Marteilia
(protist: paramyxean)

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. The Paramyxea exhibit both protistan and metazoan characters. They form unique multicellular spores where the daughter cells remain enclosed within the parent cells (up to four cells may be enclosed within each other). All species are parasitic in marine invertebrates (polychaetes, molluscs and crustaceans) and different taxa are distinguished by the number of spores and sporal cells within sporonts. Development begins with the enlargement of small amoeboid primary (stem) cells and the internal cleavage of uninucleate secondary cells; sporulation begins with internal cleavage of a third cell (spore primordium) within each secondary cell (sporont). Several species cause mortalities (QX and Abers disease) in 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)
Class:	Paramyxea (form unique multicellular spores with cells enclosed within each other)
Order:	Marteilida (internal cleavage of secondary cells then sporonts)
Family:	Marteiliidae (sporonts contain 2-4 tricellular spores)
Genus:	*Marteilia* (parasitic in tissues of oysters)
Species:	various species cause QX and Abers disease 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 (producing uninucleate spores) and Paramyxea (producing curious multicellular 'nested' spores).

The Paramyxea exhibit a curious combination of both protistan (unicellular) and metazoan (multicellular) characters in that they divide by endogeny forming unique multicellular nested spores where the daughter cells remain enclosed within the parent cells (up to four cells may be enclosed within each other). Most paramyxean developmental stages contain haplosporosomes so they have generally been regarded as a sister taxon to the Haplosporida, a placement confirmed by molecular characterization studies. Paramyxeans are endoparasitic within the tissues of marine invertebrates, including molluscs, crustaceans and polychaetes. Their developmental cycles involve the invasion and enlargement of small amoeboid primary (stem) cells which undergo internal cleavage (sporogony) to form uninucleate secondary cells (sporonts) which subsequently undergo internal cleavage (sporulation) to form tertiary cells (spore primordia), each stage nested within its predecessor. Different taxa have been distinguished primarily by the numbers and types of sporonts and spores formed, but it has proven difficult to provide consensus definitions for several genera because the cognate species vary considerably in their development. In the case of *Paramyxa* and *Paramyxoides* spp., it is relatively straightforward as their primary cells produce 2-4 secondary cells, each of which produces 4 tetracellular spores. However, species belonging to the remaining genera (*Marteilia, Martioides, Paramarteilia* and *Eumarteilia*) have been shown to produce variable numbers of secondary cells (1-16) producing variable numbers of spores (1-14) which may be bicellular or tricellular. While molecular characterization studies have helped resolve phylogenetic relationships between genera and species, they have not strongly supported any conspicuous morphotypic species groups or zoogeographic assemblages.

Family	Genus	Biodiversity	Characters	Hosts
Paramyxida (form unique multicellular spores)				
Marteiliidae	*Marteilia*	9	primary cells produce variable number (1-16) of secondary cells, each producing variable number (2-14) tricellular spores	oysters, mussels, cockles, scallops
	Marteilioides	2	primary cells produce variable number (2-12) of secondary cells, each producing one tricellular (or bicellular) spore	oysters
	Paramarteilia	2	primary cells produce variable number (2-3) of secondary cells, each producing a variable number (2-4) of bicellular spores	oysters, crabs, amphipods
	Eomarteilia	1	primary cells produce 8 secondary cells, each producing 4 tricellular spores	clams
Paramyxidae	*Paramyxa* (incl. *Paramyxoides*)	2	primary cell produces 2-4 secondary cells, each producing 4 tetracellular spores	polychaetes

The multiplication of paramyxean parasites primarily in the digestive glands of their hosts may interfere significantly with digestive processes leading to emaciation and seasonal mass mortalities, resulting in serious economic losses for both wild and farmed fisheries. *M. refringens* (O-type) causes Aber disease in flat oysters in Europe, America and Australia, *M. pararefringens* (M-type, previously *M. maurini*) causes mortalities in northern Europe, and *M. sydneyi* causes QX (= Queensland unknown) disease in Sydney rock oysters in Australia. National and international agencies have imposed quarantine regulations on stock translocations and growers have adopted a range of management procedures to mitigate outbreaks.

Parasite species	Hosts	No. secondary cells	No. spores	Spore structure	Location [Clinical signs]	Distribution
Genus ***Marteilia***						
Marteilia christenseni	Bivalvia: semelid (peppery furrow shell)	8	4	?	digestive gland	France
Marteilia cochillia	Bivalvia: cardiid (common cockle)	4-8	6	?	digestive gland [mortalities]	Spain
Marteilia cocosarum	Bivalvia: cardiid (common cockle)	1-3	?	?	digestive gland	United Kingdom
Marteilia lengehi	Bivalvia: ostreid (hooded oyster)	8	up to 14	?	digestive gland	Persian Gulf, Australia
Marteilia refringens (= O-type)	Bivalvia: ostreid (European flat oyster, Pacific oyster, dredge oyster, Australian flat oyster, Puelchean oyster, crested oyster), solenid (grooved razor shell), cardiid (common cockle), venerid (striped venus clam), mytilid (blue mussel, Mediterranean mussel)	8	4	tricellular	digestive gland [discoloration, emaciation, mortalities, Aber disease, digestive gland disease]	Europe, Americas, Australia
Marteilia octospora	Bivalvia: solenid (grooved razor shell)	4	8	?	digestive gland	Spain
Marteilia pararefringens (formerly M-type *M. refringens*, syn. *M. maurini*)	Bivalvia: mytilid (blue mussel, Mediterranean mussel, axehead mussel), solenis (grooved razor shell)	8	4	tricellular	digestive gland [mortalities]	northern Europe, Australia
Marteilia sydneyi	Bivalvia: ostreid (Sydney rock oyster, blacklip oyster); Polychaeta: nephtyid (*Nephtys australiensis*)	8-16	2-3	tricellular	digestive gland [mortalities, QX disease]	Australia
Marteilia tapetis	Bivalvia: venerid (Manila clam)	4	2-4	tricellular	digestive gland	Asia
Marteilia sp.	Bivalvia: pectinid (Atlantic calico scallop)	8	3-4	?	digestive gland [mortality]	North America

Genus ***Eomarteilia***						
Eomarteilia granula (syn. *Marteilia*)	Bivalvia: venerid (Manila clam)	8	4	tricellular	digestive gland [mortalities]	Asia
Genus ***Marteilioides***						
Marteiliodies branchialis	Bivalvia: osterid (Sydney rock oyster)	2-6 (sometimes 12)	1	bicellular	digestive gland, connective tissue cells, haemocytes [mortalities]	Australia
Marteilioides chungmuensis	Bivalvia: ostreid (Pacific oyster, Iwagaki oyster, black-lip oyster)	2 (sometimes 3)	1	tricellular	ovary [tumour-like nodules]	Australasia
Genus ***Paramarteilia***						
Paramarteilia canceri	Decapoda: cancrid (edible crab, velvet crab)	2-3	2-4	bicellular	systemic [shrunken organs]	Europe
Paramarteilia orchestiae	Amphipoda: talitrid (*Orchestia gammarellus, aestuarensis*), gammarid (*Echinogammarus marinus*)	2-3	2	bicellular	testis [males become female or intersex]	Europe
Genus ***Paramyxa***						
Paramyxa paradoxa	Polychaeta: poecilochaetid (*Poecilochaetus serpens*)	2-4	4	tetracellular	gut	Europe
Paramyxa (*Paramyxoides*) *nephtys*	Polychaeta: nephtyid (*Nephtys caeca*)	2-4	4	tetracellular	gut	Europe

Parasite morphology: Paramyxean parasites are characterized by the formation of unique multicellular spores where the daughter cells remain enclosed within the parent cells (up to four cells may be enclosed within each other, somewhat like nested Russian or babushka dolls). The developmental process varies for different taxa but involves the sequential formation of at least 4-6 cell types. Primary (stem) cells invade host epithelia and multiple by binary fission and internal cleavage producing more primary cells which disseminate throughout host tissues, some becoming nurse cells in the digestive gland where they produce spores. During sporulation, primary cells (known variously as C1 cells or sporangiosori) bud endogenously to form secondary cells (C2 cells, sporont primordia, sporonts or sporangia), in which tertiary cells (C3 cells, spore cells, spore wall cells, spore primordia or intermediate cells) bud to form the outer spore wall in which further cells bud and mature as nested spores (C4-C6). Morphological details of these small developmental stages are often difficult to determine by light microscopy so recourse is made to electron microscopy to determine their ultrastructural characteristics. The primary stem cells are amoeboid but often ovoid ranging in size from 4-16 µm. They are uninucleate and contain smooth endoplasmic reticulum, ribosomes, and unique haplosporosomes (membrane-bound organelles with a glycoprotein core). The cells sometimes develop external dendritic membranous extensions and internal multivesicular inclusion bodies. Primary cells also contain a secondary uninucleate daughter cell within a cytoplasmic vacuole. The mother cell become enlarged as the daughter cell divides by binary fission to produce 4 new daughter cells, each of which divides by internal cleavage to also contain a uninucleate cell. These new daughter cells are released and disseminate into host tissues to become new primary cells. In some species, daughter cells lodging beneath the epithelium of digestive gland tubules have been reported to form nurse cells (notably for *M. sydneyi*, but not for *M. chungmuensis* which develops in the ovary). Sporogony begins when primary cells (C1) bud endogenously to form 1-16 (generally 4 or 8) secondary cells (C2). Secondary cells are usually ovoid and grow from 5 µm up to 30 µm in diameter. They contain ribosomes, vesicles, centrioles, cytoplasmic refringent granules but consistently lack haplosporosomes (this also occurs for most haplosporidians as haplosporosome-like bodies disappear in early vegetative stages but reform in spores). Secondary cells contain tertiary cells (C3) which bud endogenously to form the outer spore wall. Tertiary cells contain ribosomes, smooth endoplasmic reticulum, vesicles, and most species have re-acquired haplosporosomes. The cells form the outer layer of the spore which is sometimes underlain by a cytoskeleton of microtubules. The spore wall varies in thickness depending on the species, and sometimes displays striated projections (often attributed to shrinkage artefacts) although *M. sydneyi* produces a thick layer of concentric membranes. Within the spore wall, further cells bud endogenously (internal cleavage) to form spore precursors (C4 cells). These cells are spherical-ellipsoidal in shape and range in size from 5-20 µm in length. They are rich in ribosomes and smooth endoplasmic reticulum, possess haplosporosomes and mitochondria, and some have numerous flattened vermiform vesicles. They divide internally (endosporulation) to form 1-8 spores (C5-C6) which are ovoid, measure from 4-10 µm and often contain spherical refringent inclusion bodies. The spores of most marteiliid species are tricellular as they contain 3 nested (outer, intermediate and inner) cells; i.e., 3 uninucleate sporoplasms of graded sizes with each smaller sporoplasm being enclosed within the cytoplasm of the next largest. In comparison, *Paramyxa* spp. form tetracellular spores and some *Paramarteilia* spp. form bicellular spores.

Site of infection: Paramyxeans are endoparasitic in aquatic invertebrates, including bivalves (oysters, mussels, clams, cockles, scallops, razor shells), decapods (crabs), amphipods and polychaetes. They invade and multiply in epithelial tissues (gills, gut) and then disseminate throughout internal tissues typically undergoing sporulation in the digestive gland (an exception is *Marteilioides chungmuensis* which develops in the gonads). Most parasite species are specific for particular hosts (oioxenous) but experimental and field studies have shown some to be able to infect closely-related hosts (stenoxenous) or rarely sympatric unrelated hosts (euryxenous). The geographic distributions of most species were initially confined to local confined regions, but subsequent studies have found some to be more widely distributed, particularly throughout oyster growing regions.

Pathogenesis: Infections vary markedly in their pathogenicity, even when parasites may be present in hosts for extended periods (years). Light infections may remain asymptomatic in some hosts, but may cause clinical signs in others. Conversely, heavy infections may cause mortalities in some hosts but not others. Such variation is thought to arise from differences in host susceptibility (e.g. innate genetic factors, immunocompetence, physiological responses to stress) and parasite virulence (invasion, reproduction, and toxicity). Infections by *M. refringens* (O-type) progress from benign to lethal in flat oysters, with rampant parasite multiplication and sporulation leading to destruction of much of the epithelium of the digestive gland resulting in host starvation and death (condition known as Aber disease, or marteiliosis). The gross signs of disease include discolouration (pale brown), inflammation and necrosis of the digestive gland, discolouration (pale yellow) of visceral tissues which appear shrunken and watery/slimy, poor condition index with significant glycogen loss (progressive emaciation), cessation of growth (including the shell), and recession of the mantle with moribund oysters appearing to gape. Infections by *M. pararefringens* (formerly M-type *M. refringens*, or *M. maurini*) also inhibit gonad regeneration after the first spawning in mussels, thus leading to population collapses. Infections by *M (Marteiliodies) chungmuensis* are restricted to the ova of oysters, where they may cause unsightly nodule formation, watery flesh from depleted glycogen reserves, and reduced reproductivity (infected oysters spawning later than normal, and infected oocytes being rendered sterile). Infections in cockles have involved the digestive gland, stomach, gills and labial palps, cumulatively resulting in poor uptake and absorption of organic matter with subsequent loss of condition and mortality. Infections by *M. sydneyi* cause seasonal mortalities (called QX disease) in Sydney rock oysters, with parasite sporulation resulting in degeneration of the digestive gland, visceral tissues becoming colourless and translucent, resorption of the gonads, poor body condition and death (sometimes within 6 weeks). The development of most paramyxean parasites appears to be directly related to water temperature (especially > 17°C), with disease becoming most severe around the end of summer coincident with parasite sporulation. Epidemiological studies have also suggested an association with sudden changes in salinity, but this may reflect host stress responses to rapid environmental changes rather than parasites succumbing to high or low salinity levels.

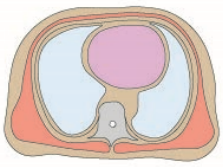
Developmental cycle and mode of transmission: The complete life-cycles of paramyxeans are not known but their development within molluscan hosts has been deduced from detailed studies on several species (notably M. *refringens, M. sydneyi* and *M.* (*Marteilioides*) *chungmuensis*). Infection begins when small amoeboid primary (stem) cells invade host epithelia (gills, palps, gut) and multiply by binary fission and internal cleavage to produce more uninucleate primary cells, each containing a secondary uninucleate daughter cell within a cytoplasmic vacuole. Following replication in epithelia, new primary cells are released into the surrounding connective tissues and haemolymph where they disseminate into host tissues, especially into digestive gland tubules (where some species (e.g. *M. sydneyi*) form nurse cells in the subepithelia). Stages in the digestive gland undergo sporogony (sporont formation) to produce prespores that undergo maturation (sporulation) to form multicellular spores. This process may vary for different species, particularly with regard to the numbers of sporonts and spores formed and their structural composition (remember that these are nested spores where cells occur within cells within cells). During sporulation, primary cells (C1) bud endogenously (unique process of internal cleavage, also known as endosporulation) to form 1-16 secondary cells (C2, sporangial primordia which mature to sporonts), in which 2-4 tertiary cells (C3, spore primordia) bud to form the outer spore wall within which further cells (C4-C6) bud by consecutive internal cleavage forming 1-8 nested spores (most tricellular and a few bicellular in marteiliid species, but tetracellular in paramyxiid species). When mature, each nested spore is surrounded by a continuous spore wall without an operculum, orifice or pore. Spores are released from the digestive tract with host faeces either free or enclosed within propagules (sporonts) singly or in clusters depending on the species (or released from the genital tract in ova in the case of *M. chungmuensis*). The transmission of infections was initially thought to be direct, but attempts to directly infect oysters by feeding or injecting infected homogenized digestive gland, by cohabitation with infected stock, or by introduction of oysters into the field after a disease event, have consistently failed to produce infections. The production of spores and their release into the water column could support either direct or indirect transmission, but the fate of free spores is unknown (although some experiments suggest they are not long-lived). Molecular screening studies have detected parasite DNA in other planktonic and benthic invertebrates (copepods, polychaetes, appendicularians, echinoderms, crustacean larvae), but it is not known whether this was the result of filter-feeding activity or actual infection (with these hosts acting as obligate, facultative, opportunistic or paratenic hosts). Further studies are required to determine the complete life-cycles of paramyxean parasites.

Differential diagnosis: Infections are generally not evident ante-mortem as they elicit few overt clinical signs in bivalves (gape and poor condition often not obvious). Infections may be suspected at post-mortem by the occurrence of watery flesh and discoloured organs (particularly the digestive gland) in moribund or dead animals, but these findings are not pathognomic for marteiliosis. Definitive diagnosis is made by the microscopic detection of parasites in host tissues, usually in impression smears of the digestive gland or in histological sections of the digestive gland, gills or palps. Differential diagnosis is made by careful examination of the

parasite developmental stages, as haplosporidian parasites infect similar tissues and may be of similar sizes. *Marteilia* spp. form multicellular nested spores ranging in size from 4-10 µm usually in digestive tubules. The spores contain haplosporosomes but electron microscopic examination is required to detect them. In contrast, haplosporidian parasites form unicellular spores or microcells ranging in size from 3-12 µm. Both *Haplosporidium* and *Minchinia* spp. form operculate spores throughout host tissues (the former bearing unique spore wall ornamentations, absent in the latter). Both *Bonamia* and *Mikrocytos* spp. form microcells within host cells (haemocytes and connective tissue cells respectively) and *Mikrocytos* spp. lack haplosporosomes. Attempts made to develop specific histological or immunological stains for these different parasites have lacked specificity and sensitivity. For example, polyclonal antibodies raised against *M. sydneyi* sporulating stages were incorporated into fluorescent-antibody assays but they failed to detect presporulation stages in connective tissues of recently infected oysters. Better success was achieved using parasite DNA markers in fluorescent *in situ* hybridization (FISH) assays to track infections in host tissues. Molecular biological studies have been used to characterize parasite species and infer phylogenetic relationships following the polymerase chain reaction (PCR) amplification of ribosomal DNA genes (with various developed for 18S, internal transcribed spacer 1 (ITS-1), 5.8S, ITS-2, 28S and intergenomic spacer (IGS) regions).

Treatment and control: There are no known treatments for *Marteilia* infections in bivalves. Nonetheless, a range of preventive measures have been employed to limit the occurrence and spread of infections in cultured stocks. National and international authorities have introduced strict quarantine protocols prohibiting the transport of oysters or spat within and between endemic areas, with regular monitoring and health surveillance mandated. Growers have adopted several farm management procedures designed to minimize risk, including not planting oysters in endemic areas during summer, harvesting large oysters prior to onset of peak transmission periods, and even holding oysters in high salinity waters where they grow more slowly but remain free of infection. To avoid QX disease, oyster growers have taken to emptying estuarine leases in late spring and restocking in early autumn. Field studies have also indicated that some oyster species appear to be more resistant to infection (e.g. Pacific oysters are more resistant to *M. refringens*) so some stock replacements have occurred. Some success has also been reported in selectively breeding Sydney rock oysters for QX resistance, with survivors having higher numbers of granulocytes in their haemolymph which appear to confer enhanced abilities to kill parasites.

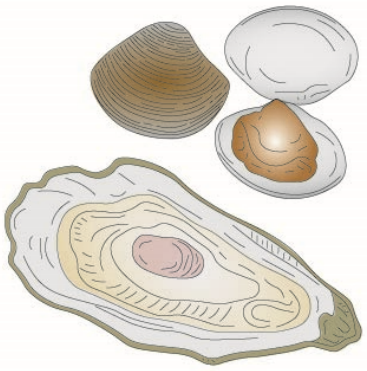
Marteilia



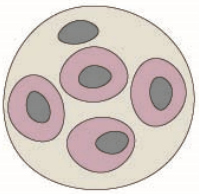
viscera, esp. digestive gland
(emaciation, mortalities)
(QX disease,
Abers disease)

form unique multicellular spores
where daughter cells remain
enclosed within parent cells

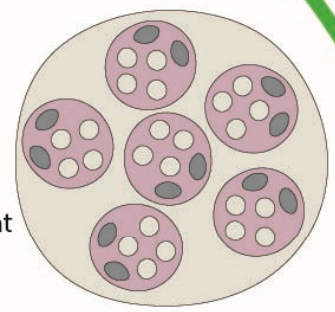
all species parasitic in marine invertebrates



Invertebrate Hosts
(bivalves)



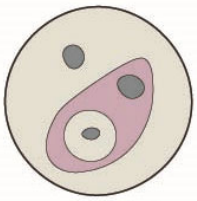
secondary cells
(sporangia)
(5-30 µm)



sporont
(refringent
bodies)

tertiary cells
(spore primordia)
(5-20 µm)

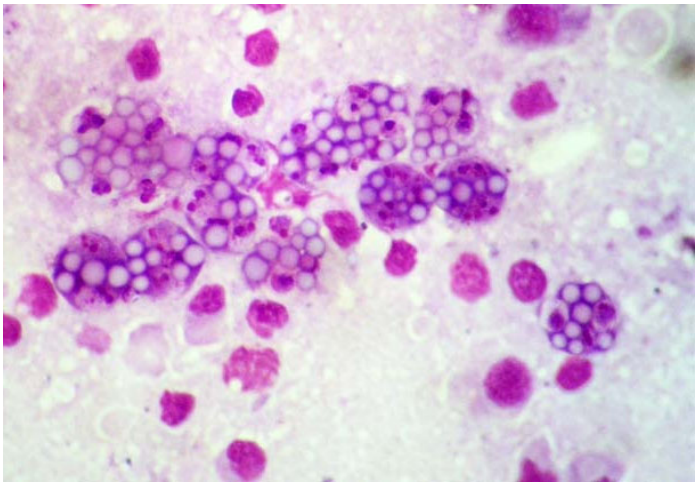
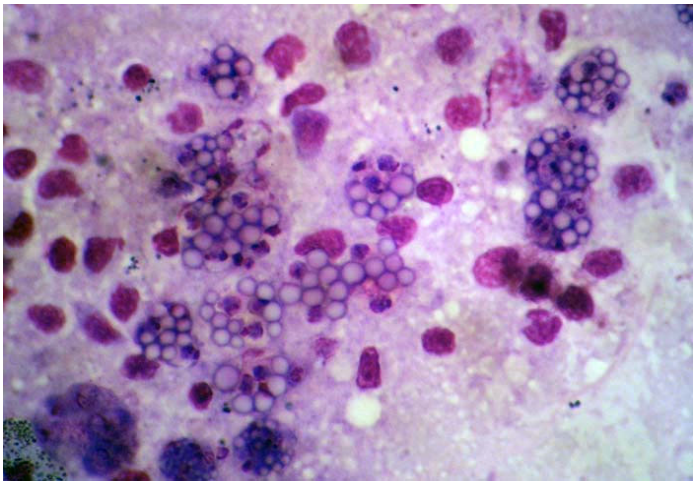
primary cells
(sporangiosori)
(4-16 µm)



multicellular
(tricellular)
spore
(4-10 µm)

?

despite spore formation and host co-habitation,
actual mode of tranbsmission between hosts unknown

	
Marteilia sporangia in oyster digestive gland	*Marteilia* sporangia in oyster digestive gland