MICROSPORA IN ARTHROPODS (insects, crustacea)

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Overview

Microsporidia are obligate intracellular parasites which lack mitochondria and form small unicellular spores. They were long considered to be a primitive basal group of Protista, but molecular phylogenetic studies have revealed many similarities in biochemical pathways and structural components to fungi, where they are now classified. Numerous species have been described in invertebrates (especially insects) and lower (rarely higher) vertebrates. The parasites proliferate in host tissues by merogony (asexual division) followed by sporogony (often involving plasmotomy prior to sporoblastogenesis). Developmental stages may be monokaryotic (single nucleus) or diplokaryotic (paired nuclei) and sporonts may be surrounded by a membranous sporophorous vesicle (pansporoblast) or lie free in the host cell cytoplasm. All spores contain a unique coiled polar tube which can be extruded to inject the infective sporoplasm into host cells. Infections may be disseminated throughout host tissues or they may cause focal lesions and inflammation involving cysts, granulomas or tumour-like xenomas (enlarged host cells). Various species are found in insects (some causing dysentery in honey bees), crustaceans (some denaturing muscles), fish (some forming lesions or deformities) and even humans (some causing diarrhoea, myositis, encephalitis or corneal lesions).

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Kingdom:	Fungi (with chitinous walls, includes microsporidia)
Division:	Microsporidia (form unicellular spores, with coiled polar tubes, amitochondriate, all parasitic)
Class:	Microsporea (polar filament well-formed, oval spores)
Order:	Microsporida (polaroplast present)
Suborder:	Pansporoblastina (sporophorous vesicle present)
Family:	Thelohaniidae (meronts diplokaryotic, spores monokaryotic, 8 spores formed in each vesicle)
Genus:	*Thelohania* (parasitic in tissues of crustaceans/insects)
Species:	various species cause cotton-tail in crayfish
Suborder:	Apansporoblastina (sporophorous vesicle absent)
Family:	Nosematidae (all stages diplokaryotic)
Genus:	*Nosema* (parasitic in gut/tissues of invertebrates)
Species:	*N. apis* (causes bee dysentery in honeybees)

Parasite biodiversity and host range: Microsporidia possess a remarkable apomorphic adaptation to life as intracellular parasites, their unicellular spores have polar tubes coiled up inside which can be forcibly everted to penetrate host cells and inject their infective germs (sporoplasm). Microsporidia have reduced cellular complexity, they have small genomes, they are amitochondriate and they have metabolomes partway between 'prokaryotes' (archae- and eu-bacteria) and eukaryotes (nucleated cells). They were long considered to be a primitive basal group of Protista but molecular phylogenetic studies have revealed many similarities in nuclear genes, biochemical pathways and structural components to fungi, where they are now classified.

The systematics of microsporidia has progressed over decades from phenotypic classifications based on spore morphology, developmental cycles and host range to genotypic classifications based on comparative gene sequences. Classical studies divided the microsporidia into those with or without an envelope (sporophorous vesicle) around sporoblasts (Pansporoblastina and Apansporoblastina respectively) and recognized families on the basis of vegetative growth, genera on the process of spore formation and species on the basis of spore morphology. The subsequent inclusion of ultrastructural features led to the recognition of three major assemblages: 'primitive' groups with rudimentary polar tubes and no polaroplasts; 'intermediate' groups with short polar tubes and rudimentary polaroplasts; and 'higher' groups with well-developed polar tubes and polaroplasts. Families continued to be identified on the basis of type of reproduction (merogony and sporogony) and nuclear condition (mono- or diplo-karyotic). More recently, chromosome cycles were used to separate microsporidia into Dihaplophasea (diplokaryon in some phase of life-cycle) and Haplophasea (unpaired nuclei in all life-cycle stages). It is thought that the developmental cycle of Dihaplophasea involves a pairing of gametes which proliferate and undergo haplosis either by meiosis (order Meiodihaplophasida) or by nuclear dissociation (order Dissociodihaplophasida), whereas the development of Haplophasea is entirely haplophasic. Molecular phylogenetic studies, however, have not provided good support for conventional classifications but have separated representative species into three major clades mostly correlated with host habitat: including the Aquasporidia (in freshwater insects, crustaceans and bryozoans), Marinosporidia (in marine fish and crustaceans) and Terresporidia (in terrestrial insects and vertebrates). Although many taxa have yet to be analysed and classified, most clades are polyphyletic with conventional genera split within and between clades. It is not known whether this reflects an evolutionary history of parasites switching hosts or habitats, or even hosts switching habitats: events quite likely to have occurred considering that many invertebrate hosts have aquatic origins and many have retained aquatic stages in their life-cycles. Further studies are required to reconcile phenotypic and genotypic classifications.

Numerous microsporidian species have been described in invertebrates (especially insects and crustaceans) and lower (rarely higher) vertebrates. Over 1,400 species belonging to ~200 genera have been described; with some 800 species in 109 genera infecting insects, 400 species in 62 genera infecting crustaceans, 120 species in 26 genera infecting fish, 17 species in 11 genera infecting mammals, 5 species in 3 genera infecting birds, reptiles and amphibians, 40 species in 27 genera infecting invertebrates (8 genera in annelids, 7 in arachnids, 4 in molluscs, 4 in bryozoans, 2 in ciliates, one in helminths and one in kinorhynchs) and another 20 species in 14 genera being hyperparasitic in other parasites (8 genera in gregarines, 3 in trematodes, 2 in myxozoans and one in mesozoa). The key characteristics of the microsporidian genera are tabulated below:

Microsporidian genera	**Hosts**	**No. nuclei (meronts, spores)** [1= monokaryotic, 2 = diplokaryotic, 1-2 = both]	**Spore types** [1 = mono-morphic, 2 = di-morphic, 3 = poly-morphic]	**Intracellular location** [CY = cytoplasm, NU = nucleoplasm, PV = parasitophorous vacuole, SP = sporophorocyst, SV = sporophorous vesicle]	**Spores per SV** [n = numerous, na = not applicable]	**Xenoma formation**
Abelspora	decapoda	1,1	1	PV	2	xenoma?
Aedispora	diptera	1-2,1-2	2	PV, SV	2-8	absent
Agglomerata	branchiopoda	1,1	1	SV	8-32	absent
Agmasoma	decapoda	2,1	?	SV	8	absent
Alfvenia	copepoda, maxillopoda	2,1	1	SV	1-2	absent
Alloglugea	anura	1,1	1	PV	na	xenoma
Amazonspora	fish	1,1	1	CY	na	xenoma
Amblyospora	copepoda, amphipoda, maxillopoda, diptera	2,1-2	3	SV	8	absent
Ameson	decapoda, diptera	2,1	1	CY	na	absent
Amphiacantha	hyperparasitic in gregarines in polychaetes	1,1	1	SV	n	absent
Amphiamblys	hyperparasitic in gregarines in polychaetes	1,1	1	SV	32-50	absent
Andreanna	diptera	2,2	1	PV, SV	8	absent
Anisofilariata	diptera	1,1	1	SV	2-16	absent
Anncaliia	diptera, coleoptera, humans	2,2	1	CY	na	absent
Antonospora	hymenoptera, psocoptera	?,2	1	CY	na	absent
Areospora	decapoda	1,1	1	SV	8	xenoma
Auraspora	collembola	2,1-2	2	CY, SV	n	absent
Bacillidium	oligochaeta, thysanura, diptera	2,2	1	CY	na	absent
Baculea	branchiopoda	1,1	1	PV, SV	n	absent
Becnelia	heteroptera	1,1	2	SV	8	absent
Berwaldia	branchiopoda	1,1	1	SV	1	absent
Binucleata	branchiopoda	1-2,1	1	SV	8	absent
Binucleospora	ostracoda	2,2	1	PV	na	absent
Bohuslavia	diptera	2,1	1	SV	8-16	absent
Brachiola	diptera, humans	2,2	1	CY	na	absent
Bryonosema	bryozoa	2,2	1	CY	na	absent
Burkea	oligochaete	1,1	1	PV	na	absent
Burunella	hymenoptera	1-2,1-2	2	PV, SV	8	absent
Buxtehudea	thysanura	1,1	1	PV	na	absent
Campanulospora	diptera	2,2	1	PV	na	absent
Canningia	coleoptera	1,1	1	CY	na	absent
Caudospora	diptera	2,2	1	CY	na	absent
Chapmanium	decapoda, diptera	2,1		SV	8	absent
Chytridiopsis	coleoptera	1,1	1	PV, SV	n	absent
Ciliatosporidium	ciliophora	1,1	1	CY	na	absent
Coccospora	diptera	2,1	1	SV	8	absent
Cougourdella	copepoda, maxillopoda, diptera	1,1	1	SV	4	absent

Crepidulospora	diptera	1,1	1	CY	na	absent
Crispospora	diptera	?,1-2	2	PV	na	absent
Cristulospora	diptera	2,1-2	2	CY, SV	8	absent
Cryptosporina	acari	1,1-2	1	SV	8	absent
Cucumispora	amphipoda	2,2	1	CY	na	absent
Culicospora	diptera	1-2,1-2	2	PV, SV	2-8	absent
Culicosporella	diptera	1-2,1-2	3	PV, SV	2-8	absent
Cylindrospora	diptera	2,1	1	PV, SV	8	absent
Cystosporogenes	lepidoptera	1,1	1	SV?	<60	absent
Dasyatispora	elasmobranch	1,1	1	SV	n	absent
Desmozoon	maxillopoda, copepoda, fish	1-2,1	1	CY	na	absent
Desportesia	hyperparasitic in gregarine in echiurid	1,1	1	CY, SV	32	absent
Dictyocoela	amphipod	2,1-2	1	SV	8	absent
Dimeiospora	diptera	1,1	2	SV	8	absent
Duboscqia	branchiopoda, copepoda, isoptera, diptera	1,1	1	SV	16	absent
Edhazardia	diptera	2,1-2	3	PV, SV	1-8	absent
Encephalitozoon	maxillopoda, orthoptera, acari, birds, mammals (incl. humans)	1,1	1	PV	na	absent
Endoreticulatus	lepidoptera, coleoptera, decapoda	1,1	1	PV	na	absent
Enterocytozoon	mammals (inc. humans), fish	1,1	1	CY	na	absent
Enterospora	decapoda, fish	2,2	1	CY, NU	na	absent
Episeptum	trichoptera	1,1	1	SV	4	absent
Euplotespora	ciliophora	1,1-2	1	SV	1	absent
Evlachovaia	diptera	2,1-2	2	PV, SV	2	absent
Facilispora	maxillopoda, copepoda	1,1	1	CY	na	absent
Fibrillanosema	amphipoda, branchiopoda	1,1	1	CY	na	absent
Flabelliforma	copepoda, ostracoda, cladocera, diptera	1,1	1	CY, SV	n	absent
Geussia	hyperparasitic in gregarine of ephemeroptera	?		SV	6-8	absent
Glugea	amphipoda, fish	1,1	1	SV	n	xenoma
Glugoides	branchiopoda	1,1	1	PV, SV	16	absent
Golbergia	diptera	2,1-2	2	CY	na	absent
Gurleya	branchiopoda, copepoda, decapoda, cladocera, diptera, ephemeroptera, isoptera, lepidoptera, odonata, trichoptera	1,1	1	SV	4	absent
Gurleyides	branchiopoda	?	2	SV	1,4	absent
Hamiltosporidium	branchiopoda	1,2	2	SV	8	absent
Hazardia	diptera	1-2,1-2	2	CY	na	absent
Helmichia	diptera	2,1-2	1	SV?	8	absent
Hepatospora	decapoda	1,1	1	PV	na	absent
Hessea	diptera	2,1-2	1	SV	n	absent
Heterosporis	fish, seasnakes	1,1	3	SP, SV	n	absent
Heterovesicula	orthoptera	2,1-2	2	SV	8-n	absent
Hirsutosporos	diptera	2,2	1	CY	na	absent
Holobispora	copepoda, maxillopoda	?,1	1	CY	na	absent
Hrabyeia	oligochaeta	2,2	1	CY	na	absent
Hyalinocysta	diptera, copepoda	2,1	1	SV	8	absent
Ichthyosporidium	fish	2,2	1	PV	na	xenoma
Inodosporus	decapoda	2,1	1	SV	8	absent
Intexta	acari	1,1	2	PV	na	absent

Intrapredatorus	diptera	2,1-2	3	SV	8	absent
Issia	trichoptera, diptera	2,2	2	SV?	2	absent
Janacekia	diptera, coleoptera	2,1-2	1	SV	1	absent
Jirovecia	fish, oligochaete	2,2	1	CY, PV?	na	xenoma
Jiroveciana	oligochaete	1,1	1	PV	na	absent
Johenrea	orthoptera	1,1	1	SV	8,16	xenoma
Kabatana	fish	1,1	1	CY	na	absent
Kneallhazia	hymenoptera	1-2,1	1	SV	8	absent
Kinorhynchospora	kinorhyncha	1,1	2	SV	n	absent
Krishtalia	diptera	2,1-2	2	CY	na	absent
Lanatospora	branchiopoda, maxillopoda, copepoda	1,1	1	SV	6-16	absent
Larssonia	branchiopoda	1,1	1	SV	4-32	absent
Larssoniella	lepidoptera	1,1	1	CY	na	absent
Liebermannia	orthoptera	2,2	1	PV	na	absent
Loma	fish	1,1	1	SV	4	xenoma
Marssoniella	maxillopoda	1,1	2	SV	4-8	absent
Merocinta	diptera	2,1-2	2	PV	na	absent
Metchnikovella	hyperparasitic in gregarine in polychaete	1,1	1	PV, SV	8-32	absent
Microfilum	fish	1,1	1	CY	na	xenoma
Microgemma	fish	1,1	1	PV	na	xenoma
Microsporidium (often used for *species inquirenda, incertae sedis*)	branchiopoda, copepoda, cirripedia, isopoda, amphipoda, mollusca, insecta, fish, mammals (incl. humans)	?	?	?	?	?
Mitoplistophora	ephemeroptera	1,1	1	PV	na	absent
Mockfordia	psocoptera	?,1	1	PV	na	absent
Mrazeckia	isopoda	2,2	1	CY	na	absent
Multilamina	isoptera, diptera	1,1	1	SV	1	absent
Myospora	decapoda	2,2	1	CY	na	absent
Nadelspora	decapoda	1,1	1	CY	na	absent
Napamichum	diptera, acari	2,1	1	SV	8	absent
Nelliemelba	copepoda, maxillopoda	1,1	1	SV	1	absent
Nematocida	nematode	1,1	1	PV	na	absent
Neoflabelliforma	oligochaete, hyperparasitic in myxozoa in oligochaete	1,1	1	SV?	?	absent
Neonosemoides	fish	2,1	1	CY	na	xenoma
Neoperezia	diptera	2,1	2	SV	2	absent
Nolleria	siphonoptera	1,1	1	PV, SV	n	absent
Norlevinea	branchiopoda	1,1	1	SV	4	absent
Nosema	branchiopoda, copepoda, decapoda, amphipoda, mollusca, hymenoptera, lepidoptera, acari, fish, mammals (incl. humans); hyperparasitic in myxozoa in fish, trematodes in snails, oysters, fish	2,2	2	CY	na	absent
Nosemoides	branchiopoda, fish, hyperparasitic in gregarine in nemertean	1,1	1	CY	na	xenoma
Novothelohania	diptera	1,1	1	SV	8	absent
Nucleospora	fish	1,1	1	NU	na	absent
Nudispora	odonata	2,1	1	CY	na	absent
Obruspora	fish	1,1	1	CY	na	xenoma
Octosporea	branchiopoda, isopoda, amphipoda, cladocera, diptera, ephemeroptera,	2,2	1	SV	8	absent

	hemiptera, lepidoptera, collembola					
Octotetraspora	diptera	2,1	1	SV	4,8	absent
Oligosporidium	acari, opiliones	1,1	2	CY	na	absent
Ordospora	branchiopoda	1,1	1	PV	na	absent
Ormieresia	decapoda	2,1	1	CY, SV	8	absent
Orthosomella	lepidoptera, coleoptera	1,1	1	CY	na	absent
Ovavesicula	coleoptera	2,1	1	CY, SV	32	absent
Ovipleistophora	fish, hyperparasitic in trematode in fish	1,1	2	CY, SV	na	absent
Pankovaia	ephemeroptera	1,1	11	CY, SV	1	absent
Paradoxium	decapoda	1-2,1	1	CY	na	absent
Paraepiseptum	trichoptera	1,1	1	SV	4	absent
Parahepatospora	decapod	1,1	1	PV	na	absent
Paranosema	coleoptera, orthoptera	1-2,2	1	CY	na	absent
Paranucleospora	maxillopoda, fish	1-2,1-2	2	CY, NU	na	absent
Parapleistophora	diptera	1,1	1	SV	48-64	absent
Parastempellia	diptera	2,1-2	2	SV	4,8,16	absent
Parathelohania	maxillopoda, diptera	2,1-2	2	SV	8	absent
Pegmatheca	diptera, tricoptera	2,1	1	SV	8	absent
Perezia	branchiopoda, decapoda, lepidoptera, coleoptera, hymenoptera, orthoptera, plus hyperparasitic in gregarine of tunicate	2,1	1	CY	na	absent
Pernicivesicula	diptera	2,1	1	SV	24-64	absent
Pilosporella	diptera	2,1-2	2	SV	8	absent
Pleistophora (Plistophora)	branchiopoda, copepoda, decapoda, blattaria, coleoptera, diptera, lepidoptera, orthoptera, mollusca, fish, mammals (incl. humans	1,1	2	SV	n	absent
Pleistophoridium	hyperparasitic in gregarine in ephemeroptera	1,1	1	CY, PV	na	absent
Polydispyrenia	diptera	2,1	2	PV, SV	n	absent
Potaspora	fish, decapod	1,1	1	CY	na	xenoma
Pseudoloma	fish	1,1	1	SV	16	atypical xenoma
Pseudonosema	bryozoa	2,2	1	CY	na	absent
Pseudopleistophora	lepidoptera, polychaete	2,2	1	PV, SV?	n	absent
Pulicispora	siphonoptera	1,1-2	1	SV	8,16,32	absent
Pyrotheca	copepoda, maxillopoda	1,1	1	CY, SV	4	absent
Rectispora	oligochaeta	2,2	1	CY	na	absent
Resiomeria	odonata	2,1	1	SV	8	absent
Ringueletium	diptera	2,2	1	CY	na	absent
Schroedera	bryozoa	1-2,2	1	CY	na	xenoma
Scipionospora	diptera	2,2	1	SV	4	absent
Semenovaia	diptera	?,1-2	2	CY	na	absent
Senoma	diptera	2,2	1	PV	na	absent
Septata	human	1,1	1	PV	na	absent
Simuliospora	diptera	2,1	2	SV	6,32	absent
Spherospora	diptera	2,1-2	2	SV	8-32	absent
Spiroglugea	diptera	?	1	SV?	8	absent
Spraguea	fish	1-2,1-2	2	CY	na	xenoma
Steinhausia	bivalve, gastropod	1,1	1	PV, NU	na	absent
Stempellia	copepoda, amphipoda, opiliones, ephemeroptera, diptera, coleoptera, isoptera	1,1	2	PV, SV	4	absent

Striatospora	diptera	2,1	1	SV	8	absent
Systenostrema	diptera	2,1-2	1?	SV	8	absent
Tabanispora	diptera	1-2,2	2	SV	1-10	absent
Takaokaspora	diptera	1-2,1-2	2	CY, SV	?	absent
Tardivesicula	trichoptera	1,1	1	SV	16-32	absent
Telomyxa	ephemeroptera, diptera, coleoptera	1,1	1	SV	2	absent
Tetramicra	fish	1,1(2?	1	PV	na	xenoma
Thelohania	branchiopoda, copepoda, decapoda, amphipoda, diptera, collembola, ephemeroptera, hemiptera, lepidoptera, hymenoptera, odonata, trichoptera, fish	1-2,1	1	SV	8	absent
Toxoglugea	branchiopoda, diptera, plecoptera, odonata, hemiptera, homoptera	2,1	1	SV	8	absent
Toxospora	diptera	?,1-2	1	SV	8	absent
Trachipleistophora	mammals (incl. human)	1,1	1	PV, SV	2-n	absent
Trichoctosporea	diptera	2,1	2	SV	8	absent
Trichodubosquia	ephemeroptera	2,1	1	SV	16-32	absent
Trichonosema	bryozoa	2,2	1	CY	na	absent
Trichotuzetia	copepoda, maxillopoda	1,1	1	SV	1	absent
Tricornia	diptera	2,1-2	1	SV	8	absent
Triwangia	decapoda	1,1	1	SV	n	xenoma
Tubulinosema	diptera, orthoptera, hymenoptera, coleoptera, mammals (humans)	2,2	1	CY	na	absent
Tuzetia	branchiopoda, copepoda, maxillopoda, ephemeroptera	1,1	1	SV	1	absent
Unikaryon	coleoptera, acari, hyperparasitic in trematode in bivalve	1,1	1	SV	2	absent
Vairimorpha	decapod, lepidoptera, hymenoptera, diptera	2,1-2	2	CY, SV	8	absent
Vavraia	ostracoda, decapoda, diptera, coleoptera, lepidoptera	1,1	1	SV	16-64	absent
Vittaforma	mammals (humans)	2,2	1	PV	na	absent
Weiseria	diptera	2,2	1	CY	na	absent
Wittmania	hyperparasitic in mesozoan in cephalopod	1-2,2	1	CY	na	absent
Zelenkaia	trichoptera	1,1	1	SV	2	absent

Although many microsporidian species have been described on the basis of presumed host specificity, recent molecular characterization studies have demonstrated that the host ranges for some species can be very broad, encompassing not only hosts from disparate taxa (e.g. insects and mammals) but also hosts from disparate environments (e.g. marine and terrestrial). While considerable work remains to determine the host ranges and phylogenetic affinities of most microsporidia, the following text considers microsporidia from one of three perspectives associated primarily, but not exclusively, with different host groups; namely, arthropods (mainly insects and crustaceans), fish (bony and cartilaginous), and tetrapods (mammals, birds, reptiles and amphibians). This section considers the microsporidia of arthropods, notably those arthropods kept and managed for economic benefit; including colonies of insects (silkworms, honeybees) and farmed shellfish (crayfish, shrimp, prawns, crabs).

Microsporidian infections are common in insect and crustacean host species throughout the world, particularly those that are aquatic (freshwater, brackish and marine) or those that complete part of their life-cycle in aquatic environments (notably freshwater diptera). Many parasite species appear to be oioxenous (host specific) or stenoxenous (infecting closely-related hosts), although a few have been found to be euryxenous (infecting a broad range of hosts). Infections have been recorded in all insect orders; notably Diptera (73 genera in flies and mosquitoes), Coleoptera (15 genera in beetles), Lepidoptera (13 genera in butterflies,

moths) and Hymenoptera (8 genera in bees). In crustaceans, infections have been recorded in the classes Malacostraca (20 genera in crabs, shrimp, lobsters, amphipods and isopods), Maxillopoda (15 in free-living cyclopid copepods and parasitic calagid copepods), Branchiopoda (13 in cladoceran hosts) and Ostracoda (2 in seed shrimp). Microsporidia in arthropods often demonstrate unique morphological and biological adaptations (including bizarre ectospore appendages, polymorphic spore development, polyxenous life-cycles) with profound impacts on their hosts (altering host behaviour, sex ratios, population dynamics). The life-cycles of arthropod microsporidia range from relatively simple with a single sporulation sequence in one host (such as *Nosema* in silkworms and honeybees) to extremely complex with multiple sporulation sequences involving more than one generation of the host and sometimes an intermediate host (as shown by polymorphic microsporidia of aquatic diptera, e.g. *Amblyospora* in mosquitoes).

Several microsporidian species are notorious pathogens of arthropods of commercial significance, causing devastating diseases in insects (collapse of European silkworm industry, dysentery in honeybees) and crustacea ('cotton-tail' in commercial shellfish species). Infections of economic significance in insects are mostly diplokaryotic and polymorphic, undergoing 2-3 sporulation sequences and being disseminated throughout host tissues without causing xenomas. Infections involve pansporoblastic (e.g. *Amblyospora*) and apansporoblastic genera (e.g. *Nosema*) which often disseminate throughout host tissues. Infections in crustacea of economic significance, including freshwater crayfish, marine prawns, shrimp and crabs, mostly form monokaryotic spores, although some species do have diplokaryotic merogonous stages (such as *Thelohania*, *Ameson*, *Agmasoma*, *Vairimorpha*). Most form monomorphic spores, except *Vairimorpha* which forms dimorphic spores, and none form xenomas but rather disseminate throughout host tissues. Microsporidia have also been used experimentally as biological control agents for some terrestrial insect pests and several species are thought to have potential for controlling aquatic insect pests or vectors of other diseases e.g. several *Amblyospora* spp. markedly reduce host fitness (vigor, longevity, fertility) in mosquitoes.

Parasite species	**Spore dimensions** (mi = microspore, ma = macrospore, me = meiospore)	**Hosts**	**Location [IH]**	**Distribution**
Suborder: Pansporoblastina (sporophorous vesicle (SV) present)				
Family: Amblyosporidae (diplokaryotic meronts, octonucleate sporonts, monokaryotic spores)				
Amblyospora connecticus	me 8-9 x 3-3.2 µm (9-10 coils) [IH 8-10 x 5-6 µm 11-12 coils]	insect (saltmarsh mosquito), crustacean IH (copepod)	fat bodies, oenocytes, ovaries, gut, muscles [ovaries]	North America
Amblyospora albifasciati	me 7.2 x 4.4 µm (4-5 coils)	insect (floodwater mosquito), crustacean IH (copepod)	ovaries, fat bodies [ovaries]	South America
Parathelohania anophelis	me 4-5.5 x 2.5-3.6 µm (3-5 coils)	insect (common malaria mosquito)	fat bodies, oenocytes, ovaries	Americas
Family: Glugeidae (monokaryotic, variable but large number spores produced)				
Vavraia parastacida	5.5 x 2.6 µm (9-11 coils)	freshwater crayfish (yabbies, marron, redclaw, gilgies)	muscles	Australia
Vavraia culicis	ma 5.9 x 3.7 µm mi 4.0 x 2.6 µm (11-13 coils)	insect (many mosquito species)	systemic	Europe, Africa, North America
Pleistophora cargoi		marine blue crab	muscles	North America
Pleistophora miyairii		freshwater shrimp	digestive tract	Japan
Pleistophora sogandaresi		freshwater swamp dwarf crayfish	muscles	North America
Family: Culicosporidae (diplokaryotic, polymorphic)				
Culicospora magna	me 12-16.5 x 4.6 µm	insect (mosquito)	fat body, oenocytes	North America
Edhazardia aedis	me 7.6 x 6 µm	insect (yellow fever mosquito)	caeca, oenocytes, fat body, ovaries	cosmopolitan
Family: Burenellidae (diplokaryotic reducing to monokaryotic, polymorphic)				
Vairimorpha austropotamobii	3.9 x 2.2 µm (11-14 coils)	freshwater crayfish (white-clawed crayfish)	muscles	Europe
Vairimorpha cheracis	3.4 x 1.9 µm (10-12 coils)	freshwater crayfish (yabbies)	muscles	Australia
Vairimorpha disparis		insect (spongy moth)		Eurasia
Vairimorpha necatrix		insect (white-specked moth)		Eurasia
Family: Thelohanidae (meronts usually diplokaryotic, spores monokaryotic, 8 spores in SV)				
Agmasoma penaei	mi 2.5-5 x 2-3.5 µm	marine white shrimp	muscles, connective	worldwide

(syn. *Thelohania*)	ma 5.5-8.2 x 3.5-4.2 µm		tissues	
Hyalinocysta chapmani jordani	me 4.5 x 2.8 µm (7-8 coils) [5.3 x 3.5 µm (6-7 coils)]	insect (black-tailed mosquito) [crustacean IH (copepod)]	fat body [ovaries]	North America
Thelohania butleri		marine shrimp		Atlantic
Thelohania californica	6 x 3 µm	insect (mosquitoes)	fat body, intestines	North America
Thelohania cambari		freshwater crayfish (Appalachian brook crayfish, Cajun dwarf crayfish, northern koura, redclaw crayfish)	muscles	North America, New Zealand
Thelohania contejeani	3.8 x 1.8 µm (9-10 coils); some diplokaryotic spores (5-6 coils)	freshwater crayfish (European crayfish, Danube crayfish, white-clawed crayfish, signal crayfish, spinycheek crayfish, virile crayfish, northern koura)	muscles	Europe, North America, New Zealand
Thelohania duorara	4.7-6.8 x 3-4.2 µm	marine brown shrimp	muscles, connective tissue	worldwide
Thelohania giardi		marine brown shrimp	muscles	North America
Thelohania macrocystis		freshwater river shrimp	muscles	North America
Thelohania maenadis		marine green crab	muscles, ovary	North America
Thelohania montirivulorum	4.9-7.2 x 2-3.1 µm (20-22 coils)	freshwater crayfish (yabbies)	muscles	Australia
Thelohania octospora		marine prawns	muscles	Europe
Thelohania paguri		hermit crab	body cavity	Europe
Thelohania parastaci	3.2-4.9 x 1.5-2.7 µm (6-8 coils)	freshwater crayfish (yabbies)	muscles	Australia
Thelohania penaei		marine white shrimp	gonad	North America
Thelohania petrolisthis		marine green porcelain crab	muscles	North America
Thelohania solenopsae		insect (fire-ants)	tissues	Americas
Suborder: Apansporoblastina (sporophorous vesicle (SV) absent)				
Family: Nosematidae (diplokaryotic, diplosporoblastic, monomorphic)				
Nosema apis	6.0 x 3.0 µm (18-44 coils)	insect (honeybees)	midgut (dysentery)	worldwide
Nosema bombi	4.2-5.4 x 2.1-3.5 µm (14-18 coils)	insect (bumblebee)	midgut	worldwide
Nosema bombycis	3-4 x 2-2.5 µm (11-12 coils)	insect (silkworms)	systemic (pebrine, pepper disease)	worldwide
Nosema ceranae	4.7 x 2.7 µm (20-23 coils)	insect (honeybees)	midgut (dysentery)	Eurasia
Nosema disstriae		insect (forest tent caterpillar moth)	body tissues	North America
Nosema empoascae		insect (potato leafhopper)	body tissues	North America
Nosema fumiferanae		insect (light brown apple moth)	body tissues	Pan-Pacific
Nosema furnacalis		insect (Asian corn borer)	body tissues	Asia
Nosema granulosis		freshwater crustacea (amphipods)		Europe
Nosema lymantriae		insect (gypsy moth)		Europe, Africa, North America
Nosema oulemae		insect (cereal leak beetle)	body tissues	cosmopolitan
Nosema pyrausta		insect (European corn borer)	body tissues	Europe
Nosema trichoplusiae		insect (Asian honeybee)	body tissues	Asia
Nosema tyriae		insect (cinnabar moth)	body tissues	Eurasia
Ameson nelsoni	2-3.5 x 1.2-2.3 µm	marine crustacea	muscles	worldwide

(syn. *Nosema, N. pulvis*)		(penaeid shrimp)		
Ameson michaelis (syn. *Nosema, N. sapidi*)	2.2 x 1.7 µm	marine crustacea (blue crabs)	muscles	North America
Anncaliia algerae (syn. *Nosema, Brachiola*)	4 x 2.7 µm (8-11 coils)	insect (mosquito), primates (human)	disseminated	North America, Australia
Family: Enterocytozoonidae (monokaryotic, develop in host cell cytoplasm, precocious development of spore organelles)				
Enterocytozoon hepatopenaei	1.1 x 0.7 µm (5-6 coils)	black tiger shrimp	hepatopancreas	South-East Asia

Parasite morphology: Microsporidia form three sequential developmental stages: meronts, sporonts and spores. The unique unicellular spores are spherical, ovoid or cylindrical, most ranging in length from 2-8 µm. They are encased within tough chitinous walls comprising a thin electron-dense exospore and a thicker electron-lucent endospore. Mature spores possess an elongate polar tube coiled up inside; most tubes being isofilar (of uniform diameter) although some are anisofilar (tapered, showing a reduction in diameter over length). The polar tubes are attached to an anterior anchoring disc enveloped by a membranous polar sac. For most microsporidian genera, the wall of the polar tube and its central canal are inserted into the polar sac, but for chytridiopsid genera (*Buxtehudea, Chytridiopsis* and *Nolleria*), only the central canal surrounded by a honeycomb layer is inserted into the polar sac. The anterior section of the polar tube is straight and surrounded by the polaroplast, which may be lamellar, tubular or both. Mature spores contain a prominent posterior vacuole (often visible by light microscopy) and an amoeboid nucleated sporoplasm. Spores may be monokaryotic (uninucleate) or diplokaryotic (with two closely-appressed nuclei). Many microsporidia form only one type of spore (monomorphic), while others are heterosporous (dimorphic or polymorphic) forming several different types (usually micro-spores and macro-spores, sometimes meio-spores). Following host cell invasion, parasites undergo asexual merogony (schizogony) and then 1-3 sporulation (sporogony) sequences forming sporoblasts (sporoblastogenesis) which mature to form infective spores (sometimes referred to as germination). Meronts are located either directly within the host cell cytoplasm (often surrounded by host endoplasmic reticulum and sometime host mitochondria) or bound within parasitophorous vacuoles (membranous envelopes of host origin). They appear as clusters of small nucleated intracellular parasites that have divided by binary or multiple fission, although several species form multinucleated plasmodial stages. Sporonts also appear as small clusters of parasitic cells but are characterized by thickened plasmalemmas due to the deposition of parasite secretions on their surface membranes. Pansporoblastic species also form an isolating envelope (membranous sporophorous vesicle) whereas apansporoblastic species lie direct in the host cell cytoplasm or within parasitophorous vacuoles. Sporonts divide internally one or more times by binary or multiple fission or plasmotomy to form sporoblasts which then mature into spores. Sporonts of a few species (mostly in insects) are also thought to divide by meiosis to form uninucleate meiospores.

Site of infection: All arthropod microsporidia are histozoic parasites with obligative intracellular development within host cells; either being in direct contact with the host cell cytoplasm (a few even occurring in the nucleoplasm) or being enclosed within parasitophorous vacuoles (membrane of host origin) or sporophorous vesicles (envelopes of parasite origin). Infections in arthropods often involve cells in the midgut (including the hepatopancreas), skeletal musculature, connective tissues (fibrous and/or spongy) and/or gonads (ovaries). Various parasite species demonstrate some tissue tropism within their hosts, particularly within the midgut (*Nosema*), fat bodies and ovaries (*Amblyospora*) of insects and the muscles (*Thelohania*, *Ameson*) of crustacea. Infections of the gut are common for those microsporidian species relying on horizontal transmission, while infections of the gonads are mandatory for those relying on vertical transmission.

Pathogenesis: Microsporidian infections cause a wide variety of diseases in arthropods, most being chronic in nature (slow onset, long duration) and rarely acute (rapid onset, short duration). During development, the parasites are metabolically dependent on host cells and are able to mobilize host cell organelles to meet their demands. Infected cells become swollen and the host cell nucleus becomes enlarged or fragmented. The parasites ultimately cause lysis of infected host cells, and the spores replace host tissue resulting in both structural and functional deficits for the host. Heavily infected tissues often become opaque in appearance due to the presence of numerous refractile spores (hence the condition being colloquially called porcelain-, cotton-, milk- or cooked-tail in crustaceans). Infections may be disseminated throughout host tissues leading to systemic degenerative changes although some species cause focal lesions, inflammation and granulomas. Only a few species form tumour-like xenomas (hypertrophic host cells) in insects: either syncytial-like (fusion of infected host cells to form large multinucleated plasmodia) or neoplastic (parasite-induced increase in number of infected cells (hyperplasia) with hypertrophic, fragmented or branched nuclei and highly modified cell surface). Both adult and pre-adult arthropods (larvae, pupae, nauplius, zoea) may be clinically affected, particularly when parasites compete for nutrients during critical stages of host development (moulting and pupation). Clinical signs of disease are often progressive in severity and include conspicuous tissue manifestations (opacities, inflammation, deformities, lesions, cysts) and parasite-mediated changes in host phenotype, development and behaviour; all serving to diminish host fitness (reduced vigour, poor stress resistance, lower fertility (sometimes sterility) and poor survival, especially following capture/handling).

In insects, infections of the midgut and fat bodies results in swollen distended tissues that are porcelain white in appearance due to large masses of refractile spores. Infected larvae and pupae are lethargic, stunted and deformed, while infected adults show loss of vigour, shortened lifespans and lower fertility. *Nosema* infections in silkworms have high mortality in late larval instars and survivors spin poor quality silk. *Nosema* infections in worker bees cause dysentery with high mortalities at the onset of winter. Infections in queen bees cause atrophy of the ovaries and reduced fecundity. *Nosema* infections in bumblebees may result in smaller adults, queens with distended abdomens, fewer gynes (reproductive females), crippled and/or infertile males, and reduced hibernation survival. *Amblyospora* spp. vary in their pathogenicity at different stages of their life-cycles in order to complete their transmission: infections in larval mosquitoes and copepod intermediate hosts often being fatal so that spores are released into water for horizontal transmission; whereas infections in adult mosquitoes are not fatal so that vertical (transovarial) transmission may occur. In aquatic crustaceans (marine shrimp, prawns, crabs, freshwater crayfish), microsporidian infections may cause significant economic losses in commercial aquaculture or fisheries due to unsightly tissue lesions, production-limiting diseases and mortalities. Infections of the musculature by various genera (*Ameson, Nadelspora, Thelohania, Myospora*) have been associated with progressive opacity in myofibers due to sarcoplasm replacement by spores, rupture of infected muscle fibres, infiltration by host haemocytes, granulomatous lesions, generalized lethargy and reduced tail-flick responses (poorer avoidance of predators). Heavily infected muscles become porcelain white in appearance, unsightly and unpalatable, thus being rejected from human consumption. Infections of both fibrous and spongy connective tissues in the interstitial spaces of crustacea have been associated with the formation of syncytia (multinucleated giant cells) rather than xenomas. Infections of the midgut and hepatopancreas may cause intracellular inclusions, disruption of hepatopancreatic tubules and pronounced haemocytic infiltration. Some infections in the gonads have also been associated with reduced fecundity as well as distorting host sex ratios through male killing or feminization. Invertebrates often respond to infections by melanization processes involving the formation of dark-pigmented foci within the tissues and haemolymph. Microsporidia have also developed sophisticated mechanisms to evade host responses, including modification of phenol-oxidase cascades, inhibition of apoptosis, accumulation in specialized haemocytes and adipocytes, and stimulating host cells to grow into giant cells with prolonged cell cycles (cysts in insects, xenomas in fish) apparently by differentially influencing gene expression and metabolism.

Developmental cycle and mode of transmission: The life-cycles of microsporidia vary considerably. Most species have simple asexual monoxenous life-cycles, although some species in insects have complex heteroxenous life-cycles with both asexual and sexual reproduction involving successive host generations or crustacean intermediate hosts. Mature spores have tough resistant walls which allow them to survive moderate ranges of various physical and chemical factors encountered in host environments (temperature, moisture, UV radiation, salinity, pH), but they are still susceptible to extremes of heat, cold, desiccation, sunlight, hypersalinity, acidity or alkalinity. Each spore contains an infective sporoplasm which is injected into a host cell through the polar tube when it is forcibly everted by swelling of the posterior vacuole and polaroplast (triggered by changes in calcium influx, osmotic pressure, pH, mechanical compression, etc.). The parasites then undergo vegetative reproduction by merogony (schizogony) by binary or multiple fission, although cytokinesis is sometimes delayed thus forming multinucleate plasmodia which divide by plasmotomy to form more plasmodia or segment into uninucleate meronts again. Meronts may lie direct in the host cell cytoplasm or be contained within membranous parasitophorous vacuoles. The parasites then form thickened cell membranes and/or enveloping sporophorous vesicles and undergo further division called sporogony. Sporonts divide by binary or repeated fission or plasmotomy to form sporoblasts (sporoblastogenesis) which subsequently mature to form spores (sporulation). The latent period may be as short as 1-2 weeks but could last the entire lifespan of the arthropod (whose longevity may be significantly reduced by infection). The developmental stages of many microspora have single isolated nuclei throughout their development, ultimately giving rise to uninucleate sporoblasts and spores. Others have paired nuclei which divide synchronously and remain closely appressed throughout their development into diplokaryotic sporoblasts. Some diplokaryotic sporonts also appear to undergo meiotic reduction forming monokaryotic spores. The number of spores formed by each sporont (di-, tetra-, octo-, poly-sporous) can be a defining characteristic for many genera, particularly those developing within sporophorous vesicles. Merogony and sporogony may take place within the same or different tissues of individual hosts. Many microsporidian species are monomorphic (forming only one type of spore) while other species may produce different types of spores (macro-, medi-, micro-, meio-spores) at different stages of development, each type probably having a different function, such as autoinfection (facilitating dissemination within the same host), horizontal transmission (from host to host) and vertical transmission (from mother to offspring). The polymorphic microsporidia of aquatic diptera (esp. mosquitoes) have complex life-cycles involving asexual (merogony and sporogony) and sexual (karyogamy, gametogenesis and plasmogamy) reproduction, the formation of multiple spore types, and both horizontal and vertical transmission. Most species require two successive host generations to complete their life-cycles, and at least three genera (*Amblyospora, Hyalinocysta* and *Parathelohania*) require obligatory development in an intermediate copepodid host. Copepods acquire infections through the ingestion of spores released into water following the death of infected mosquito larvae. Different types of spores formed in copepods are released into water following the death of the copepods and are infective to mosquito larvae.

Horizontal transmission between arthropod hosts occurs predominantly by contact with infectious spores contaminating the immediate environment (water, soil and plant materials). This may occur by oral ingestion of foodstuffs (including scavenging of carcases and cannibalism of weak or moribund individuals), by trophallaxis (where adults pass food directly to other adults) or during grooming behaviours (especially in eusocial insects). Aquatic hosts may also be infected by spores contacting respiratory surfaces. Infective spores have been shown to be voided into the environment with host faeces, oral secretions (including regurgitates), larval silk as well as from decomposing carcasses. Spores are not motile so their dispersal is dependent on host

behaviours (sometimes including parasitoids and predators) and physical factors (wind and water movements). Indeed, water is vitally important for many microsporidian species of invertebrates, particularly hosts that are aquatic or have aquatic larval stages. Some aquatic microsporidia even have 'floatation' devices (coverings or appendages) that allow them to remain suspended in the water column. Spores have been found to remain viable under favourable environmental conditions for extended periods (weeks to months, but usually not longer than a year). Viability is significantly reduced by exposure to high temperatures (over 35°C), low moisture (to the point of desiccation) and direct sunlight (high UV radiation). Spores remain viable and infective for longer when kept in shaded aqueous or semi-solid media (water, faeces, cadavers, etc.) at low temperatures just above freezing (2-10°C). Mature spores of microsporidia infecting terrestrial invertebrates typically have thick chitinous endospore layers that provide protection against environmental insults, while those of microsporidia infecting aquatic invertebrates have thinner endospore walls and are not tolerant of drying or freezing. Some microsporidia have developed more sophisticated strategies to survive in hosts of limited availability (e.g. hosts in ephemeral pools or those with prolonged diapause): including delayed development (overwintering), synchronizing their development to host reproduction (using hormonal cues), and adopting vertical transmission (usually maternally-mediated by trans-ovum contamination of eggs during ovipositioning or transovarial infection within oocytes, rarely paternally-mediated via venereal transmission during mating). Parasite virulence also appears to be associated with transmission modalities: species undergoing horizontal transmission being more virulent so hosts die to release spores; while species undergoing vertical transmission are less virulent so female hosts survive to reproduce.

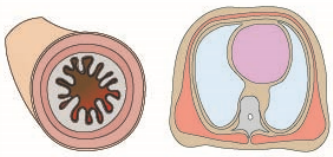
Differential diagnosis: Infections are best diagnosed by the direct detection of parasites within host tissues, using both macroscopic techniques to detect visible cysts/xenomas and microscopic techniques to detect spores. Visual examination of tissues may reveal the presence of distinctive white cysts, tumour-like xenomas, unnatural tissue opacities (due to presence of refractile spores) or focal dark discolourations (due to focal melanization deposits). Microscopic examination of wet tissue mounts, squash preparations, impression smears or histological sections may reveal the presence of characteristic microsporidian spores. Unstained samples are best examined at medium to high power (400X magnification) by bright-field microscopy with a suboptimal illumination system (introduce diffraction/contrast through specimen by racking down condenser and/or partially closing diaphragm) or by using phase-contrast or differential interference-contrast microscopy. Alternatively, fluorescence microscopy may be used as the spores are autofluorescent at particular ultraviolet (UV) wavelengths. Spores may be concentrated from tissue homogenates by centrifugation and microscopic examination of the spore pellet, which can be quantitated by haemocytometer counts. Mature spores are highly refractile, phase-bright, Gram-positive, acid-fast, and have a PAS-positive polar granule. Treatment of spores with dilute hydrogen peroxide can induce mature spores to evert their polar tubes. Histological sections are best stained using Ziehl- Neelson acid-fast, Periodic acid Schiff, Giemsa or trichrome stains to highlight merogonous and sporogonous stages. Given the small spore size and their homogenous appearance, transmission electron microscopy is often conducted to reveal the presence of the coiled polar tube within spores and/or the presence of a sporophorous vesicle. Various insect microsporidia have also been established in tissue culture systems using established cell lines or tissue explants. More recently, immunodiagnostic procedures have been developed to detect host antibodies or parasite antigens, including the use of polyclonal and monoclonal antibodies, fluorescent-labels and enzyme immunoassays. Considerable success has been achieved in detecting parasite DNA by polymerase chain reaction (PCR) amplification of nuclear gene sequences (notably, small subunit ribosomal RNA and internal transcribed spacers, large subunit RNA polymerase II). Molecular characterization techniques are facilitating not only more sensitive and specific diagnoses but also allowing more comprehensive phylogenetic analyses of relationships between taxa.

Treatment and control: Infections in colonies of cultured arthropods have been ameliorated (but often not eradicated) by treatment with antimicrobial (fumagillin, fumidil, benomyl), antiprotozoal (quinine, toltrazuril, buquinolate) and even anthelmintic drugs (albendazole, thiabendazole). Insects are often treated with drugs administered in sugar solutions, while aquatic arthropods are treated with drugs added to water baths or aquaria. Some success has been reported using heat therapy where insect hosts exhibit a higher heat tolerance than the parasites, despite poor host survival at temperatures required (e.g. 47°C for 30-45 minutes). Recourse is made to the control of outbreaks by the complete destruction of infected colonies and the disinfection of all equipment. Spores in bee hives have been killed by acetic acid fumigation, gamma radiation, ultrasound and heat treatment (49°C for 24 hours). In aquaculture, contaminated aquaria and tanks need to be drained, cleaned with disinfectants or steam cleaned, or even limed and dried over summer before restocking. Colonies should be constantly monitored and screened for the presence of disease. Infected individuals should be isolated or culled as soon as possible after disease presentation to prevent environmental contamination by spores from faeces, necrotic lesions or decaying carcases. When processing shellfish, it is recommended not to dispose of shells, entrails or offcuts into source waters, but to dispose of them by burning or burying in landfill. Translocations of hosts between geographic zones should be avoided where possible, and all new stock introduced into culture should be held in quarantine pending satisfactory screening. Colonies can be initiated by selecting uninfected progeny (Pasture method) and instituting good sanitation procedures. There is little evidence that arthropods develop any resistance to infections so the prospect for vaccines is poor. Nonetheless, recent studies have indicated that some future novel therapies may be based on gene silencing using small interfering RNA molecules (siRNAs) possibly involving ADP/ATP transporter genes.

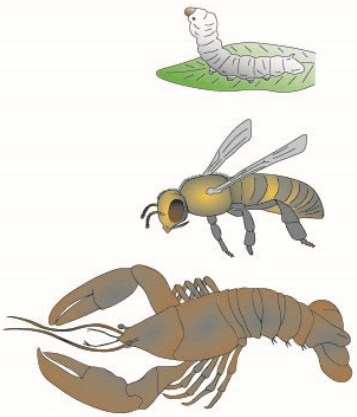
Microspora (arthropod hosts)
e.g. *Thelohania*

most diplokaryotic at some stage
most with monoxenous life-cycles
some with complex heteroxenous cycles

form unicellular spores
with unique polar tubes



histozoic (gut, viscera)
(lesions, cysts, mortalities)



Invertebrate Hosts
(insects, crustaceans)

development may occur direct in host cell cytoplasm
or in parasitophorous vacuole (membrane of host origin)
or in sporophorous vesicle (envelope of parasite origin)

meronts

multiplication by merogony
(binary or multiple fission, although
several form multinucleated plasmodia)

spore formation by sporogony
(binary or multiple fission,
a few by plasmotomy)

sporont

most cycles
direct

some cycles
indirect
(via IH)

some cycles
involve sexual
development
in mosquitoes

diplokaryotic
(paired nuclei)

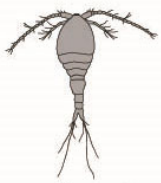
polar tube
eversion

coiled
polar
tube

microspore
(2-8 µm)

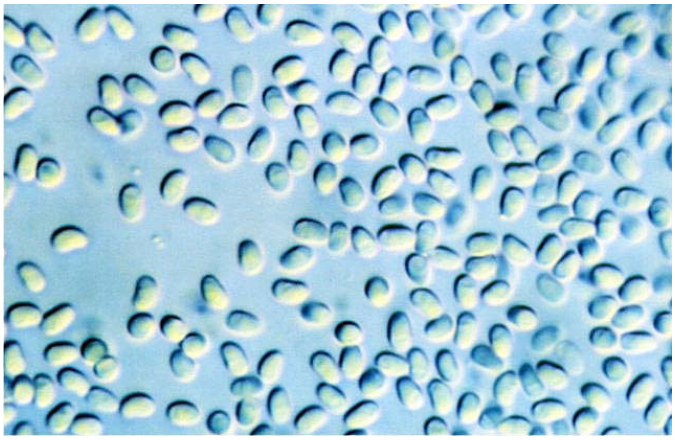
spores
ingested

mature spores
released

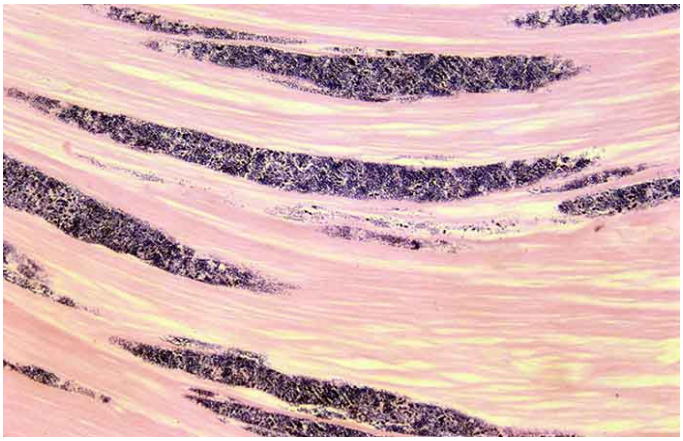


Intermediate Hosts (IH)
(copepods)

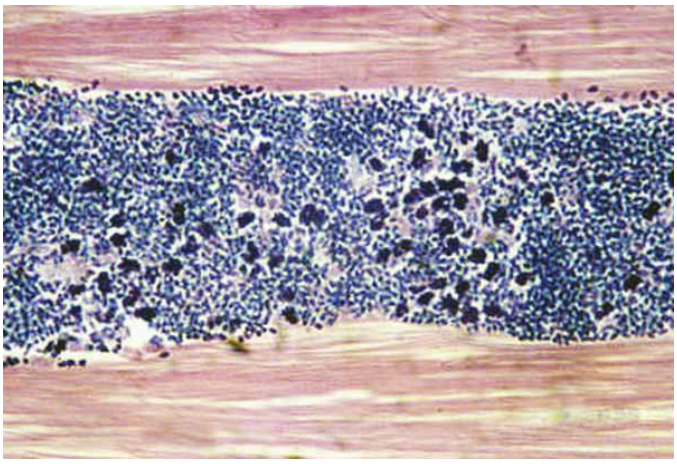
most transmission direct (horizontal, sometimes vertical) via microspores,
some cycles indirect involving copepod IHs, and some in aquatic Diptera
very complex (with sexual development in alternate mosquito generations)



Thelohania microspores from yabby

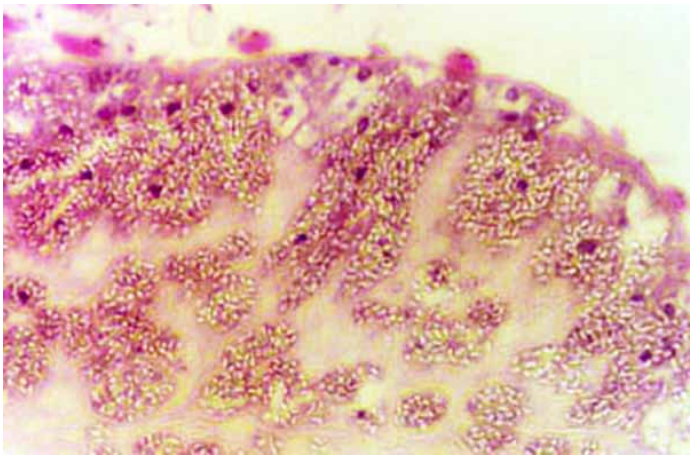


Thelohania cysts in yabby muscle

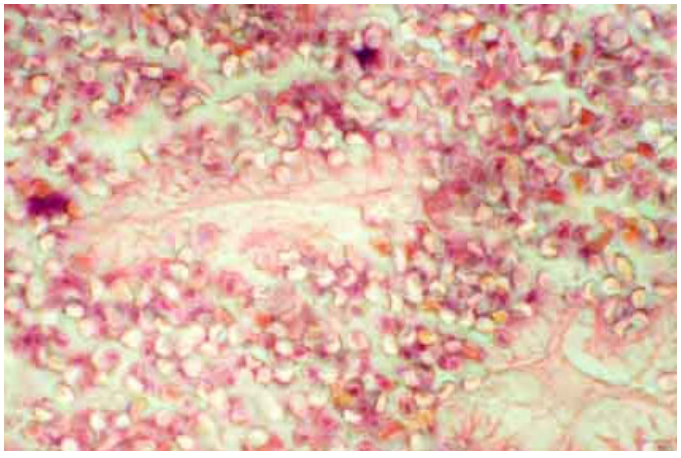


Thelohania cyst in yabby muscle

Thelohania cotton-tail appearance of yabby musculature (L)



Nosema disseminated infection in in bee gut



Nosema refractile spores in bee gut