

## ***Hexamita* and *Spiroucleus***

(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 (amoebflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). The metamonads comprise fornicates (diplomonads), parabasalians (trichomonads, hypermastigids, retortamonads) and preaxostylans (oxymonads). Diplomonads have complex nucleus-associated karyomastigonts and are defined by the possession of two nuclei and unique bilateral symmetry (diplozoic appearance). They typically form reproductive cysts (each containing four nuclei) which facilitate their transmission between hosts. Hexamitids are parasites or commensals in mammals, birds, reptiles and fish. All species are characterized by their pyriform shape and bilateral symmetry with two equal nuclei lying side by side, two intracytoplasmic granular axonemes and 6-8 flagella. *Hexamita* and *Spiroucleus* species are parasitic in the intestines of vertebrates and insects and several species cause enteric diseases in birds and fish.

### **Classification:**

Domain: Eukaryota (membrane-bound nucleus)  
Supergroup: Excavata (with conspicuous ventral feeding groove)  
Group: Metamonad (amitochondriate flagellates with karyomastigonts)  
Phylum: Fornicata (diplomonads)  
Order: Diplomonadida (with 1-2 karyomastigonts (each with 4 basal bodies/flagella associated with nucleus))  
Family: Hexamitidae (2 karyomastigonts arranged in binary axial symmetry)  
Genus: *Hexamita* and *Spiroucleus* (parasitic in small intestines/organs/skin of vertebrates and insects)  
Species: various species cause hexamitosis in birds or 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 (amoebflagellates), 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>Crepidoodinium</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

Metamonads are a group of excavates (with ventral feeding groove) that have several subcellular elements associated with their flagella forming a unique mastigont (an ultrastructural complex of organelles and cytoskeletal fibrils (incl. dictyosomes (Golgi bodies), centrioles (basal bodies) and a microtubular axostyle)). The metamonads comprise fornicates (diplomonads), parabasalians (trichomonads, hypermastigids, retortamonads) and preaxostylans (oxymonads). Most metamonads are amitochondriate but have retained reduced organelles of mitochondrial origin (fornicates containing mitosomes while parabasalians possess hydrogenosomes). Members of the phylum Fornicata are characterized by the possession of a distinct feeding groove or cytopharyngeal tube. Two classes are recognized: Eupharyngea commonly found in the digestive tracts of animals; and Carpediemonadea found in anaerobic intertidal sediments. The class Eupharyngea contains 2 orders: Diplomonadida with paired karyomastigonts; and Retortamonadida with single karyomastigonts. The diplomonads (meaning 'double monads') are binucleate and most species are found in the alimentary tracts of vertebrate hosts, although several free-living species occur in organically rich waters. Diplomonads typically form reproductive cysts (each containing 4 nuclei) to facilitate their transmission. Their motile vegetative forms (trophozoites) contain 1-2 kinetids, each with 4 kinetosomes but sometimes not all flagellated, and at least one flagellum per kinetid is directed posteriorly and may be associated with the cytopharyngeal tube or groove. Two families are generally recognized, 'monozoic' Enteromonadidae containing *Enteromonas*, *Caviomonas* and *Trimitus*; and 'diplozoic' Hexamitidae containing the genera *Giardia*, *Hexamita*, *Spironucleus*, *Trepomonas* and *Octomitus*. Some consider the enteromonads to be a regressive group with progressive reduction in the number of flagella while others consider them to be ancestral giving rise to the diplozoic hexamitids through the failure of cytokinesis. More recently, it was proposed that the order Diplomonadida be replaced by two new orders, Distomatida

(containing *Trepomonas*, *Hexamita* and *Spiroucleus*) and Giardiida (containing *Giardia* and *Octomitus*), both in a novel subclass Diplozoa. Differences in the placement of families and genera within the Diplomonadida (or Fornicata) remain to be reconciled.

Family	Genus	Hosts	Pathogenicity
Enteromonadidae (monozoic)	<i>Enteromonas</i>	mammals	nonpathogenic
	<i>Trimitus</i>	frogs, reptiles, fish, insects	nonpathogenic
	<i>Caviomonas</i>	rodents	nonpathogenic
Hexamitidae (diplozoic)	<i>Trepomonas</i>	frogs, fish, tortoises	nonpathogenic
	<i>Octomitus</i>	frogs, rodents	nonpathogenic
	<i>Spiroucleus</i>	rodents, birds, fish	pathogenic in birds, fish
	<i>Hexamita</i>	birds, frogs, fish, tortoises, insects	pathogenic in birds, fish, tortoises
	<i>Giardia</i>	mammals, birds, frogs, reptiles	pathogenic in mammals, birds

The genera *Hexamita* and *Spiroucleus* infect a wide range of vertebrate hosts, with some species causing enteric and/or systemic diseases in birds, fish and reptiles. It is often difficult to differentiate between these genera using morphological criteria because the pyriform trophozoites change shape prior to division (rounding up to become more spherical). Many species have been transferred between genera which has led to considerable taxonomic confusion. Nonetheless, ultrastructural and molecular characterization studies have confirmed that the genera should be kept separate rather than merged. Both genera form cells with 2 karyomastigonts, thus possessing 8 flagella (6 anterior flagella used for locomotion and 2 recurrent flagella in longitudinal canals (flagellar pockets)). The genus *Hexamita* forms cells with elongate-spherical bodies, large cytostomes, spherical nuclei with shallow kinetosomal pockets, kinetosomal complexes located anterolaterally, and the recurrent flagella associated with the surface of the nuclei. In contrast, the genus *Spiroucleus* forms cells with pyriform bodies, smaller cytostomes, elongated nuclei with deep kinetosomal pockets, kinetosomal complexes located anteromedially, and the recurrent flagella passing between the nuclei. Both genera form cysts which are passed into the external environment contaminating food and water supplies. Molecular characterization studies are now being used to determine phylogenetic relationships between clinical isolates, but it is apparent that more detailed morphotypic and more extensive genotypic studies are required to help resolve the current taxonomic confusion. Several species are recognized as significant pathogens causing clinical disease in their hosts: including *Spiroucleus salmonis* causing enteritis in salmonid fishes, *Spiroucleus meleagridis* causing enteritis in galliform birds, *Spiroucleus vortens* causing hole-in-the-head lesions in aquarium fishes, *Spiroucleus muris* causing digestive problems in rodents, and *Hexamita parva* causing tubulointerstitial nephritis in tortoises.

Parasite species	Size (µm)	Vertebrate Hosts	Location	Clinical signs	Distribution
Family: <b>Hexamitidae</b> (with functional feeding apparatus)					
Genus: <b>Hexamita</b> (pyriform body with 2 tube-like cytopharynxes run through body)					
<i>H. africanus</i>		freshwater Siluriformes: mochokid (wahrindi)	stomach, intestines		Africa
<i>H. axostylus</i>	8-10 x 5-10	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<i>H. capellani</i> (syn. <i>capelani</i> )		marine Gadiformes: gadid (poor cod)	intestines		Europe
<i>H. capsularis</i>		Cypriniformes: cyprinid (yellowfin)	intestines		China
<i>H. cryptocerci</i>		Blattodea: blaberid (wood-feeding roach)	hindgut		North America
<i>H. furcata</i>		free-living			Europe
<i>H. giganti</i>	10-14 x 10-14	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<i>H. gigas</i>		Clitellata: haemopid (horse-leech)	gut		Europe
<i>H. globulus</i>	4-6 x 4-6	Cypriniformes: cyprinid (yellowtail, yellow-tailed pomfret)	posterior intestine		China
<i>H. gracilima</i>		free-living in water			Europe
<i>H. guanqiaoensis</i>		Cypriniformes: cyprinid (smallscale yellowfin)	intestines		China
<i>H. gyrans</i>		free-living in water			Europe
<i>H. hemiculteri</i>	9-12 x 4-7	Cypriniformes: cyprinid (sharpbelly)	posterior intestine		China
<i>H. hollandei</i>		free-living in water			Europe
<i>H. honghuensis</i>	8-10 x 5-9	Cypriniformes: cyprinid (yellowtail, yellow-tailed pomfret)	posterior intestine		China
<i>H. inflata</i>		free-living in water			Europe

(= <i>Bodo inflatus</i> )					
<i>H. insana</i>		free-living in water			Europe
<i>H. intestinalis</i>		Anura: ranid (pond frogs), hylid (peepers), bufonid (toads); Urodela: ambystomatid (mole salamanders), plethodontid (dusky salamanders), salamandrid (crested newts)			North America, Eurasia
<i>H. jiangxiensis</i>	8-11 x 3-6	Cypriniformes: cyprinid (grass carp)	posterior intestine		China
<i>H. liangzihuensis</i>		Cypriniformes: cyprinid (smallscale yellowfin, xenocyprinids)	intestines		China
<i>H. longifila</i>		free-living in water			Europe
<i>H. longiformis</i>	32-40 x 3-5	Cypriniformes: cyprinid (yellowtail, yellow-tailed pomfret)	posterior intestine		China
<i>H. megalobramae</i>	8-10 x 4-7	Cypriniformes: cyprinid (Wuchang bream, goldfish, qingbo)	posterior intestine		China
<i>H. minor</i>		free-living in water			Europe
<i>H. motellae</i>		marine Gadiformes: lotid (shore rockling)	intestines		Europe
<i>H. mutabilis</i>		free-living in water/soil			Europe
<i>H. nelsoni</i>	8-16 x 3-8	Bivalvia: ostreid (eastern oyster, Pacific oyster, Sydney rock oyster, Olympia oyster, European flat oyster)	stomach		Europe, North America, Australia
<i>H. nobilis</i>	12-17 x 9-12	Cypriniformes: cyprinid (yellowtail, yellow-tailed pomfret)	posterior intestine		China
<i>H. nodulosa</i> (= <i>Bodo nodulosus</i> )		free-living in water			Europe
<i>H. ophiocephala</i>	8-11 x 4-5	Anabantiformes: channid (northern snakehead)	caecum, posterior intestine		China
<i>H. oviformis</i>	12-17 x 7-11	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<i>H. parva</i>	3-4	Testudines: testudinid (yellow-footed tortoise, red-eared slider, leopard tortoise, Forsten's tortoise, Chaco tortoise, Asian forest tortoise), emydid (eastern box turtle, diamondback terrapin, wood turtle, pond turtles, American box turtles), geoemydid (Asian spiny turtle, Asian box turtle, leaf turtles, spotted turtle); Anura: ranid (pond frogs), bufonid (toads); Urodela: ambystomatid (mole salamanders), plethodontid (dusky salamanders), salamandrid (crested newts), Sauria: chameleonid (veiled chameleon, common chameleon, panther chameleon), gekkonid (African fat-tailed gecko), agamid (frilled dragon), scincid (broad-headed skink)	intestines, kidneys	nephritis	worldwide
<i>H. periplanetae</i> (syn. <i>Octomitus</i> )		Blattodea: blattid (cockroaches)	gut		Europe
<i>H. pitheci</i> (syn. <i>Octomitus</i> )		Primates: cercopithecoid (rhesus macaque)	intestines, gall bladder		Asia
<i>H. polymorphola</i>	9-14 x 4-7	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<i>H. pulcher</i>		Rodentia: sciurid (thirteen-lined ground squirrel)	caecum		North America
<i>H. rodiformis</i>	15-22 x 4-	Cypriniformes: cyprinid (yellowtail,	posterior		China

	5	yellow-tailed pomfret)	intestine		
<i>H. rostrata</i> (now <i>Urophagus</i> )		free-living in water			Europe
<i>H. salpae</i>		marine Perciformes (salema porgy)			
<i>H. spiralis</i>		free-living in water			Europe
<i>H. symphysodonis</i>		Cichliformes: cichlid (blue discus)	intestines		Europe
<i>H. skujai</i>		free-living in water			Europe
<i>H. truncatus</i>		free-living in water			Europe
<i>H. truttae</i>	7-12 x 3-6	freshwater Cichliformes: cichlid (angelfish, banded cichlid), Cypriniformes: cyprinid (goldfish, barbs, golden rudd), Cyprinodontiformes: poeciliid (least killifish, mosquitofish), Characiformes: characid (black widow tetra)	gut, gallbladder		
<i>H. variformis</i>		Cypriniformes: cyprinid (grass carp, yellowfin)	intestines		China
<i>H. vesiformis</i>	7-9 x 5-7	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<i>H. wuchangensis</i>	8-9 x 3-4	Cypriniformes: cyprinid (yellowtail)	posterior intestine		China
<b>Genus: <i>Spiroucleus</i> (elongate body with 2 tube-like cytopharynxes run through body) [8F]</b>					
<i>S. anguillae</i> (syn. <i>S. mirabilis</i> )		freshwater Anguilliformes: anguillid (eels)	intestines		Eurasia
<i>S. barkhanus</i>	6-20 x 4-14	marine Salmoniformes: salmonid (Atlantic salmon, Arctic char, grayling)	intestines, liver, spleen, kidneys	enteritis, lesions	Europe
<i>S. carassii</i>	7-9 x 3-4	Cypriniformes: cyprinid (goldfish, Eurasian carp, crucian carp)	posterior intestine		Eurasia
<i>S. columbae</i>		Columbiformes: columbid (pigeons)	intestines		
<i>S. ctenopharyngodoni</i>	7-9 x 3-4	Cypriniformes: cyprinid (grass carp)	posterior intestine		China
<i>S. elegans</i> (syn. <i>Hexamita sinensis</i> ?)	5-11 x 2-5	freshwater Anura: ranid (frogs); Urodela: salamandrid (salamanders); Cypriniformes: cyprinid (grass carp); Cichliformes: cichlid (angelfish)	lower intestines, rectum	emaciation	France
<i>S. granularis</i>		Cypriniformes: cyprinid (yellowfin)	intestines		China
<i>S. meleagridis</i> (syn. <i>Hexamita</i> )	8 x 3	Galliformes: phasianid (peafowl, turkey, pheasant, quail, chukar partridge)	intestines		worldwide
<i>S. minutus</i>		Cypriniformes: cyprinid (yellowfin)	intestines		China
<i>S. mobilis</i>		Anguilliformes: anguillid (eel)			Asia
<i>S. muris</i>	7-9 x 2-3	Rodentia: murid (rats, mice, hamsters)	small intestines, caecum	digestive disorders	worldwide
<i>S. phycidis</i> (syn. <i>Hexamita</i> )		marine Gadiformes: lotid (shore rockling)	intestines		Europe
<i>S. salmonicida</i> (separated from <i>S. barkhanus</i> on basis of molecular studies and pathogenicity)		Marine Salmoniformes: salmonid (Arctic char, Atlantic salmon, brown trout); plus aquarium fish?	muscles, organs	abscesses	Europe
<i>S. salmonis</i> (syn. <i>Octomitus</i> , <i>Hexamita</i> )	7-14 x 3-10 cysts 10 x 7	freshwater and marine Salmoniformes: salmonid (brown trout, rainbow trout, brook trout, lake trout, steelhead trout, grayling, coho salmon); Gadiformes: gadid (Atlantic cod), lotid (burbot)	intestines, other organs	enteritis	Eurasia, North America
<i>S. salpae</i> (syn. <i>Hexamita</i> )		marine Perciformes: sparid (salema porgy)	intestines		Europe
<i>S. sinensis</i>	10-12 x 5-	Cypriniformes: cyprinid (yellowtail)	posterior		China

	7		intestine		
<i>S. torosus</i> (syn. <i>torosa</i> )	10-19 x 3-13	marine Gadiformes: gadid (Atlantic cod, haddock, saithe, merling), lotid (burbot, cusk, fourbeard rockling)	lower intestines		Canada, Europe
<i>S. vortens</i>	5-21 x 3-11	Cichliformes: cichlid (freshwater angelfish, red discus); Cypriniformes: cyprinid (ide)	gastro-intestinal tract, head	hole-in-head disease, lateral line erosion	North America, Europe
<i>S. xenocyprini</i> (syn. <i>Hexamita</i> )		freshwater Cypriniformes: cyprinid (xenocyprinids, smallscale yellowfin)	intestines		China

**Parasite morphology:** Hexamitids (*Hexamita* and *Spiroucleus*) form 2 different types of stages in their developmental cycles: namely flagellated trophozoites and encapsulated cysts. While trophozoites of the 2 genera do exhibit some structural differences, they are remarkably similar in morphology. They are dorsoventrally flattened and oval-pyriform in shape but become rounded just before they divide. They range in size from 3-22 µm in length by 2-13 µm in width depending on the species. Trophozoites exhibit binary axial symmetry in that they possess 2 round-elongate anterior nuclei that are laterally apposed. Each nucleus is adjacent to a cluster of 4 flagellar basal bodies (kinetosomes) collectively forming a karyomastigont unit. The kinetosomes in each cluster are arranged in 2 pairs, with 2 anterior (K1, K2) and 2 posterior (K3, R). Each kinetosome gives rise to a flagellum (eukaryotic-type, with 2 central microtubules and 9 peripheral doublets), 3 flagella (K1-3) project forwards from the cell body, while the fourth flagellum (R) is recurrent and passes through the body in a flagellar pocket emerging posteriorly as a free flagellum. Each trophozoite therefore has paired karyomastigonts collectively giving rise to 8 flagella (6 anterior and 2 recurrent). The recurrent flagella are also associated with 2 long cytopharynxes running longitudinally through body and opening as cytostomes at the posterior end. Trophozoites lack mitochondria, dictyosomes (Golgi bodies) and microbodies, but possess glycogen granules as their main carbohydrate storage products. The genus *Hexamita* forms cells with elongate-spherical bodies, tube-like cytopharynxes with large funnel-like cytostomes, spherical nuclei with shallow kinetosomal pockets, kinetosomal complexes located anterolaterally, and the recurrent flagella associated with the surface of the nuclei. The genus *Spiroucleus* forms cells with pyriform bodies (some tapering to caudal projections), narrow cytopharyngeal tubes with smaller cytostomes (not funnel-like), elongated nuclei (often sausage U-shaped) with deep kinetosomal pockets, kinetosomal complexes located anteromedially, and the recurrent flagella passing between the nuclei. Some species (e.g. *Spiroucleus vortens*) have complex adorned surfaces while others (e.g. *S. salmonis*, *S. barkhanus*, *S. salmonicida*) are unadorned. Both genera form cysts which are rarely observed. The cysts are ovoid, measuring 10 x 7 µm, thick-walled and possess 2-4 nuclei, glycogen rosettes and flagellar sheaths comprising partially dissembled microtubular ribbons and striated rootlet fibres.

**Site of infection:** Some 19 *Spiroucleus* spp. have been described, 16 species in fish (both marine and freshwater), 2 in birds and 1 in mammals, while 48 *Hexamita* spp. have been recorded; 26 species in fish, 2 in mammals, 1 in reptiles, 1 in amphibia, 1 in oysters, 1 in leeches, 2 in insects, and 14 free-living species in water and soils. Trophozoites of both genera occur extracellularly in the gastrointestinal tract, often in the upper intestines and pyloric region, sometimes in the gall bladder, caecum or rectum, but they may also occur in internal organs or in lesions on the skin. Infections in fish generally involve the intestines and sometimes internal organs, while infections in birds and mammals are confined to the small intestines and caeca.

**Pathogenesis:** Most hexamitid infections are asymptomatic or subclinical and have not been associated with any clinical disease, but infections by a few species (particularly heavy infections) may cause mild to severe disease: e.g. *Spiroucleus salmonis* causing enteritis in salmonid fishes, *Spiroucleus vortens* causing hole-in-the-head lesions in aquarium fishes, *Spiroucleus meleagridis* causing enteritis in galliform birds, *Spiroucleus muris* causing digestive problems in rodents, and *Hexamita parva* causing tubulointerstitial nephritis in tortoises. The parasites feed on solutes, particulate material and even bacteria by cytostomal endocytosis and phagocytosis, some cells becoming intimately associated with host cells suggesting they may feed on host tissues. Infections may cause irritation, inflammation and cellular destruction leading to organ dysfunction. Infections in birds cause bulbous fluid-filled dilatations of the small intestine (especially duodenum and upper jejunum) and the crypts become filled with parasites which attach to epithelial cells by their posterior flagella. This results in catarrhal enteritis (disease known as hexamitosis or spironucleosis) with profuse watery (sometimes yellowish) diarrhoea, cachexia, rapid weight loss without loss of appetite, emaciation, ruffled appearance with dry unkempt feathers, listlessness, weakness, and death (birds may die in coma or convulsions). Surviving birds develop some protective immunity to infection and disease but may be stunted in growth. Asymptomatic infections in adult birds may be protracted such that they act as long-term reservoirs of infection. Infections in rodents cause duodenal inflammation with the crypts becoming cystic and filled with trophozoites resulting in diarrhoea, weight loss, listlessness and weakness. Persistent infections in the kidneys of tortoises may cause tubular dilation, epithelial destruction, metastatic calcification, hyaline casts and tubulointerstitial nephritis, with the excretion of unusually concentrated urine smelling strongly of ammonia. Infections in fish may cause intestinal and/or systemic disease, the severity of which depends on parasite pathogenicity, intensity of infection, host susceptibility and tissues affected. Intestinal infections (e.g. *S. salmonis* in upper intestines, *S. elegans* in lower intestines, *S. torosa* in rectum) are often chronic but may cause damage to the mucosal epithelia resulting in catarrhal enteritis, yellowish watery or gelatinous intestinal contents, pale stringy faeces, faecal pseudocasts, excessive mucus, ascites with abdominal

distension, intestinal haemorrhages, red haemorrhagic vent, anaemia, pale gills, weakness, weight loss, anorexia, emaciation with retention of large head ('pinhead' disease) and sometimes exophthalmia and/or dark body coloration. Systemic infections occur when trophozoites secrete histolytic enzymes facilitating their invasion into a variety of tissues/organs (blood, heart, liver, gall bladder, spleen kidney, eye, brain, mesenteries, abdominal cavity, musculo-skeletal system and skin). Many systemic infections are acute with rampant cellular destruction, necrosis, inflammation and congestion. Organ-specific pathologies include kidney necrosis, renal tubule atrophy, anaemia, oedema, ascites, hepatocellular necrosis, cholecystitis, cholangiohepatitis, perihepatitis, encephalitis, meningitis, dermatitis, fin discoloration, fibrosis, granuloma formation, haemorrhages, ulceration, abscess formation, skin lesions releasing yellow strings of mucus, head and lateral line erosion (hole-in-head syndrome), and cartilaginous erosions leading to locomotory disorders (corkscrew motion, swimming on one side). Infections have occasionally been associated with low reproduction rates due to the reduced hatchability of eggs and higher mortalities in young fry. Disease is rarely found in wild fish populations but can commonly occur in cultured fish when subject to stressful conditions. Predisposing factors include inadequate diet, low oxygen content, poor sanitation, over-crowding, transportation, smoltification, poor water quality, changes in temperature, concomitant infections and reduced immunocompetence. Sporadic outbreaks have been reported in many aquarium fish, with different species varying considerably in their susceptibility, but young fish (fry, fingerlings, yearlings and smolts) being most susceptible.

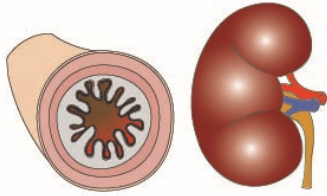
**Developmental cycle and mode of transmission:** Hexamitids form motile trophozoites that multiply by binary fission within vertebrate hosts, with some ultimately forming encapsulated cysts that are passed in host excretions. Infections are transmitted between hosts by the ingestion of cysts or trophozoites passed into the external environment where they contaminate food and water sources. Trophozoites are highly susceptible to external environmental conditions but may survive passage between hosts involved in intimate associations. Cysts are highly resistant to external environmental conditions and may survive for long periods in contaminated food and water supplies. Infections in birds and mammals typically exhibit faecal-oral transmission where cysts excreted with host faeces are ingested by new susceptible hosts. Many birds may act as long-term carriers and persistently shed cysts in their droppings. Infections in fish undergo horizontal transmission in water when stages (cysts and trophozoites) are shed in faeces, mucus strings or from haemorrhagic lesions and ingested by new hosts. It has been suggested that mucus and/or faecal material protected trophozoites from osmotic stress in aquatic environments. Infections are more prevalent in cultured fish, particularly those co-habiting aquaria.

**Differential diagnosis:** Some infections may be suspected on clinical grounds, mostly those causing gross lesions, such as pinhead or hole-in-head disfigurement in fish. However, most clinical infections cause nonspecific signs which could be attributed to a range of other aetiological agents. Diagnosis is conventionally confirmed by the direct detection of hexamitids in clinical samples. Parasites are usually identified by observations of motility patterns, body morphometrics (shape, size) and subcellular features (notably number and location of flagella) using high-contrast bright-field, phase-contrast or interference-contrast microscopy. It is advised that viscous media (Protoslo, methyl cellulose) be used to prepare wet mounts to slow down energetic trophozoites. Clinical samples from birds, mammals, reptiles or fish usually include fresh faeces or gastrointestinal content, while those from fish may also include skin scrapings, gut scrapings, blood smears, impression smears or tissue imprints of internal organs. Blood samples may be subject to microhaematocrit tube centrifugation to concentrate trophozoites in the buffy coat layer. Smears may be fixed and stained with Giemsa, while fixed tissues may be processed for histological examination after staining with haematoxylin and eosin, Giemsa or periodic acid Schiff's reagent. Several in vitro culture techniques have been used to grow *Spironucleus salmonis* in balanced organic medium (199) supplemented with serum and lactalbumin hydrolysate, Eagle's minimum essential medium (MEM) supplemented with foetal bovine serum, or TYI-33 medium supplemented with bile. Isolates of *S. meleagridis* have also been grown in the allantoic cavities of developing chicken and turkey embryos. An enzyme immunoassay was developed to detect host antibodies against *Spironucleus barkhaus* in Atlantic salmon, but little is known about test sensitivity or specificity. More recently, molecular biological techniques have been used to detect, identify and characterize isolates from a range of hosts by the polymerase chain reaction (PCR) amplification of nuclear gene sequences (small subunit ribosomal RNA, alpha-tubulin, and glutamate dehydrogenase 1 (GDH1)).

**Treatment and control:** Different strategies have been used to treat and control infections depending on the hosts involved. Infections in birds may be treated with nitroimidazoles (dimetridazole, metronidazole) given in feed or drinking water for several days, but their application may cause some adverse side-effects. There are also growing concerns about their toxicity and residue levels (particularly in commercial production flocks) and the potential for the development of drug resistance by parasites. It is often recommended that concurrent antibiotic treatment (e.g. oxytetracycline, chlortetracycline) be used to help control secondary bacterial infections. Preventive measures depend on regular health surveillance (testing, quarantine, culling), strict hygiene (disinfect equipment and fomites, water treatment, clean foods), good sanitation (remove litter, use raised or wiremesh floors), biosecurity (prevent contact with wild birds, personnel to take precautions) and sensible husbandry (separate host species and cohorts, avoid over-crowding, provide good nutrition, treat concomitant infections). Infections in mice that have become problematic in rodent colonies have proven difficult to treat, with the administration of metronidazole, fenbendazole or both in food, water or medicated gels not clearing infections. Some efforts to establish parasite-free colonies based on regular testing and culling infected individuals were not completely successful due to false-negative tests due to low parasite burdens or intermittent shedding. It was found necessary to re-derive mouse colonies from scratch to completely eliminate parasites. Clinical infections in the renal and urinary

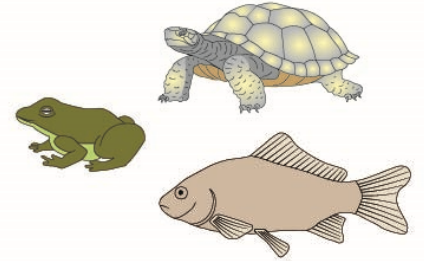
systems of tortoises responded well to oral dosing (via stomach tube) with metronidazole or ronidazole, although some treatments induced vomiting in several individuals. Infections in fish have been treated with a variety of chemicals administered either as bath treatments or added to foodstuffs, including the anti-protozoal nitroimidazoles (metronidazole, benznidazole, ronidazole, secnidazole, dimetridazole), nitrofurans (furazolidone), nitrothiazoles (enheptin-A), phenanthrolines (entobex), antibiotic aminoglycosides (aminosidine) and chlortetracyclines (aureomycin), and even some anthelmintics (albendazole, diethylcarbamazine, nitroscanate). Magnesium sulphate has also been used as a purgative to expel flagellates from the intestines of salmonids. The efficacy of chemical treatment was highly variable depending on host species, age cohorts, host stressors (dietary, physiological, immunological), culture conditions (facilities, crowding), concomitant infections and parasite species/strains. Producers often make recourse to preventive strategies to reduce parasite prevalence and transmission in aquaculture systems. Infections are usually controlled by avoiding the introduction of parasites (through regular testing, quarantine and culling), reducing contamination (good sanitation, cleaning tanks, gravel beds and filters), maintaining water quality (regular flushing, removing accumulated wastes, avoiding fluctuations in temperature, dissolved oxygen and pH), and providing good husbandry (separating cohorts, sensible stocking rates, good nutrition). Infections have become less problematic in salmonid hatcheries since the implementation of moist pellet diets.

# Hexamita



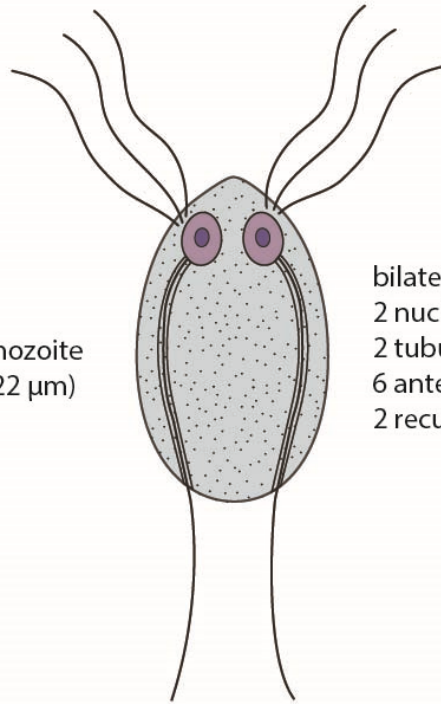
intestines, kidney  
(diarrhoea, nephritis)

division by  
longitudinal  
binary fission



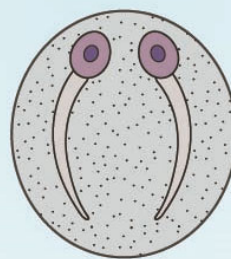
Vertebrate Hosts  
(fish, tortoises, frogs)

trophozoite  
(3-22  $\mu\text{m}$ )



bilateral symmetry  
2 nuclei  
2 tubular cytopharynxes  
6 anterior flagella  
2 recurrent flagella

cysts passed in excreta

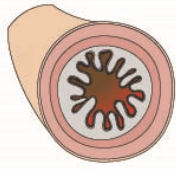


2-4 nuclei

cyst  
(~ 10  $\mu\text{m}$ )

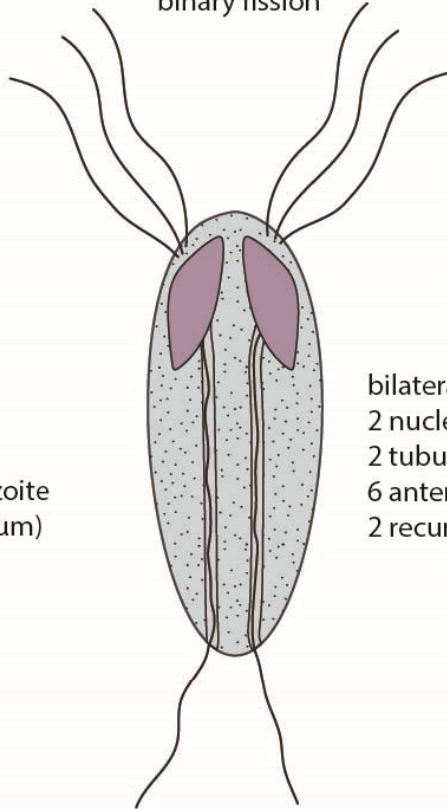
direct transmission between hosts  
by cysts (sometimes trophozoites)  
contaminating environment

# Spironucleus



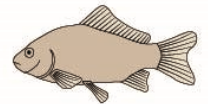
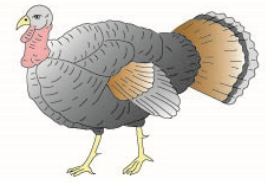
intestines  
(enteritis, lesions,  
diarrhoea)

division by  
longitudinal  
binary fission



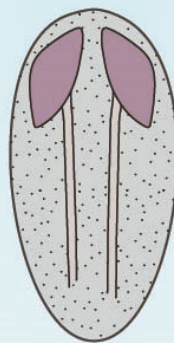
trophozoite  
(5-20  $\mu\text{m}$ )

bilateral symmetry  
2 nuclei  
2 tubular cytopharynxes  
6 anterior flagella  
2 recurrent flagella



Vertebrate Hosts  
(fish, birds, mammals)

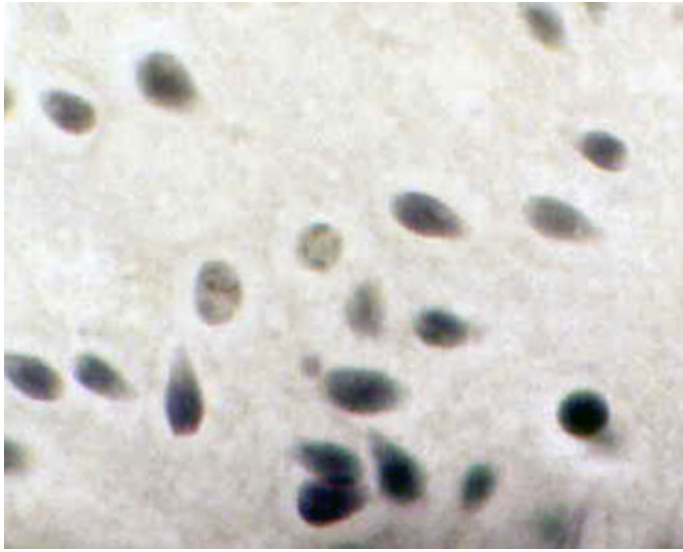
cysts passed in excreta



2-4 nuclei

cyst  
(~ 10  $\mu\text{m}$ )

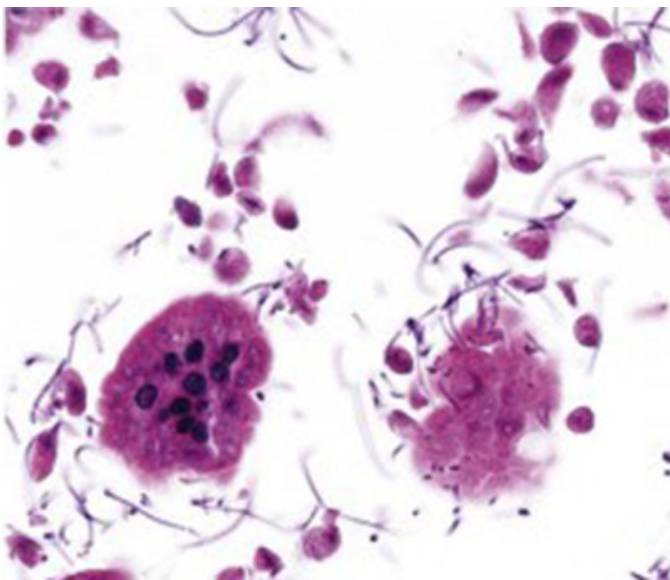
direct transmission between hosts  
by cysts (sometimes trophozoites)  
contaminating environment



*Hexamita* trophozoites



*Hexamita* trophozoite



*Spirotrichia* trophozoites in gut