Perkinsus
(protist: flagellate)

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). One small alveolate group comprises the Perkinsozoa (Protalveolata) which includes the unique perkinsids that parasitize molluscs. Perkinsids form endogenous vacuolated trophonts in the tissues of bivalves and abalone and exogenous biflagellated zoospores with apicomplexan and dinoflagellate affinities. Infections have caused significant mortalities in commercial and wild shellfish populations.

Classification:
Domain: Eukaryota (membrane-bound nucleus)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria)
Group: Alveolata (with cortical alveoli)
Phylum: Perkinsozoa/Protalveolata (perkinsids parasitic in molluscs)
Class: Perkinsea (parasitic in marine bivalves and abalone, form biflagellated zoospores)
Order: Perkinsorida (with characters of class)
Family: Perkinsidae (with characters of order)
Genus: *Perkinsus* (parasitic in tissues of abalone)
Species: various species cause lesions and mortalities in shellfish

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)	Trans-mission*
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)	*Amyloodinium* *Crepidoodinium* *Piscinoodinium*	fish (skin)	direct (w)
	Order: Syndiniales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	*Haematodinium* *Ichthyodinium*	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	*Perkinsus*	gastropods, bivalves (tissues)	direct (w)

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)	*Giardia*	vertebrates (gut)	direct (f-o)
			Hexamita Spironucleus	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)	*Histomonas*	birds (gut, liver)	direct (f-o)
			Dientamoeba	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	*Trichomonas*	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	*Cochlosoma*	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)	*Ichthyobodo* (= *Costia*)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	*Cryptobia*	fish (gills, skin)	direct (w)
			Trypanoplasma	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	*Trypanosoma*	vertebrates (blood, tissues)	indirect (v-b)
			Leishmania	vertebrates (blood, tissues)	indirect (v-b)

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

The group Alveolata in the supergroup SAR is characterized by the possession of cortical membranous alveoli (vesicles or sacs) underlying and supporting the cell wall, and includes many otherwise disparate groups comprising 3 large assemblages (Apicomplexa, Ciliophora, Dinoflagellata) and 6 smaller taxa (Acavomonidia, Chromerida, Colpodellida, Colponemidia, Perkinsozoa and Voromonadida). The phylum Perkinsozoa (syn. Protalveolata) was erected to accommodate members of the unique dinoflagellate-like genus *Perkinsus* which are parasitic in marine molluscs, but which form planktonic biflagellated zoospores involved in transmission. Classification of the group has a convoluted history. The causative agent for a disease in oysters was first reported as a fungus belonging to the genus *Dermocystidium*. It was subsequently placed in various fungal and protozoal groups depending on which phenotypic characters were used. Ultrastructural studies then revealed the presence of subpellicular membranes, micropores and putative (incomplete) conoids in trophonts and it was renamed *Perkinsus* and transferred to the phylum Apicomplexa. More recently, however, molecular biological studies on several independent genes suggested strong affinities between *Perkinsus* and dinoflagellates; with both groups assigned to the clade Alveolata. Several *Perkinsus* spp. have been described as parasites of gastropods and bivalves, especially abalone, oysters and clams. Infections were first linked to massive oyster mortalities in the Gulf of Mexico and subsequently to mortalities (or die-back) in gastropods (abalone) and other bivalves (oysters and clams) around the world. Recent surveys have revealed perkinsids to be common parasites in numerous bivalve species, especially in tropical waters.

***Perkinsus* species**	**Hosts**	**Location**	**Clinical signs**	**Distribution**
P. beihaiensis	Bivalvia: ostreid (Hong Kong oyster, Suminoe oyster), pteriid (Akoya pearl oyster, blacklip pearl oyster)	connective tissues, gut epithelia, digestive gland	lesions	China
P. chesapeaki (syn. *P. andrewsi*)	Bivalvia: myid (soft-shell clam), solecurtid (saltwater clam), tellinid (Baltic clam), venereid (hard clam), cardiid (small giant clam, crocus clam), ostreid (eastern oyster)	gills	-	United States
P. honshuensis	Bivalvia: venerid (Manila clam, variegated carpet shell clam)	connective tissues, digestive gland	-	Asia
P. karlssoni (dubious record)	Bivalvia: pectinid (bay scallop)	cultured soft tissues	-	Canada (hatchery)

P. marinus (syn. *Dermocystidium marinum, Labyrinthomyxa marina*)	Bivalvia: ostreid (eastern oyster, mangrove cupped oyster); Gastropoda: pyramidellid (impressed odostome sea snail, ectoparasitic on eastern oysters)	soft tissues	mortalities	Atlantic, Gulf of Mexico
P. mediterraneus	Bivalvia: ostreid (European flay oyster)	soft tissues	-	Europe
P. olseni (syn. *P. atlanticus*)	Gastropoda: haliotid (blacklip abalone, greenlip balone, whirling abalone, staircase abalone), Bivalvia: arcid (ark cockle), chamid (reflexed jewel box), venerid (New Zealand little neck clam, Palourde clam, Manila clam, pullet carpet shell)	soft tissues	pustular lesions, mortalities	Pacific, Atlantic, Mediterranean
P. qugwadi	Bivalvia: pectinid (Japanese weathervane scallop)	internal tissues	mortalities	Canada

Parasite morphology: *Perkinsus* spp. form 4 different morphological stages during their development, each stage given different names over time; namely, trophozoites (aplanospores, meronts); schizonts (sporangia, tomonts, rosettes); zoosporangia (including prezoosporangia or hypnospores); and zoospores (considered to be equivalent to dinoflagellate dinospores). Trophozoites are ovoid stages measuring 2-20 µm in diameter that may be found throughout molluscan tissues. Smaller forms are also often observed within phagosomes of haemocytes. Mature trophozoites (10-20 µm) have a large eccentric vacuole occupying up to 90% of the cell volume, thereby displacing the nucleus to the cell periphery and producing a typical signet-ring appearance. The vacuole sometimes contains a large polymorphic inclusion body (termed a vacuoplast), and the nucleus contains a prominent nucleolus. Parasites undergo asexual multiplication (vegetative proliferation known as palintomy) whereby mature trophozoites undergo successive bipartitioning (alternating karyokinesis and cytokinesis) to form schizonts ranging in diameter from 15-100 µm (usually < 25 µm). Schizonts contain 2-64 (usually 8-32) daughter trophozoites which often stay together in a rosette-like arrangement inside a dense wall 2-4 µm thick. The contained trophozoites are coccoid or cuneiform in shape measuring 2-4 µm along their long axis. They are liberated when the wall ruptures allowing them to invade adjacent tissues where they grow becoming larger and developing vacuoles. When trophozoites are trapped in anoxic tissues from dead or dying hosts, or cultured in fluid thioglycolate medium, they become enlarged and appear as thick-walled ovoid prezoosporangia measuring 30-80 µm in diameter (some extremes up to 480 µm). When these stages are released into seawater, they differentiate into flask-shaped zoosporangia with 1-2 lateral discharge tubes and undergo zoosporulation by successive karyokinesis and cytokinesis producing hundreds of zoospores. Zoospores are biflagellated ellipsoidal uninucleate cells measuring 4-6 x 2-3 µm, and they contain several cytoplasmic vacuoles, lateral mitochondria, and a rudimentary apical complex with a conoid, polar ring, subpellicular microtubules (up to 39), micronemes and rhoptries (similar to that possessed by apicomplexan parasites). The 2 flagella are inserted laterally, with the longer anterior flagellum bearing a row of long filamentous mastigonemes (tinsels) and the shorter posterior flagellum being naked. Mature zoospores escape from zoosporangia via the discharge tube, and they may initiate infection in new hosts via epithelia of the gills, mantle or gut.

Site of infection: Screening surveys have detected *Perkinsus* spp. in over 67 molluscan species, with clinical infections found primarily in bivalves (oysters) and gastropods (abalone) from temperate and tropical regions in Atlantic, Pacific and Mediterranean waters. Trophozoites have been detected in stomach and intestinal epithelial cells, digestive gland epithelia, haemocytes, adductor muscles, and connective tissues associated with viscera, mantle and gills.

Pathogenesis: Clinical infections have been detected in conjunction with mass mortalities in cultured oysters as well as population declines (die-back) in wild shellfish. The disease is generally known as perkinsosis (although *P. marinus* infections in oysters were initially known as ‘dermo’) and is characterized by gross signs of severe emaciation, digestive gland pallor, pustular-like lesions in soft tissues, shrinkage of the mantle from the shell, retarded growth and inhibition of gonad development. Trophozoites invading host tissues evoke haemocytic infiltrates of epithelia, connective tissue, muscle fascicles and blood spaces, with parasites occurring both within haemocytes and free within tissues. While haemocytes may actively phagocytose parasites, they are not destroyed but rather multiply within haemocytes ultimately causing their lysis (thus contributing to the systemic spread of infections). Advanced infections are characterized by extensive haemocyte activation and recruitment, with concomitant exuberant production of oxygen intermediates (free oxygen radicals). Host cells in the immediate vicinity are destroyed leading to structural losses through focal necrotic and/or inflammatory lesions. Pathogenesis appears to be associated with various host-parasite molecular interactions involving agglutinins (enhancing phagocytosis) and the release of enzymes (facilitating tissue invasion and necrosis) and antiproteases (inhibiting humoral responses). The lesions sometimes become so extensive that they appear as cream-yellow pustular abscesses up to 10 mm in diameter, making the flesh unacceptable for marketing. Infections spread and damage gut epithelia, adductor muscles, connective tissues throughout the viscera and gills, and sometimes occlude haemolymph sinuses. Molluscs

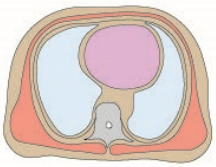
become moribund and lose condition with reduced growth, weight loss and even impaired fecundity due to depleted energy reserves. Heavy infections have been associated with high mortalities in specific regions, both within cultured shellfish and in wild populations. Disease outbreaks have often been reported in conjunction with stressors affecting host susceptibility to infection, including the weakened condition of hosts following spawning, pollution and reduced water quality leading to hypoxia, over-crowding and poor nutritional status.

Developmental cycle and mode of transmission: These enigmatic organisms have life-cycles involving the repeated schizogonous multiplication of endoparasitic trophozoites in the tissues of live molluscs, followed by the liberation of free-swimming biflagellated zoospores from zoosporangia forming when lesions rupture or when hosts die. Trophozoites invade tissues and grown from small uninucleate ovoid stages to larger vacuolated forms which have a typical signet-ring appearance due to peripheral displacement of the nucleus by the eccentric vacuole. Mature trophozoites then multiply by forming schizonts which exhibit successive internal partitioning (alternating nuclear and cytoplasmic divisions) to form numerous (2-64) round to wedge-shaped trophozoites which are liberated when the mother schizont ruptures. The parasites may undergo multiple cycles of schizogony in live hosts leading to heavy disseminated infections. When the host dies and infected tissues decompose becoming anoxic, trophozoites transform into large ovoid prezoosporangia. This transformation also occurs *in vitro* when infected tissues are cultured in thioglycolate medium. When prezoosporangia are then released into seawater, they differentiate into flask-shaped zoosporangia within which numerous biflagellated zoospores develop by palintomy over 4 days. The zoospores are liberated into the marine environment through discharge tubes which develop in the sides of the zoosporangia. The free-swimming zoospores may then infect new hosts with the primary portal of entry apparently being the gut epithelium, although it may also occur via epithelia of the gills, labial palps and mantle. The zoospores lose their flagella when they invade host tissues and round up to become small trophozoites, thus completing the cycle. Transmission between hosts is therefore direct and may be accomplished without vectors or alternative hosts. However, it is also thought that trophozoites multiplying within live hosts may also be released by diapedesis and in faeces, and may constitute a source of infection for hosts in the immediate vicinity (thus accounting for the rapid spread of infections in beds/racks of live oysters). There are also some suggestions that scavengers may be involved in the dissemination of infections as prezoosporangia have been detected in the faeces of fish, oyster drills and crabs feeding on dead or moribund oysters. One study also suggested that *P. marinus* found in pyramidellid snails ectoparasitic on oysters may be injected during feeding or contaminate the surrounding water. Epizootiological studies have consistently shown correlations between parasite development, prevalence, intensity and geographic distribution, particularly with respect to water temperature and salinity. More infections and disease have been associated with warmer temperatures (over 20°C for at least one month) and higher salinities (over 15 parts per thousand), often manifesting in annual seasonal patterns of infection and disease (peaking in summer).

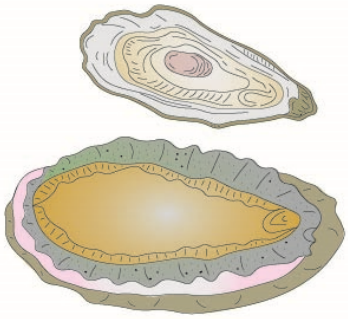
Differential diagnosis: Infections are generally detected postmortem by the light microscopic observation of vacuolated trophozoites in tissue sections taken from moribund shellfish or those with visible cyst-like pustular lesions. Alternatively, prezoosporangial stages may be detected in tissue samples cultured for several hours-days in fluid thioglycolate medium (FTM) or Ray's FTM supplemented with nystatin (RFTM). These stages stained well with Lugol's iodine and were evident as large blue-black spheres. Cultures may be incubated for as little as 4-8 hours but are often extended up to 7 days to promote better growth and development. Prezoosporangia are induced to transform to zoospore-producing zoosporangia by transferring them from RFTM to seawater. Various agencies recommend culturing postmortem samples from the heart, rectum, mantle and gills (sometimes antemortem samples of haemolymph) and semi-quantitating the results by counting the numbers of organisms and ranking them numerically (1-5) for infections ranging from very light to heavy. Researchers have also successfully raised polyclonal and monoclonal antibodies against prezoosporangia and incorporated them into fluorescent and enzyme immuno-assays to detect infections, but encountered some problems with specificity and cross-reactivity against some dinoflagellate species. More recently, molecular biological techniques have been used with considerable success to detect infections and examine parasite phylogenetic relationships following the polymerase chain reaction (PCR) amplification of several DNA sequences (small and large subunit ribosomal RNA plus internal transcribed spacers regions, and type I actin genes).

Treatment and control: Experimental studies on controlling infections in cultured oysters revealed some apparent benefits (reduced mortality and lesion scores) using bath treatments with chlorine, cycloheximide, N-halamine disinfectants, freshwater flushes, and water filtration followed by ultraviolet irradiation. However, such treatments are impractical under field conditions, and prezoosporangial stages have been found to have a high tolerance to chlorine, low temperatures and low pH. Control has therefore relied primarily on modifying shellfish management and culture procedures to reduce the incidence and spread on infections. Recommended strategies include regular and intensive monitoring (particularly over summer), the removal of infected stock, the isolation of seed stock, transplanting seeds from low salinity areas to higher salinity grow-out areas, avoiding over-crowding, harvest early if infections detected before mass mortalities occur, avoiding "shucking" shellfish over commercial beds, and fallow beds after harvest. Several mathematical models based on host, parasite and environmental factors have been devised to help growers predict and avoid outbreaks. There has also been some success in selectively breeding resistant hosts, including allochthonous species, triploid oysters, hybrid scallops, and first-generation scallop progeny, most of which grow faster but are not as fecund (although some say they taste better as they have higher glycogen contents due to gametogenesis arrest).

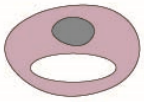
Perkinsus

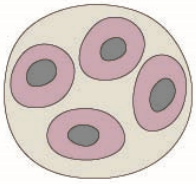


soft tissues
(pustular lesions,
emaciation,
mortalities
(die-back))



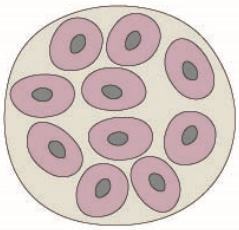
Hosts
(marine molluscs)

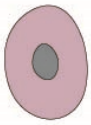




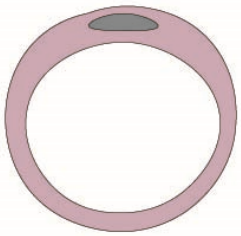
trophozoites
(2-20 µm)

schizonts
(15-100 µm)

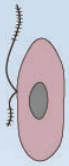




prezoosporangia
(30-80 µm)

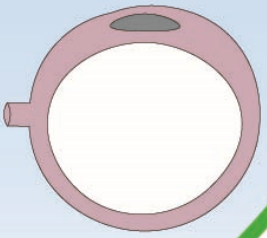


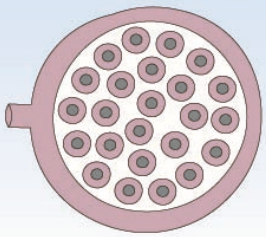
formed on death of host
and released into water



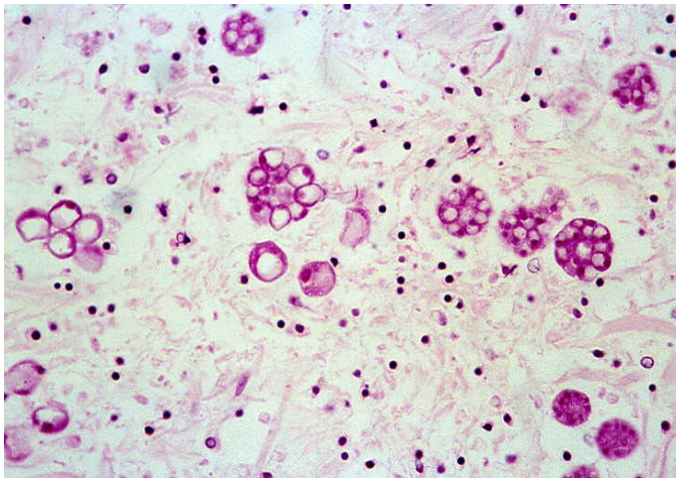
biflagellated
zoospores
(4-6 µm)

zoosporangia
(100-200 µm)

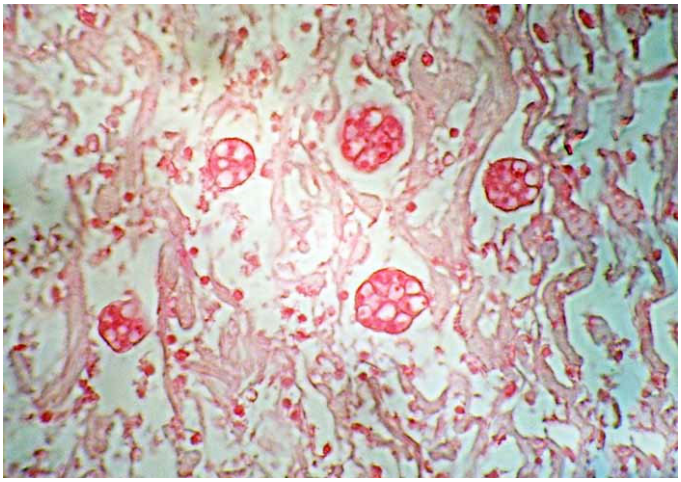




direct transmission via
free-swimming zoospores

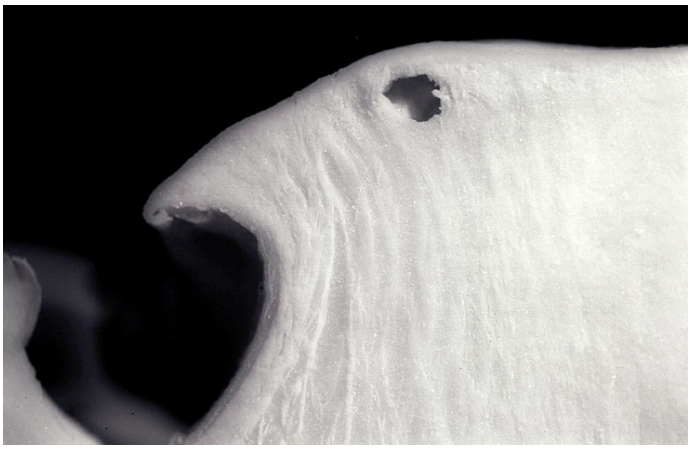


Perkinsus trophozoites in abalone muscle



Perkinsus trophozoites in abalone muscle

Perkinsus pustules in abalone muscle



Perkinsus lesion in abalone muscle