Mikrocytos
(protist: microcells)

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). Two major rhizarian groups are recognized: retarians (with reticulopodia) and cercozoans (with filopodia). An enigmatic cercozoan group is the Ascetospora which contains the spore-forming Haplosporidia and Paramyxea. *Mikrocytos mackini* is an unusual amitochondriate protistan previously thought to be related to the haplosporidia, but it does not form spores or multinucleate plasmodia. The only known stages are uninucleate microcells that do not contain haplosporosomes. Infections have been associated with pustule formation and mortalities (Denman Island disease) in commercial oyster beds in North America.

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	SAR (Stramenopiles + Alveolata + Rhizaria)
Group:	Rhizaria (various amoebae and flagellates)
Division:	Cercozoa (biflagellated and/or amoeboid, usually with filopodia, plus ascetospora)
Phylum:	Ascetospora (haplosporidian and paramyxean parasites forming unique spores/microcells)
Class:	Haplosporea (haplosporosomes present)
	incertae sedis
Genus:	*Mikrocytos* (parasitic in tissues of bivalves, spores not formed, no haplosporosomes)
Species:	various species cause mortalities in oysters

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). Two major rhizarian groups are recognized: retarians (with reticulopodia) and cercozoans (with filopodia). An enigmatic cercozoan group is the Ascetospora which contains the spore-forming Haplosporidia and Paramyxea that lack flagella and pseudopodia. Haplosporidia are usually characterised by the formation of unicellular spores (without polar capsules or polar filaments) that contain a single sporoplasm and several membrane-bound electron-dense organelles (known as haplosporosomes), while paramyxeans form unique multicellular (nested) spores. Most species occur as histozoic or coelozoic parasites of aquatic invertebrates (including molluscs, annelids, crustaceans, ascidians and helminths), and several species cause significant diseases and mortality in oysters around the world.

The identification and classification of haplosporidians has a complex and turbulent history. The complete developmental cycles of most species are not known, but various members of the genera *Haplosporidium*, *Minchinia* and *Urosporidium* were shown to form 2 developmental stages: multinucleate plasmodia which undergo modified schizogony to form sporonts that eventually differentiate into uninucleate spores. The spores were surrounded by a spore wall with an orifice (micropyle) and the enclosed endosporoplasm contained a complex membranous system known as a spherulosome (or spherule). Members of several other genera (*Bonamia* and *Mikrocytos*) which produced microcells (but not spores) were nonetheless recognized as haplosporidians on the basis of perceived structural and biological similarities. However, subsequent ultrastructural and molecular characterization studies have now removed *Mikrocytos* to a separate order Mikrocytida as they do not produce plasmodia, spores or haplosporosomes, while *Bonamia* was retained with the Haplosporida as they produced haplosporosomes.

Family	Genus	Biodiversity	Characters	Hosts
Order: Haplosporida (form unicellular spores/microcells with haplosporosomes)				
Haplosporidiidae (spores with operculum)	*Haplosporidium*	33	spores with external hinged lids, spores with filaments of spore wall material	oligochaetes, molluscs, crustacea, urochordates, echinoderms, polychaetes
	Minchinia	6	spores with external hinged lids, some spores with transient extensions of epispore cytoplasm	oysters, chitons, clams, tusk shells, shipworms
	Bonamia	4	microcells formed, one species forming spores with external hinged lids and filamentous projections of spore wall material	oysters
Urosporidiidae (spores without operculum)	*Urosporidium*	7	spores occluded with internal lingula (plug, flap, diaphragm)), spores with tapering extensions of epispore cytoplasm	hyperparasitic in helminths of molluscs and crabs
Order: **Mikrocytida** (form microcells without haplosporosomes)				
Mikrocytidae	*Mikrocytos*	5	form microcells, no spores, no plasmodia, no haplosporosomes	oysters, clams
	Paramikrocytos	1	form microcells, no spores, no plasmodia, no haplosporosomes	crabs

Mikrocytos spp. produce uninucleate microcells (2-4 µm) that appear as quiescent, vesicular or endosomal cells in connective tissue cells, myocytes and haemocytes of oysters. Infections by *M. mackini* have been associated with pustule formation and mortalities (Denman Island disease) in commercial oyster beds in North America. Several others *Mikrocytos* spp. and isolates have also been associated with lesions and mortalities in other oysters and clams. Novel isolates from crabs were found to produce multinucleate plasmodia (but not spores) and they were named *Paramikrocytos canceri*. [Note that the species *Mikrocytos roughleyi* associated with winter mortality in Sydney rock oysters has recently been transferred to the genus *Bonamia* on the basis of ultrastructural and molecular studies. However, the aetiology of winter mortality may be more complex than previously thought, as recent studies have indicated that some isolates of *B. roughleyi* may actually be mis-identified *B. exitiosa,* while other isolates may not be *Bonamia* or *Mikrocytos* at all, thus necessitating further study].

Parasite species	Hosts	Microcell size	Location [Clinical signs]	Distribution
Mikrocytos boweri	Bivalvia: ostreid (Olympia oyster, Pacific oyster)	2.0-3.8 µm	connective tissue cells	North America
Mikrocytos donaxi	Bivalvia: donacid (wedge clam)	1.8-3.2 x 1.1-2.4 µm	muscles, nerves, digestive gland, gut, gills, gonads [mortality]	Europe
Mikrocytos mackini	Bivalvia: ostreid (eastern oyster, European flat oyster, southern Olympia oyster, Pacific oyster, Kumamoto oyster)	2-4 µm	connective tissue cells, myocytes, haemocytes [abscess/pustule formation, mortalities, Denman Island disease]	North America
Mikrocytos mimicus	Bivalvia: ostreid (Pacific oyster)	2-5 µm	connective tissue cells, muscles, haemocytes	Europe
Mikrocytis mytilicoli	Hyperparasitic in Copepoda: mytilicolid (*Mytilicola intestinalis*) from gut of Bivalvia: mytilid (Mediterranean mussel)	1.3-3.5 µm	somatic organs	Europe
Mikrocytos veneroides	Bivalvia: donacid (wedge clam)	2.0-4.7 x 1.3-4.0 µm	muscles, digestive gland, gut, gills, gonads, neurons, haemocytes [mortality]	Europe
Mikrocytos sp.	Bivalvia: venerid (Manila clam)	2.4-3.8 µm	muscles, gills, siphon, visceral mass	Asia
*Paramikrocytos canceri**	Decapoda: cancrid (edible crab)	3 µm	antennal gland, bladder, gills [hypertrophy]	Europe

*Only species for which plasmodial stages have been detected. Molecular screening studies have also detected DNA sequences in other decapods (shore crabs), molluscs (mussels, nudibranch), and annelids (tubificids).

Parasite morphology: *Mikrocytos* spp. are unusual haplosporidian parasites that form unicellular microcells in host tissues, but other developmental stages such as plasmodia and spores have not been detected. The microcells are small spherical or ovoid stages measuring 2-5 µm in diameter. They are uninucleate with each containing an eccentric polymorphic nucleus ranging in size from 1.3 x 1.0 µm (occasionally binucleate forms may be found). The microcell cytoplasm contains round vesicles, Golgi bodies, both smooth and rough endoplasmic reticulum, but haplosporosomes and mitochondria are absent (note that some cells possess small membrane-bound organelles ~0.5 µm in diameter which are thought to be putative mitochondria-related organelles (MROs)). It is noteworthy that intracellular microcells are often surrounded by host cell mitochondria, suggesting that they may usurp host organelles for energy production. Many microscopic studies have described 2 morphological forms of microcells: small dense spheroidal forms predominating (with eccentric nuclei and dark granular cytoplasm); and some larger clear forms (with lighter granular cytoplasm). Several electron microscopic studies have also described 3 ultrastructural forms of microcells (especially for *M. mackini* and *M. mimicus*): namely quiescent, endosomal and vesicular cells. Quiescent cells (apparently corresponding to the small dense morpho-forms) are found predominantly in haemocytes and myocytes and are characterized by the presence of a nucleus with a granular nucleolus as well as a Golgi apparatus that is not budding. In contrast, endosomal cells have well-developed anastomosing endoplasmic reticulum arising from the nuclear membrane budding into the cytoplasm. Vesicular cells (corresponding to the larger clear morpho-forms) are usually found in vesicular cells and myocytes and typically have some dilation of the nuclear membrane forming cisternae as well as large vesicles scattered throughout the cytoplasm.

Site of infection: Microcells of *Mikrocytos* spp. have been detected in oysters and clams mainly in coastal waters around Europe and North America. The parasites may be located intracellularly in haemocytes, vesicular connective tissue cells and myocytes, or extracellularly in connective tissues or sinuses (presumably following host cell rupture). Infections have been recorded in the palps, gills, digestive gland, gut epithelia, mantle epithelia, heart and kidney. Most parasite species have only been detected in individual host species (oioxenous), but laboratory and field challenges have found that the species infecting oysters may occur in a small range of related host species (stenoxenous).

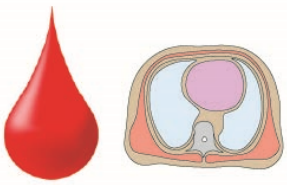
Pathogenesis: Infections by *M. mackini* may cause a disease in oysters known as Denman Island disease (or mikrocytosis). Parasites form microcells within host cells which ultimately rupture resulting in focal green lesions or abscesses in host tissues, mainly the mantle, body wall, labial palps and adductor muscles. Histologically, lesions are sometimes associated with intense inflammatory reactions particularly in connective tissues and parasites are often found adjacent to focal haemocytic infiltrates. Oysters may recover from mild infections but their market value is significantly reduced due to the presence of unsightly discoloured lesions. Heavy infections, however, have been associated with high levels of morbidity and mortality. Although juvenile oysters have been shown to be susceptible to infection, they usually do not show any signs of disease until they are older (2-3 years old). Epidemiological studies demonstrate a strong seasonal pattern to infections and disease apparently linked to water temperature (with peak disease occurring in cooler spring months). Infections by *M. donaxi* and *M. veneroides* have been associated with mortalities in wedge clams, with parasites detected throughout most host tissues.

Developmental cycle and mode of transmission: The complete life-cycles of *Mikrocytos* spp. are not known. Infections in oysters and clams involve up to 3 types of microcells, with quiescent, endosomal and vesicular cells possibly representing invasive, multiplicative and disseminating stages. There is no evidence of spore formation nor spore-like stages that would facilitate parasite survival outside of hosts. Infections appear to be transmitted directly between hosts via close contact (cohabitation), and experimental studies have successfully transmitted infections between oysters by the intramuscular inoculation of purified parasites. It has been noted that disease will only develop in oysters held in cooler waters <10°C for several months, and water temperatures >15°C effectively prevent disease development even though parasites persist within host tissues.

Differential diagnosis: Infections may be strongly suspected by gross signs of abscesses or green pustules on the palps and mantle, but these lesions are not pathognomonic. Diagnosis is generally made by the direct detection of parasites in host tissues, usually by microscopic examination of haemolymph smears, tissue/lesion imprints or histological sections of tissues. When stained with haematological stains (Wright's, Giemsa or equivalent Romanowsky stains such as Hemacolor and Diff-Quik), parasites are evident as small basophilic cells (2-4 µm) with eosinophilic nuclei. Transmission electron microscopy is required to confirm that *Mikrocytos* microcells do not possess haplosporosomes (whereas those of *Bonamia* do). *Mikrocytos* spp. also do not form unicellular spores (like typical haplosporidians such as *Haplosporidium* and *Minchinia*) nor multicellular nested spores (like the paramyxean genus *Marteilia*). Researchers have developed monoclonal antibodies against *M. mackini*, but immunodiagnostic tests have not been developed commercially. Molecular biological techniques have been used to detect infections, characterize species and determine phylogenetic relationships following the polymerase chain reaction (PCR) amplification of ribosomal RNA gene sequences (including 18S-ITS1-5.8S-ITS2-28S) and the incorporation of specific probes into restriction fragment length polymorphism (RFLP) and fluorochrome- and chromogenic-based *in situ* hybridization (ISH) assays.

Treatment and control: There is no known effective treatment for *Mikrocytos* infections in oysters or clams. Various preventive strategies have been utilized to limit disease spread amongst shellfish stocks, based on regular screening programmes with strict quarantine of translocated stocks, destroying infected stock with waste material not being returned to the water, timing plantings with respect to season and tide levels to avoid cooler waters, and harvesting stocks when they are <3 years old.

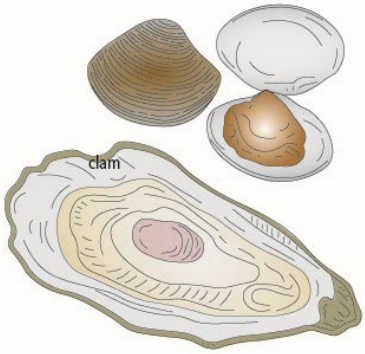
Mikrocytos



haemozoic/histozoic
(haemocytes, viscera)
(pustular lesions,
mortalities)

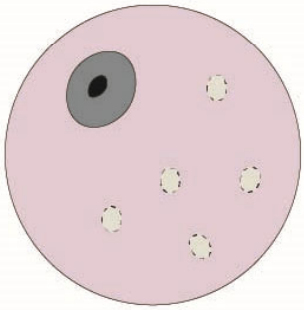
all species form micro-cells within hosts

parasites lack haplosporosomes
spore formation not observed
plasmodial stages not observed



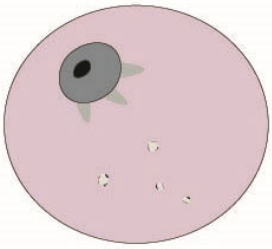
Invertebrate Hosts
(bivalves)

uninucleate
micro-cells
(2-5 µm)



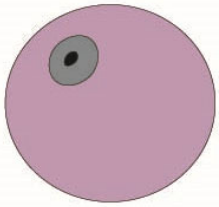
vesicular form
(large clear)

[invasive
stage?]



endosomal form
(plentiful endo-
plasmic reticulum)

[multiplicative
stage?]

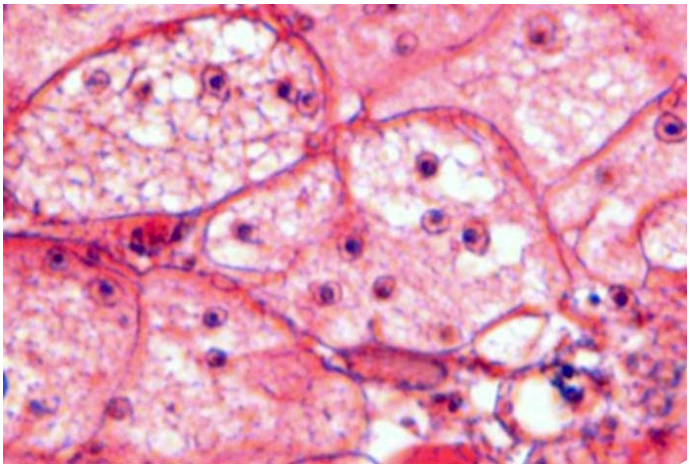


quiescent form
(small dense)

[disseminating
stage?]

?

life-cycles not known
transmission between hosts apparently occurs direct
via close contact (co-habitation)



Mikrocytos microcells in oyster tissues