Bonamia
(protist: haplosporidian)

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. Haplosporidia are characterised by the formation of unicellular spores (without polar capsules or polar filaments) that contain a single sporoplasm and several dense organelles (known as haplosporosomes). The complete developmental cycles of most species are not known but most have two developmental stages: multinucleate plasmodia and spores. *Bonamia* (and *Mikrocytos*) are unique in that they form microcells and most species do not form spores. Most are histozoic or coelozoic parasites of aquatic molluscs, annelids, crustaceans and helminths. *Bonamia* spp. infect the haemocytes of oysters and may cause wasting disease and mortality in cultured oysters.

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:	*Bonamia* (parasitic in tissues of bivalves, most species do not form spores)
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. Recent studies have since shown that one *Bonamia* species (*B. perspora*) does produce spores, and conversely, that a few *Haplosporidium* spp. do not actually produce spores as supposed. Ultrastructural studies conducted on spores demonstrated that the orifice was covered either by an external hinged operculum (adopted as a key characteristic for the family Haplosporidiidae containing the genera *Haplosporidium*, *Minchinia* and *Bonamia*) or occluded by an internal flap-like lingula (taken to characterize the monotypic family Urosporidiidae). Many spores also possessed elaborate external ornamentations, described variously as tails, filaments, folds, projections, extensions or wrappings. It was initially thought that their presence and structure could be used to differentiate taxa, but studies demonstrated considerable variation both between and within taxa. Most *Minchinia* spores lacked ornaments, but some isolates were found with epispore cytoplasmic extensions, albeit transiently as they disappeared during the spore maturation process. It was speculated that genera could be discerned by the origins (rather than the structure) of the ornamentations, being derived from either spore wall material (as found for many *Haplosporidium* spp. and one *Bonamia* sp.) or from epispore cytoplasmic extensions (observed for *Urosporidium* spp. and a few *Minchinia* isolates). However, molecular characterization studies did not reveal any strong correlations with morphotypic (phenetic) characters. Instead, phylogenetic analyses revealed a series of polyphyletic clades, including: a basal

clade of diverse *Haplosporidium* isolates; a more derived clade containing most *Urosporidium* isolates from helminths; 2 sister clades containing *Minchinia* and *Bonamia* isolates respectively; and a clade containing many *Haplosporidium* isolates from gastropods. [Note that various spore-forming organisms detected in cockroaches, copepods, rotifers, polychaetes and toads that were originally thought to be other haplosporidian genera (e.g. *Bertramia, Coelospora, Coelosporidium, Nephridiophaga*) are now considered to be fungi on the basis of molecular phylogenetic studies.]

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

Haplosporean infections have been detected throughout the world in aquatic invertebrates in coastal waters (freshwater, marine and brackish waters). While *Haplosporidium* and *Minchinia* spp. have been recorded from a wide range of hosts (e.g. molluscs, crustacea, oligochaetes, polychaetes), infections by *Bonamia* spp. have only been recorded in oysters (both flat and cupped oysters). Two species (*B. ostreae* and *B. exitiosa*) have been associated with mortalities in farmed and wild oysters in the Northern and Southern Hemispheres, with the parasites forming microcells (but not plasmodia or spores) within host haemocytes. Another species (*B. perspora*) has recently been found to form microcells, plasmodia and operculate spores in the tissues of crested oysters, thus being unique as the only *Bonamia* species exhibiting the basic haplosporean convention of extracellular infection and spore formation. On the basis of ultrastructural and molecular characterization studies, another microcell parasite (*Mikrocytos roughleyi*) is now considered to be *Bonamia roughleyi*, with parasites infecting haemocytes in farmed Sydney rock oysters apparently causing winter mortalities (Note that the aetiology of this disease 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).

***Bonamia* species**	**Hosts**	**Microcell size**	**Location [Clinical signs]**	**Distribution**
B. ostreae	Bivalvia: ostreoid (European flat oyster, Australian flat oyster, dredge oyster, Puelchean oyster, Olympia oyster, Pacific oyster, Portuguese oyster, Suminoe oyster)	2-3 µm	haemocytes [haemocyte lysis]	North America, Europe, New Zealand
B. exitiosa	Bivalvia: ostreoid (European flat oyster, Australian flat oyster, dredge oyster, Puelchean oyster, Olympia oyster, Suminoe oyster, crested oyster, eastern oyster, Sydney rock oyster)	2-7 µm	haemocytes [haemocyte lysis]	New Zealand, Australia, Americas, Europe
B. perspora	Bivalvia: ostreoid (crested oyster)	2.4-5.6 µm [spores 4.3-6.4 µm]	connective tissues, haemocytes [tissue congestion, infiltration]	North America
B. roughleyi (syn. *Mikrocytos roughleyi*)	Bivalvia: ostreoid (Australian flat oyster, Sydney rock oyster)	2-5 µm	haemocytes [haemocyte lysis, winter mortality]	Australia

Parasite morphology: *Bonamia* is an unusual haplosporidian representative as most species have developmental stages restricted to the formation of 'microcells' that do not produce typical haplosporidian spores. Microcells are typically uninucleate, small (2-7 µm in diameter), irregular in shape, and may occur singly or in groups of up to 10 per host cell. Two morphological forms are often evident: the most frequent being small dense forms (2-3 µm) with basophilic cytoplasm forming a pale halo around the nucleus; and larger clear forms (3-7 µm) with less dense cytoplasm. The former usually occurs extracellularly within host tissues, whereas the latter is found intracellularly mostly within host haemocytes and branchial epithelial cells. Both forms have a single nucleus which is eccentric in *B. ostreae* and centrally-located in *B. exitiosa* (imparting a fried-egg appearance). The microcells possess several mitochondria and they have Golgi bodies arising from the nuclear envelope and associated with a *trans*-Golgi network from which haplosporosomes form. It is thought that microcells may divide by binary fission as binucleate (diplokaryotic) cells are sometimes observed. There have also been rare reports of multinucleate stages measuring from 7-18 µm and containing around 5 nuclei. The uninucleate and multinucleate stages closely resemble the presporogonic stages of other haplosporidians. Recent studies on one species (*B. perspora*) have described 4 different morphological stages: microcells, plasmodia, sporonts and spores. The microcells occurred within connective tissues and measured 2-6 µm and had central to slightly eccentric nuclei. The plasmodia varied in size from small (<8 µm) to large (up to 16 µm) and they contained <5 nuclei per electron microscope (EM) section. The sporonts (also called sporocysts) measured up to 26 µm and possessed 4-14 sporoblasts. The unicellular spores measured 4.3-6.4 x 2.8-4.6 µm and contained an orifice with a hinged operculum and unique spore wall ornamentation comprising numerous short thin flat ribbons projecting from the spore wall and terminating in a capped structure. Spore formation has not been observed for other *Bonamia* spp.

Site of infection: *Bonamia* spp. infect oysters, forming microcells in haemocytes which typically accumulate in the vascular sinuses around the digestive gland, intestine and stomach. In heavy infections, microcells may be found throughout most host tissues, especially connective tissues in the mantle and gills. Following haemocyte lysis, parasites may be observed free (extracellular) amongst host tissues. *Bonamia* spp. were initially thought to exhibit high zoogeographic specificity, with *B. ostreae* infecting flat oysters in the Northern Hemisphere and *B. exitosa* infecting flat and rock oysters in the Southern Hemisphere. However, field and experimental studies have now found infections to be more cosmopolitan than previously thought with most species being able to infect a small range of sympatric related hosts.

Pathogenesis: Most infections are asymptomatic or subclinical with no or few gross or clinical signs present. Heavy infections, however, may cause disease (known as bonamiosis) resulting in high mortalities (up to 60%) in certain oyster beds. Concurrent infections with more than one *Bonamia* spp. may also occur. Parasites infect host haemocytes forming microcells within cytoplasmic vacuoles where they are able to survive and multiply. Even though haemocytes are phagocytic immune cells, the parasites appear to be able to down-regulate reactive oxygen species (ROS) production thus partly disabling their destructive metabolic capability. In susceptible oysters, infections often become systemic when infected haemocytes gather in vascular sinuses where they ultimately lyse releasing microcells into necrotic host tissues, often coincident with host death. In oysters thought to be partly resistant, infections are usually restricted to epithelia demarked by conspicuous haemocyte responses. Clinical signs of disease are inconspicuous with the first indication of infection being increased mortality with dead gaping oysters observed at the farm, tank or pond level. Detailed studies have shown gross pathological signs to include thin watery flesh, pale-yellow atrophied digestive glands, mantle recession with weakened shell closure leading to slight gaping accentuated by deformities to the gill margins and algae-covered shell lips. Infected oysters show stunted growth (including reduced shell growth), poor condition, emaciation and death. Infections are often accompanied by dense focal haemocyte infiltrates into the connective tissues of the gills and mantle and around the gut, sometimes resulting in tissue lesions with occasional perforated ulceration of the gills and mantle. The heart often appears enlarged and darkened by the presence of numerous granulocytes. Experimental studies have shown that lethal infections may develop within 3-6 months, but often require 2 years for disease development. Infections may be detected in spat and larvae, but older or larger oysters have the heaviest infections. Mortalities may occur year-round, but are more prevalent in spring and summer. Epidemiological studies indicate that mortality rates may be influenced by genetic differences in host susceptibility, environmental conditions (particularly those leading to host stress) as well as by differences in parasite virulence (both inter- and intra-specific). Mortalities have been correlated with higher water temperatures (heavier infections in spring when waters are warming) and oysters seemed to be more susceptible to infection after seasons with lower food availability, lower salinity levels or following stressful situations such as dredging. Infections by *B. roughleyi* may cause winter mortality in Sydney rock oysters with disease characterized by focal abscess-like lesions in the gills, connective and gonadal tissues and alimentary tract, especially in high salinity waters (30-35 parts per thousand).

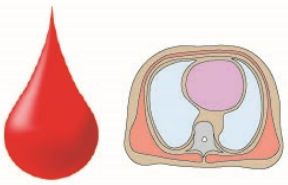
Developmental cycle and mode of transmission: The life-cycles of most *Bonamia* spp. are not known as only microcell formation has been observed in all but one species. Nonetheless, it has been mooted that 2 sequential stages may be involved in parasite development: firstly, uninucleate amoeboid cells (large clear morphological forms) occurring within host haemocytes (possibly representing multiplicative stages); followed by free microcells (small dense forms) being released into host tissues (possibly transmissive stages). There is some evidence that intracellular stages may multiply by fission, as some diplokaryotic forms and a few multinucleate forms (with up to 5 nuclei) have occasionally been observed. However, the occurrence of large multinucleate plasmodia (20-60 nuclei) and the formation of spores typically found for other haplosporidians (e.g. *Haplosporidium*, *Minchinia*)

have not been observed for *Bonamia*, except for one species (*B. perspora*) described from crested oysters in North America. In this species, microcells formed comparatively small multinucleate plasmodia (with <5 nuclei per EM section) which divided to form sporonts/sporocysts with 4-14 sporoblasts that matured to operculate ornamented spores. Despite the formation of spores by one *Bonamia* species, the transmission of most infections appears to be via direct contact. Experimental studies have successfully transmitted infections between oysters held in the same aquaria (cohabitation) and by the *in vivo* inoculation of infected haemocytes or purified parasite suspensions. Transmission apparently occurs year-round, but is greatest during the summer. Epidemiological studies have indicated that human activities may facilitate transmission, particularly when oysters are translocated by farmers, growers or scientists, and possibly via biofouling of ship hulls or ballast waters.

Differential diagnosis: Infections often only become apparent when oysters die and postmortem examination reveals tissue discolouration and sometimes ulcerative lesions. Few clinical signs develop prior to death other than poor condition and sometimes gaping exacerbated by mantle recession. However, similar lesions and signs may be found in oysters infected with other haplosporidian parasites, especially *Mikrocytos* spp. Definitive diagnosis requires the direct detection of parasites in host tissues, usually by microscopy of hemolymph smears, tissue imprints (including ventricular heart imprints) or histological sections of tissues. Parasites are usually found in haemocytes, but may also occur in or near epithelia, and in vesicular connective tissues. They are evident as small (2-5 μm) basophilic spherical-ovoid uninucleate microcells. Transmission electron microscopy is required to detect haplosporosome organelles (absent in *Mikrocytos* microcells). Other haplosporidian parasites (such as *Haplosporidium* and *Minchinia*) typically form multinucleate plasmodia and operculate spores within host tissues (similar stages have only been observed for one *Bonamia* species, *B. perspora* in crested oysters). In comparison, paramyxean parasites of oysters (including *Marteilia* spp.) form multicellular nested spores (4-10 μm) usually in digestive tubules. Several studies have developed monoclonal antibodies against *B. ostreae* and incorporated them into immunological assays (fluorescent-antibody and enzyme-linked assays) to detect parasites in oyster tissues and extracts. More recently, molecular biological studies have been used to diagnose infections, characterize parasites and infer phylogenetic relationships following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (especially ribosomal RNA) and the incorporation of specific probes into restriction fragment length polymorphism (RFLP), realtime PCR, and fluorochrome- and chromogenic-based *in situ* hybridization (ISH) assays.

Treatment and control: There is no effective treatment known for bonamiasis in oysters. Although disease outbreaks may occur rapidly (developing over several weeks to months), various preventive measures have been used to great effect to mitigate the occurrence and spread of infections. *Bonamia* spp. are generally considered to be notifiable pathogens and strict quarantine regulations have been applied to limit translocations of oysters or spat from known endemic areas. Zoning systems have been implemented around foci, with disease-free areas regarded to be protected areas. Regular screening programmes have been recommended and any infected stocks should be destroyed with waste going into landfill rather than being returned to the water. Experimental and field observations have also led to several management strategies being adopted to help control infections, including reducing stocking densities, using suspension cultures, overwintering smaller oysters on up-river leases where lower salinities and higher racks protect them, on-growing in deeper waters, harvesting oysters at a young age (15-18 months), culling and fallowing beds where infection prevalence exceeds 10%, and thoroughly cleansing sites and fishing gear. Studies have also indicated that some oyster species (e.g Pacific oysters) or strains (Rossmore flat oysters) may be more resistant to infection, but inbreeding and population bottlenecks have proven problematic.

Bonamia

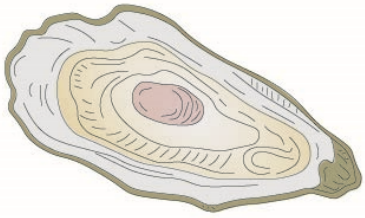


haemozoic/histozoic/coelozoic
(haemocytes, soft tissues)
(lesions, wasting disease,
mortalities)

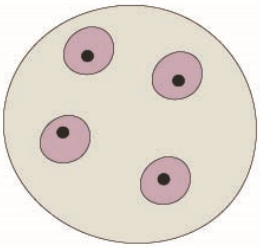
all species form micro-cells within hosts

one species forms unicellular spores
with haplosporosomes and a sporoplasm

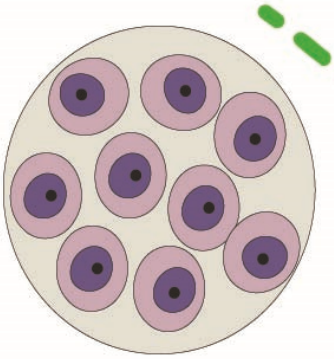
apparently multiplies by modified schizogony
within multinucleate plasmodia



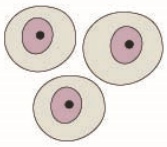
Invertebrate Hosts
(oysters, flat
and cupped)



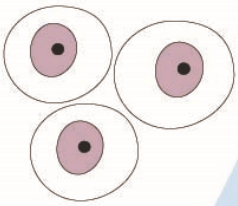
multinucleate
plasmodium
(7-18 µm)



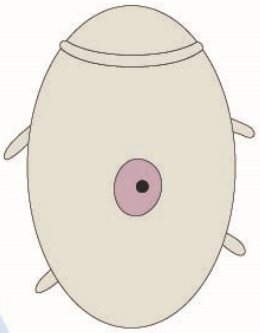
sporont (~ 20 µm)
with uninucleate
sporoblasts



extracellular
(small dense)
(2-3 µm)



intracellular
(large clear)
(3-7 µm)



hinged lid

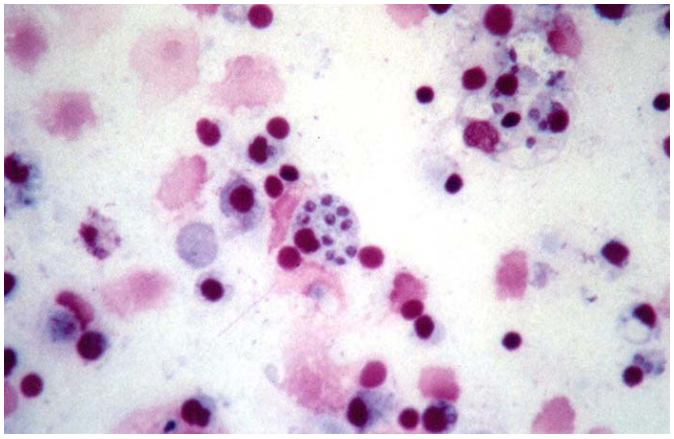
filamentous
projections

spores (4-6 µm)

uninucleate
micro-cells

?

most life-cycles not known
but transmission between hosts is often direct
via close contact (co-habitation)



Bonamia microcells in oyster haemolymph