllae leading to the cytostome located around the mid-body. The genus includes free living species as well as some that are facultative, and even obligate, parasites on the gills and/or skin of freshwater fishes, including farmed, ornamental and wild fish species in warm and cold waters.

<i>Chilodonella</i> species	Trophont size (µm)	Life-style (Hosts) [Clinical signs]	Distribution
<i>C. acuta</i>	30-49	terrestrial and freshwater, free-living on bladderwort (<i>Utricularia</i>) (recently reported as epizoic on skin/gills of freshwater fish Perciformes: latid (barramundi))	Saudi Arabia (Australia)
<i>C. aplanata</i>	40-60	terrestrial and freshwater, free-living on mosses and bladderwort (<i>Utricularia</i>)	Europe, America
<i>C. calkinsi</i> (syn. <i>C. pediculatus</i>)	40-60	brackish water (salt lake), free-living	Europe
<i>C. capucina</i>	40-70	freshwater, epizoic on carapace of Amphipoda: gammarid (<i>Gammarus</i>); Isopoda: asellid (<i>Asellus</i>)	Eurasia
<i>C. caudata</i>	42	freshwater, free-living on duckweed (<i>Azolla</i>)	Europe
<i>C. crebricostata</i>	57-76	brackish water, free-living	?
<i>C. cucullulus</i> (syn. <i>Trithigmostoma</i> , <i>Kolpoda</i> , <i>C. steini</i>)	130-300	fresh and brackish water, free-living	cosmopolitan
<i>C. elongata</i> (syn. <i>Phascolodon</i>)	130	freshwater, free-living	New Zealand
<i>C. fluviatilis</i>	50	freshwater, free-living	Europe
<i>C. gouraudi</i> (syn. <i>Odontochlamys</i> , <i>Chilodon geographicus</i>)	30-50	freshwater and terrestrial, free-living and on mosses	Europe, America
<i>C. granulata</i>	30-35	freshwater, epizoic on carapace of Amphipoda: gammarid (<i>Gammarus</i>); Isopoda: asellid (<i>Asellus</i>)	Eurasia
<i>C. helgolandica</i>	55-90	marine, free-living (benthic)	Europe
<i>C. hexasticha</i>	30-65	freshwater, epizoic on skin and gills of fish (Anabantiformes: nandid (Gangetic leaf-fish); Atheriniformes: melanotaeniid (eastern rainbowfish); Cichliformes: cichlid (redbelly tilapia, redbreast tilapia, mango tilapia, banded tilapia, red discus, southern mouthbrooder, Mozambique tilapia, Nile tilapia); Clupeiformes: dorosomatid (bony bream); Cypriniformes: cyprinid (goldfish, Eurasian carp, crucian carp, grass carp, bighead carp, straightfin barb, rohu); Gymnotiformes: gymnotid (naked-backed knifefish); Perciformes: latid (barramundi), percid (European perch), siniperid (mandarin fish), terapontid (silver perch, spangled grunter, barred grunter); Salmoniformes: salmonid (rainbow trout, brown trout, Atlantic salmon); Siluriformes: bagrid (yellowhead catfish), clariid (African sharptooth catfish), ictalurid (channel catfish), plotosid (eeltail catfish)) [skin lesions, epithelial hyperplasia, infiltrates, petechia, oedema, necrosis]	worldwide
<i>C. labiata</i>	40-50	freshwater, free-living	Europe
<i>C. longipharynx</i>		freshwater, epizoic on carapace of Amphipoda: talitrid (<i>Orchestia palustris</i> , <i>Talorchestia longicornis</i>)	Eurasia
<i>C. megalotrochae</i>	45-60	freshwater, epizoic on carapace of Rotifera: flosculariid (<i>Megalotrocha</i>)	Europe
<i>C. nana</i>	15-20	marine, free-living	
<i>C. parauncinata</i>		freshwater, free-living	China
<i>C. piscatoris</i> [now <i>Pseudochilodonopsis</i>]	65-80	freshwater, free-living on plants (<i>Lemna</i> , <i>Hottonia</i>)	Europe
<i>C. piscicola</i> (syn. <i>C. cyprini</i> , <i>Chilodon</i>)	30-80	freshwater (occasionally estuarine and brackish waters), epizoic on skin and gills of fish (Characiformes: characid (neon tetra); Cichliformes: cichlid (redbreast tilapia, banded tilapia, redbelly tilapia, mango tilapia, blue tilapia, Mozambique tilapia, Nile tilapia, southern mouthbrooder); Cypriniformes: catostomid (longnose sucker), cyprinid (goldfish, grass carp, rock carp, naked schizothoracin, yarlungi marinka, karestikumarinka); Perciformes: perichthyid (Murray cod), percid (European perch); Salmoniformes: salmonid (brown trout, masu salmon, Atlantic salmon, rainbow trout); Siluriformes: ictalurid (channel catfish))	worldwide

		[skin lesions, epithelial hyperplasia, infiltrates, petechia, oedema, necrosis]	
<i>C. porcellionis</i>	30	terrestrial, endozoic in genital spaces of Isopoda: porcellionid (<i>Porcellio</i> woodlice)	Europe
<i>C. propellens</i>	~50	freshwater, free-living (planktonic?)	Europe
<i>C. psammophila</i>		euryhaline, free-living	Europe
<i>C. rotunda</i>		freshwater, epizoic on carapace of Amphipoda: talitrid (<i>Orchestia agilis</i>)	Eurasia
<i>C. schewiakoffi</i>	250	free-living, freshwater	Europe
<i>C. subtilis</i>		freshwater, free-living	Europe
<i>C. turgidula</i>	50-55	freshwater, free-living (benthic)	Europe
<i>C. uncinata</i> [type species] (syn. <i>C. algivora</i> , <i>curvidens</i> , <i>dentata</i> , <i>dorsisuprema</i> , <i>rigida</i> , <i>Chilodon uncinatus</i>)	50-90	freshwater, free-living (recently reported as epizoic on skin/gills of fish (Perciformes: latid (barramundi), Cypriniformes: cyprinid (carp, goldfish))	cosmopolitan (Australia, Mexico)
<i>C. wisconsinensis</i>	50	terrestrial, free-living on mosses	America

A small range of other *Chilodonella* spp. have been re-examined and re-assigned to other sister genera: with *C. bavariensis*, *C. chattoni*, *C. hyalina*, *C. marginata*, *C. schedoeublepharis* and *C. steini* (syn. *Chilodontopsis bengalensis*) transferred to the genus *Thigmogaster*; *C. convexa* to the genus *Odontochlamys*, *C. minuta* to the genus *Chilodonatella*, and *C. plurivacuolata* to the genus *Alinostoma*.

Parasite morphology: *Chilodonella* spp. form motile trophic stages (trophozoites) that use cilia to swim with a distinctive gliding movement in fluid media or to browse over inert or living substrates. Trophozoites have oval, heart- or kidney-shaped bodies (many with an anterior notch) and they are dorsoventrally flattened. The cells vary in size depending on the species and their state of nutrition, most ranging from 27-85 x 13-62 µm (*C. piscicola* 30-80 x 20-62 µm, *C. hexasticha* 30-65 x 20-50 µm, *C. acuta* 30-49 x 16-30 µm, *C. uncinata* 27-42 x 13-29 µm). The dorsum is slightly convex and non-ciliated (although sometimes appearing to have a short anterior ciliary row, which is usually apical or subventral). The ventrum is flat or slightly concave and has a distinctive subapical buccal opening (mouth) consisting of a circular depressed cytostome with a tube- or funnel-like cytopharyngeal apparatus formed by a cirlet of long rod-like nematodesmata (phyllae) surrounding the cytopharyngeal tube (consistent with other 'leaf-throated' phyllopharyngid ciliates). The ventral surface is ciliated with 3 short oral rows located anterior to the cytostome, and with 2 large conspicuous ciliary belts running longitudinally down the left and right sides close to the body margins (straight left belt, arched right belt). The number of kineties (ciliary rows) in each belt varies according to species, with most ranging from 3-14 rows in the left belt and 4-15 rows in the right belt (*C. piscicola* (7-15 right, 8-14 left); *C. hexasticha* 5-7 right, 5-9 left; *C. acuta* 4 right, 5 left; *C. uncinata* 4 right, 3 left). Trophozoites have a single large ovoid macronucleus with a small adjacent micronucleus, both often located midbody or in the posterior half of the cell. Trophozoites usually possess 2 contractile vacuoles located diagonally across the midbody. Some early studies conducted in Russia observed cyst formation in '*Chilodon cyprini*' (now a junior synonym of *C. piscicola*). An attached figure illustrated the cysts as rounded uninucleate stages (30-40 µm in diameter) with no cilia or kineties, but with a central mass not quite extending to the peripheral membranous wall. There have since been few observations of cysts.

Site of infection: Most *Chilodonella* spp. are free-living, with some 27 species found in aquatic or moist terrestrial habitats (most freshwater, some brackish and saltwater) where they are free-swimming or may browse on plants (e.g. bladderworts, duckweed, mosses) or occur as ectocommensals on invertebrates (e.g. amphipods, isopods, rotifers). Two free-living species (*C. acuta* and *C. uncinata*) may also become facultatively or opportunistically parasitic and invade the gills or skin of freshwater fish. Another 2 species (*C. hexasticha* and *C. piscicola*) are obligate parasites on the gills, skin and fins of fish (mostly freshwater species but also some estuarine species). Infections have been recorded on over 40 fish species belonging to 18 families in 10 orders (mainly Cypriniformes and Perciformes, but also Cichliformes, Siluriformes, Salmoniformes, and occasionally Anabantiformes, Atheriniformes, Characiformes, Clupeiformes, and Gymnotiformes).

Pathogenesis: Infections in fish may be asymptomatic or subclinical when parasite numbers are low, but under favourable condition, parasite populations can build in number and cause mild-severe clinical disease (chilodonellosis). Infections generally cause disease and mortalities when fish are physiologically stressed (and thus immunocompromised) due to environmental changes in water quality (rapid temperature fluctuations and extremes, low sunlight, reduced oxygen levels, high/low pH, organic overloads), age (young), diet (inadequate nutrition) and poor husbandry (overcrowded unhygienic conditions). Clinical disease has been recorded globally in many freshwater fish species, particularly in food or ornamental fish culture. *C. hexasticha* and *C. piscicola* may cause significant epidemics in wild and farmed freshwater fish when they attach to external surfaces (skin, fins and gills) using their cytopharyngeal apparatus and ventral cilia. Feeding trophozoites disintegrate surface epithelial tissues and take up the resultant cellular debris, mucus and organic material, as well as any attached bacteria, diatoms and filamentous algae. The presence of

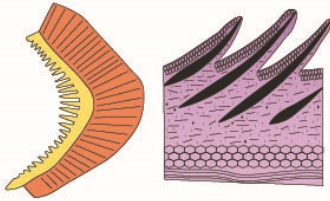
feeding organisms causes epithelial irritation, hyperplasia, inflammation with cellular infiltrates, excessive mucus production and dystrophic changes. Skin lesions may develop with pigment and scale loss, lymphocytic dermatitis or necrosis, pale mottled gray appearance, ulcerations, petechiae and abrasions. Gill filaments may become clubbed, fused, clogged with mucus, or even completely destroyed. Fish become lethargic, cease feeding, lose condition and show signs of hypoxia with breathing difficulties such as gaping, gasping and slow swimming at the water surface. They often exhibit uncoordinated swimming and flashing behaviours (attempting to dislodge parasites). Heavy infections can be fatal with many epidemics occurring in culture facilities resulting in high mortalities. Opportunistic infections by *C. acuta* and *C. uncinata* are usually not as severe and cause inflammatory foci on the skin or in the gills, but some have been associated with mortalities in goldfish.

Developmental cycle and mode of transmission: Trophozoites reproduce asexually by transverse binary fission, and repeated division may occur within several hours under favourable conditions, particularly in waters with low dissolved oxygen (often associated with high stocking densities), accumulated organic material (associated with overfeeding) and abundant anaerobic bacteria (considered to be a favoured food). Under adverse conditions, particularly when food is limited, the ciliates may undergo sexual reproduction by conjugation (pairing and exchanging genetic material (micronuclei)). Direct transmission typically occurs with free-swimming ciliates moving between hosts and attaching to gills and body surfaces. Trophozoites may infect fish of all ages, especially when they are stressed (immunocompromised) due to sudden changes in environment (e.g. temperature transitions from summer to autumn) or improper culture conditions (poor diet, low oxygen, overstocking). The involvement of possible cyst formation in transmission cycles is unclear due to their infrequent occurrence, but the source report stated that “in the presence of adverse conditions, chiefly during separation from the host, large specimens were able to form resting cysts that were able to survive for a long period on the bed of the pond and in open water”. Further studies are required to determine the significance of any cyst formation with respect to parasite survival, viability and infectivity.

Differential diagnosis: Infections are diagnosed by the direct microscopic detection of ciliates in wet mounts or scrapings taken from the gills, skin or fins. Motile ciliates exhibit a gliding and circular rotating motion over substrates and they have a distinctive dorsoventrally-flattened ovoid body with an anterior notch. Ciliates may be enrobed on albuminized slides, fixed and stained with a variety of silver stains (silver nitrate, silver carbonate or silver proteinate (protargol) to highlight their nuclei and kineties (argentophilic basal bodies (kinetosomes))). Ciliates may also be cultured in the dark in liquid media, including filtered water with added rice grains or cereal (wheat) infusions with feeder bacteria (e.g. *Klebsiella*). Molecular biological techniques have been used to characterize ciliates following the polymerase chain reaction (PCR) amplification of nuclear (small subunit ribosomal DNA, 5.8S rDNA, internal transcribed spacer regions 1 and 2, beta-tubulin P3 (protein coding locus)) and mitochondrial (cytochrome c oxidase subunit 1) gene sequences.

Treatment and control: Clinical infections in fish have responded well to bath treatments with salt, formalin, hydrogen peroxide, potassium permanganate or copper sulphate solutions, but the fish become rapidly re-infected when returned to their source ponds or tanks. Where possible, aquaculture systems should be regularly flushed with clean water to remove planktonic ciliates as well as accumulated organic wastes, debris, and bacteria. Producers have sometimes resorted to draining and drying ponds to curb persistent infections. Parasites can multiply rapidly in favourable conditions and they infect fish more readily when hosts are stressed by suboptimal culture (e.g. crowded ponds, poor water quality, organic enrichment and bacterial blooms usually due to overfeeding). It is essential that fish be kept healthy in clean ponds so they are less susceptible to infection, and that any infected individuals be quickly identified and removed for treatment or culling.

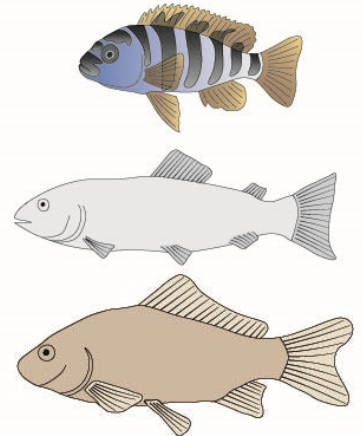
Chilodonella



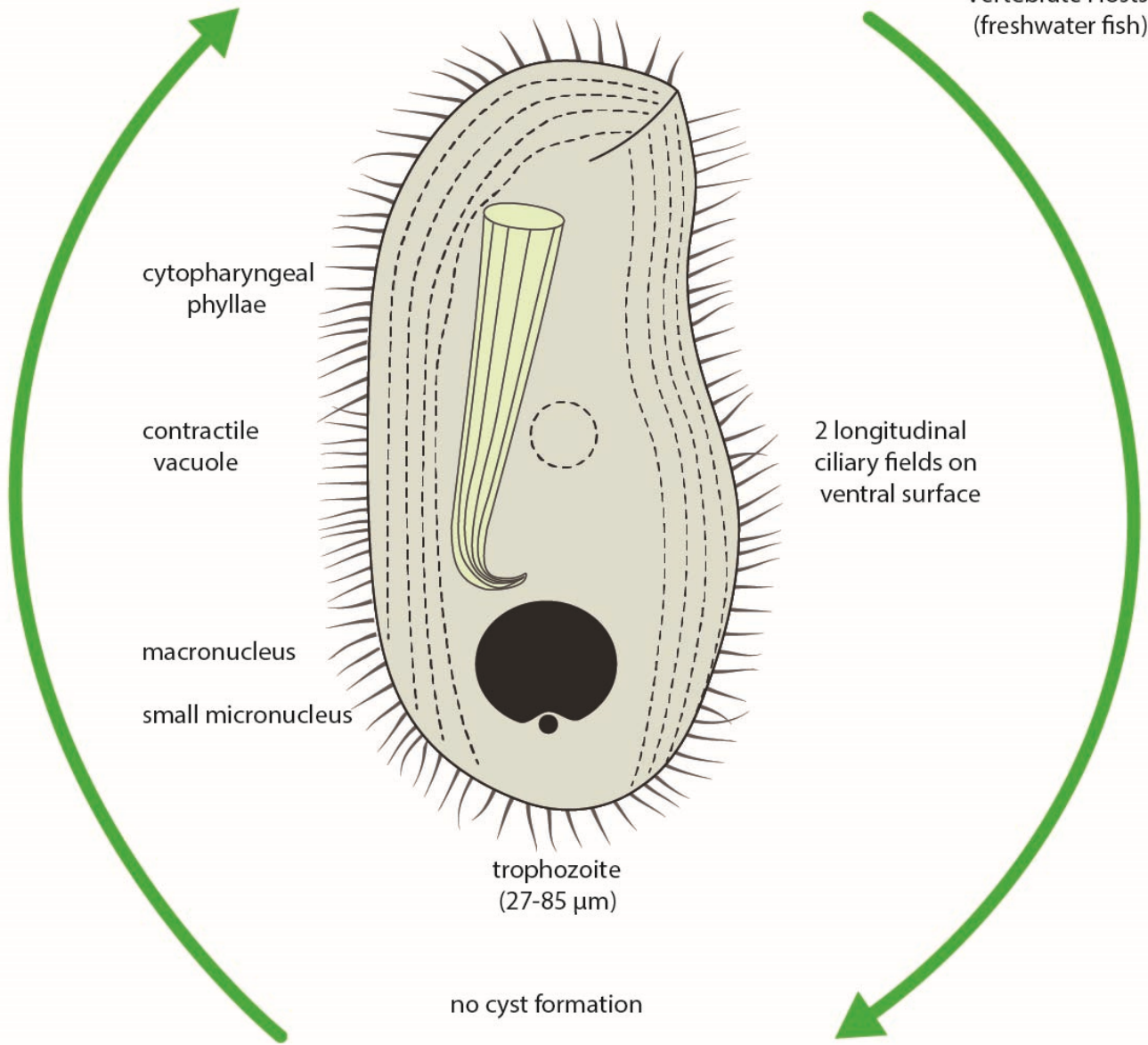
gills, skin
(irritation, inflammation,
congestion, lesions)

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

many free-living aquatic species
some facultatively/opportunistically parasitic
a few obligate parasites



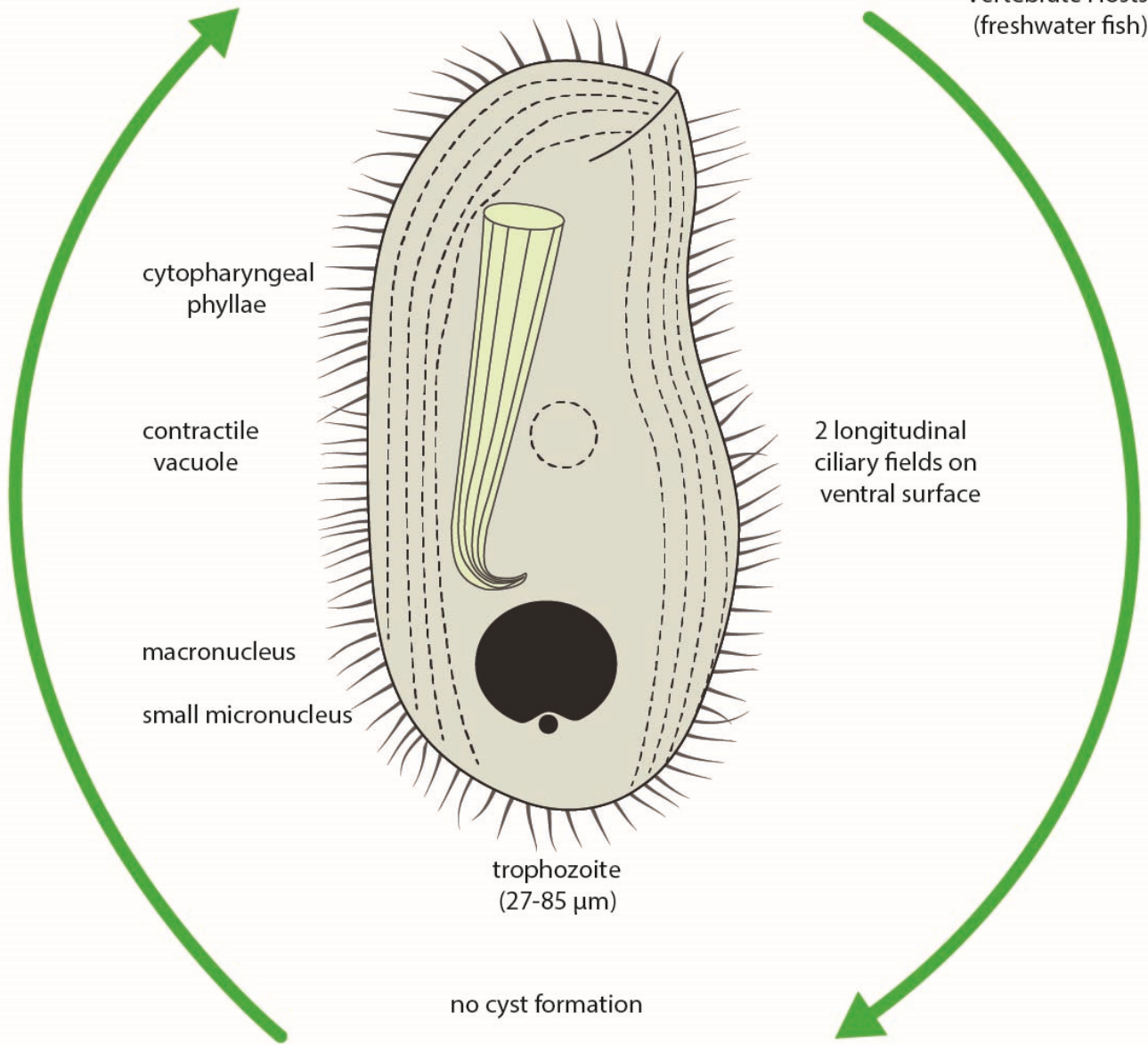
Vertebrate Hosts
(freshwater fish)



trophozoite
(27-85 μ m)

no cyst formation

direct transmission between hosts
via free-swimming trophozoites in water column





Chilodonella trophozoites on fish gill