Chapter 2

Protistan parasites and Myxozoa

Coverage of 'lower' organisms in this book is restricted to protistan and metazoan parasites and excludes fungi and other organisms such as bacteria and viruses, many of which are *parasitic* as defined in the Introduction. The boundary between fungi and protistans is ill-defined, however. Protistan taxonomy, based on molecular phylogeny, is continually changing. For example, the 'Sarcomastigophora' (still treated as such in this chapter), has disintegrated into many separate, high ranking taxa. Among them, the Opalinata belongs to a class of Heterokonta, these themselves are a subphylum of the phylum Chromista. This Chapter includes groups that have commonly been considered to be protistan. The Chapter also includes the Myxozoa, which have the appearance of protistans and were for a long time considered to belong to that group. Recent DNA and ultrastructural studies have shown that they are metazoan, although their exact position within the metazoans has not been resolved. Like the Cnidaria, they possess nematocysts (or nematocyst-like structures), but molecular evidence points to a position among the bilaterian metazoan. All the groups discussed in this Chapter have considerable importance as agents of disease, particularly in aquacultured fish and molluscs. Some of them, in particular the Microsporidia and Apicomplexa, are important as infective agents in immunocompromised people. Species richness is practically unknown for Sarcomastigophora, Microsporidia, Ciliophora and Myxozoa: new species are being described continually. In Australia, for example, less than 5% of the thousands of fish species have been examined for these parasites, and sample sizes of those which have been examined, were small. Fish of African, South American and many Asian countries have been examined even less. It may well be that these parasites belong to the most speciose groups of parasites in the marine environment and, as such, have considerable ecological importance. Also, at least some of the vast number of marine invertebrates that are yet to be examined are likely to have protistan parasites, probably including large numbers of sarcomastigophorans and ciliophorans.

In view of the great morphological and taxonomic diversity of the protistans, this chapter begins with a brief overview of this kingdom before proceeding to the various sections dealing with the groups in greater detail.

Protistan biodiversity

Peter O'Donoghue

Introduction

The kingdom Protista (syn. Protoctista) comprises unicellular eukaryotic organisms which exist as structurally and functionally independent individual cells (including those species which are gregarious or form colonies). None have adopted multicellular somatic organisation characteristic of

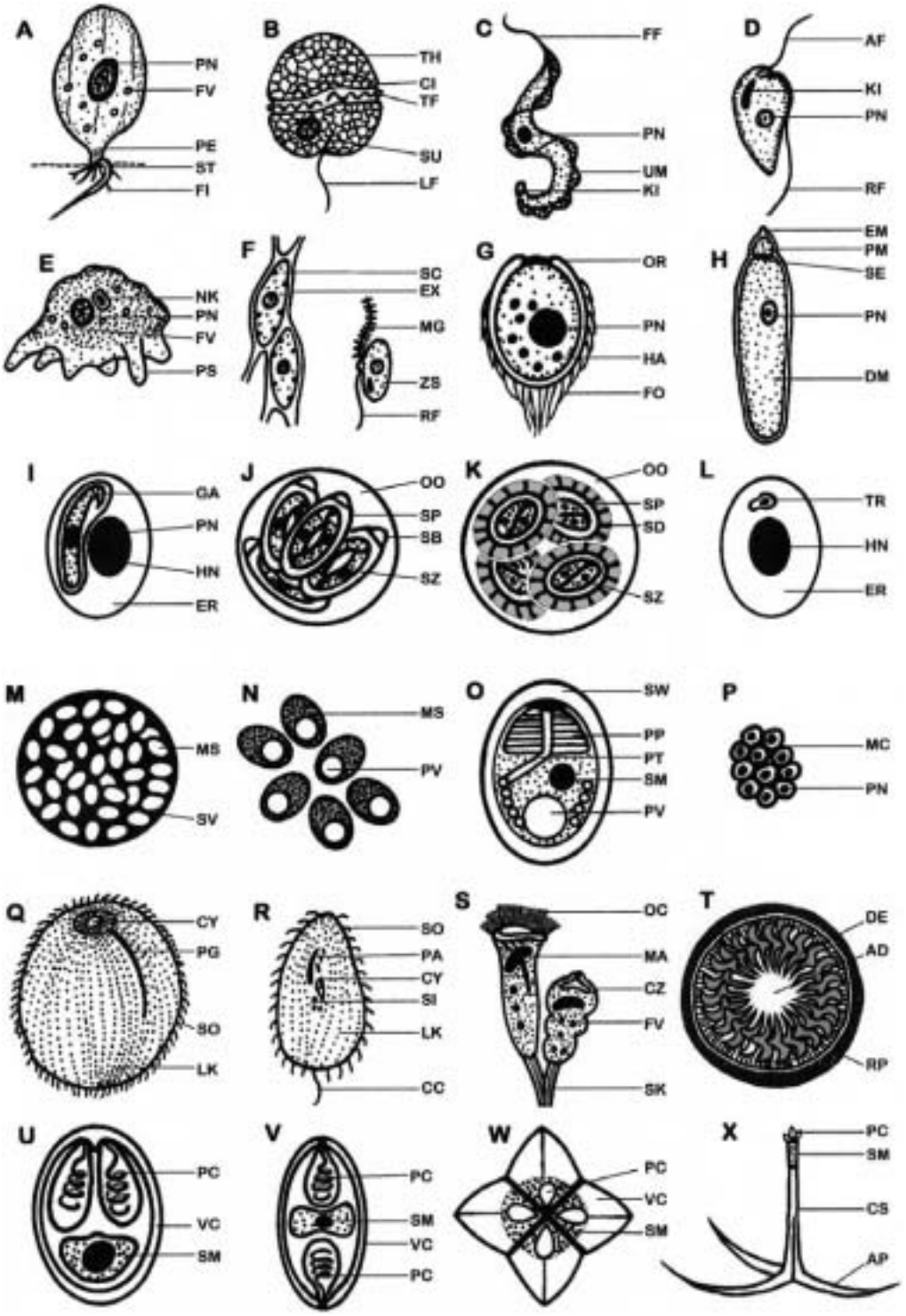
metazoan organisms. Instead, Protista have developed relatively complex subcellular features (membranes and organelles) which enable them to survive the rigours of their environments. The kingdom is not considered a natural assemblage of organisms but rather a classification of convenience, containing motile protozoal protists as well as non-motile algal and fungal protists. They exhibit enormous diversity in form and function, and some 40 phyla have been recognised on the basis of their unique morphology and biology. It has been conservatively estimated that there are about 100 000 extant species of Protista and they are considered ubiquitous as free-living organisms in terrestrial and aquatic habitats and as commensals, mutualists or parasites of most animals and many plants. A brief guide to the major protistan parasites of marine hosts is given below.

Mastigophora (flagellates)

The Mastigophora exhibit locomotion by flagella for most of their life-cycle. They possess one or more flagella (sometimes called kinetids, mastigonts or undulipodia); each arises from a small basal body (centriole or kinetosome) and contains two single central microtubules and nine peripheral doublets (2 + 9 configuration). Multiflagellated forms may be isokont (equal flagella) or heterokont (unequal flagella) and some species have a trailing (recurrent) flagellum often associated with an undulating membrane. Flagellates reproduce usually by longitudinal binary (symmetrogenic) fission between, rather than across, rows of flagella. Most flagellates are free-living aquatic organisms but representatives of several families (esp. dinoflagellates and kinetoplastids) parasitise marine hosts. Dinoflagellates have a distinctive haploid (dinokaryon) nucleus where the chromosomes remain condensed during interphase and appear as beaded threads as a result of low levels of histones and associated proteins. Over 4000 species have been identified, most being free-living pelagic or planktonic autotrophs, while some 140 species are

Figure 2.1 (see opposite) Stylised drawings of representative protista parasitic in marine organisms (figures vary in scale, approximate length or diameter indicated). **A**. *Amyloodinium* trophont from fish skin (100 µm). **B**. Free-swimming gymnodinid-type dinospore (20 µm). **C**. *Trypanosoma* trypomastigote from fish blood (40 µm). **D**. *Cryptobia* trophozoite from fish gills (10 µm). **E**. *Neoparamoeba* trophozoite from fish gills (30 µm). **F**. *Labyrinthula* spindle cells from marine plant and free swimming zoospore (15 µm). **G**. *Haplosporidium* spore from hepatopancreas from oyster (5 µm). **H**. *Nematopsis* gamont from gut of crab (80 µm). **I**. *Haemogregarina* gamont in fish erythrocyte (12 µm). **J**. *Eimeria* oocyst from gut of fish (15 µm). **K**. *Calyptospora* oocyst from hepatocytes of fish (25 µm). **L**. *Haemohormidium* trophozoites in fish erythrocyte (2 µm). **M**. *Glugea* cyst from subcutaneous tissues of fish (50 µm). **N**. *Loma* spores from gills of fish (4 µm). **O**. Ultrastructure of *Pleistophora* spore from muscles of fish (4 µm). **P**. *Mikrocytos* microcells from digestive gland of oyster (1 µm). **Q**. *Cryptokaryon* trophont from skin of fish (100 µm). **R**. *Uronema* trophont from brain of fish (30 µm). **S**. *Epistylis* zooids from exoskeleton of decapod crustacean (50 µm). **T**. *Trichodina* trophont from skin of fish (60 µm). **U**. *Myxobolus* spore from gills of fish (15 µm). **V**. *Myxidium* spore from kidney of fish (10 µm). **W**. *Kudoa* spore from muscles of fish (10 µm). **X**. Triactinomyxid actinospore released from oligochaete (100 µm).

Key to abbreviations used in Figure 2.1: AD, adhesive disc; AF, anterior flagellum; AP, anchor-like protrusions; CC, caudal cilium; CI, cingulum; CS, central stem; CY, cytostome; CZ, contracted zooid; DE, denticles; DM, deuteromerite; EM, epimerite; ER, erythrocyte; EX, extracellular matrix; FF, free flagellum; FI, filiform projections; FO, filamentous ornaments; FV, food vacuole; GA, gamont; HA, haplosporosomes; HN, host cell nucleus; KI, kinetoplast; LF, longitudinal flagellum; LK, longitudinal kineties; MA, macronucleus; MC, microcells; MG, mastigonemes; MS, microspores; NK, Nebenkörper (parasome, a kinetoplastid endosymbiont); OC, oral ciliature; OO, oocyst; OR, orifice; PA, paroral membrane; PC, polar capsule; PE, peduncle; PG, postoral groove; PM, protomerite; PN, parasite nucleus; PP, polaroplast; PS, pseudopodia; PT, polar tube; PV, posterior vacuole; RF, recurrent flagellum; RP, radial pins; SB, Stieda body; SC, spindle cell; SD, sporopodia; SE, septum; SI, scuticum; SK, stalk; SM, sporoplasm; SO, somatic cilia; SP, sporocyst; ST, stomopode; SU, sulcus; SV, sporophorous vesicle; SW, spore wall; SZ, sporozoite; TF, transverse flagellum; TH, theca; TR, trophozoite; UM, undulating membrane; VC, valve cell; ZS, zoospore.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parasitic in zooplankton, filamentous algae or on the external surfaces (and gills) of crustaceans and fishes (Fig. 2.1A). Parasitic trophonts often develop elaborate holdfast attachments and may become macroscopic as they feed to repletion. They then drop from the host and form motile biflagellated dinospores, with a transverse flagellum lying in an equatorial cingulum and a posterior flagellum often lying in a longitudinal ventral sulcus (Fig. 2.1B). Many dinospores are encased in armour (theca) composed of cellulosic plates. Most species contain chloroplasts while others have coloured pigments; some pigments are neurotoxic to mammals when concentrated in the tissues of fish or filter-feeding shellfish.

Kinetoplastid flagellates are characterised by the possession of extranuclear DNA (kinetoplast) within the single large mitochondrion usually associated with the flagellar basal body. Over 500 species have been described and many species are parasitic in vertebrate and invertebrate hosts with simple monoxenous (one-host) or more complicated heteroxenous (two-host) life-cycles. Trypanosomes found in the bloodstream of fishes are transmitted by leech vectors. They form characteristic trypomastigotes with a single recurrent flagellum that adheres to the cell body and becomes an undulating membrane (Fig. 2.1C). Infections are usually chronic but some have been associated with tissue pathology and mortality. Bodonids have two unequal flagella arising from a deep flagellar pocket (Fig. 2.1D) and several species are endozoic or ectozoic parasites in fishes where they cause local irritation, degenerative changes and erratic behaviour.

Sarcodina (amoebae)

Amoebae exhibit locomotion by the formation of pseudopodia (false feet) or by distinct protoplasmic flow. Amoeboid movement is also used by many species to engulf and ingest food items (phagocytosis). They reproduce by binary fission where trophozoites undergo nuclear division (karyokinesis) then cytoplasmic division (cytokinesis). Rhizopod amoebae form broad lobopodia, filamentous filopodia or reticular anastomosing reticulopodia and they may be testate (producing a shell or test) or naked (without a test). Most species are free-living aquatic or terrestrial organisms although a small number of naked gymnamoebae are parasitic in animals (often as opportunistic histophages). Several *Neoparamoeba* and *Paramoeba* species, with a unique parasome (or Nebenkörper, recently revealed to be a kinetoplastid endosymbiont) adjacent to the nucleus (Fig. 2.1E), have been linked to disease and death in marine fish and invertebrates.

Labyrinthomorpha (slime nets)

Labyrinthomorpha do not produce orthodox pseudopodia but form elaborate networks where trophozoites are associated with, sometimes appearing to glide along, ectoplasmic slime channels (Fig. 2.1F) secreted by special organelles (called sagenogenetosomes or sagenogens). Many have recently been shown to undergo reproductive cycles involving the formation of heterokont biflagellated zoospores (Fig. 2.1F). About 30 species have been described as saprobic or parasitic on marine molluscs, algae and vascular plants, sometimes in association with wasting diseases.

Haplosporidia (haplosporidians)

Haplosporidia are characterised by the formation of unicellular spores (without polar filaments) that contain a single sporoplasm and several dense organelles (known as haplosporosomes). The spore wall has an orifice covered by an operculum or occluded by a lingula plug. The spores are covered with filamentous ornaments that sometimes appear as tails (Fig. 2.1G). No complete life cycle has been elucidated for any haplosporidian and the fate of spores is unknown. In the final host uninucleate cells undergo modified schizogony that gives rise to multinucleate plasmodia which develop into sporonts and eventually differentiate into spores. Some 40 species are found as histozoic or coelozoic parasites of aquatic molluscs, annelids, crustaceans and helminths, and several species cause significant oyster diseases throughout the world.

Apicomplexa (sporozoans)

The spore-forming parasites Apicomplexa possess a distinctive apical complex of organelles, comprising a conoid, polar ring, rhoptries, micronemes and subpellicular microtubules, which facilitate entry into host cells as they are obligate intracellular parasites for most of their life cycles. They undergo cyclic development involving three divisional processes: merogony (schizogony), gamogony and sporogony. Cell division may occur by fission (splitting of the maternal cell) or endogeny (internal formation of daughter cells). Over 8000 species have been described as monoxenous or heteroxenous parasites of vertebrate and invertebrate hosts. Representatives of most apicomplexan groups (gregarines, haemogregarines, coccidia and haematozoa) are found in marine hosts.

Gregarines form large extracellular gamonts which may be septate (cephaline) or aseptate (acephaline); the former being divided by a septum into an anterior protomerite and a posterior deutomerite (Fig. 2.1H). The conoid is modified, forming an anterior holdfast organelle (epimerite in septate species or mucron in aseptate species). Equal numbers of gametes are produced by male and female gamonts. Most species have monoxenous life cycles in the digestive tracts or body cavities of invertebrates and lower chordates although some have heteroxenous life-cycles cycling between molluscan and crustacean hosts involved in predator–prey relationships.

Haemogregarines are adeleorin coccidia which form small intracellular gamonts; microgamonts producing from 1 to 4 non-flagellated microgametes which associate pairwise with macrogametes (syzygy). Over 400 species have been recorded as heteroxenous parasites in vertebrate leucocytes and erythrocytes (Fig. 2.1I) with haematophagous invertebrates acting as vectors. Species in fish are transmitted by leeches and infections are usually mild and chronic although some have been associated with severe disease.

Coccidian parasites form non-motile resistant oocysts that contain infective sporozoites usually confined within secondary spores (sporocysts). The gamonts of eimerian coccidia develop separately and many flagellated microgametes are produced. Over 200 species have been described in fish predominantly on the basis of oocyst morphology and host occurrence. Most fish coccidia sporulate endogenously and the oocyst envelope is thin and fragile and never contains a micropyle. The sporocysts vary markedly in appearance, some with Stieda bodies (Fig. 2.1J), finger-like sporopodia (Fig. 2.1K), gelatinous coverings or geometric shapes. Infections may be confined to the gut or undergo extra-intestinal development leading to marked histopathological changes.

Haematozoa are small blood-borne parasites which undergo merogony and gamogony in vertebrate blood cells (Fig. 2.1L). They are transmitted by blood-sucking invertebrates where fertilisation occurs forming a motile zygote (ookinete). Gamonts do not exhibit syzygy and sporozoites are not enclosed within sporocysts. Two main groups are recognised in terrestrial vertebrates: pigment-forming haemosporidia with insect vectors and non-pigment forming piroplasms with arachnid vectors. Only around 10 species have been found in fish and they are transmitted by leech vectors.

Microsporidia (microsporans)

Microsporidia (also known as Microspora) are obligate intracellular parasites which lack mitochondria and form small unicellular spores. Over 1300 species have been described in invertebrates (especially insects) and lower (rarely higher) vertebrates. The parasites undergo cyclic merogony within host tissues followed by sporogony (often involving plasmotomy prior to sporoblastogenesis). Developmental stages may have single or paired nuclei (diplokaryotic) and they may be surrounded by a membranous sporophorous vesicle (pansporoblast) (Fig. 2.1M) or lie free in the host cell cytoplasm (Fig. 2.1N). All spores contain a unique coiled polar tube

which can be extruded to inject the infective sporoplasm into host cells (Fig. 2.1O). Nearly 100 species occur in fish and infections may be disseminated throughout the tissues or they may cause focal lesions, inflammation and granulomas. Some species cause extensive hypertrophy of the host cell producing large xenomas.

Mikrocytos (microcells)

These enigmatic organisms are unicellular parasites characterised by the formation of small (1–2 μm) ovoid microcells with central nuclei (Fig. 2.1P). The classification of *Mikrocytos* is uncertain but they demonstrate many similarities to the haplosporidia. Several species have been described from the palps and mantle of molluscs (sometimes systemic) and infections have been associated with focal necrotic lesions and winter mortality in several oyster species.

Ciliophora (ciliates)

Ciliates are unique in that they possess two different types of nuclei (vegetative macronuclei and reproductive micronuclei), cilia (undulipodia) at some stage in their life cycle (kinetosomes and associated fibrils are organised into an infraciliature, even when cilia are absent) and the cell membrane is supported internally by membrane-bound alveoli. Asexual reproduction occurs by transverse (homothetogenic) binary fission across rows of cilia and some species exhibit sexual reproduction by conjugation. Most species are free-living aquatic or terrestrial organisms but many are commensals in vertebrate or invertebrate hosts and some are parasitic. About 150 species occur in fish, most as ectoparasites causing fouling, irritation and local lesions (occasionally penetrating wounds) and some as endoparasites causing variable damage at the tissue/organ level. Classification systems are based on multiple characters, including cilia organisation, kinetid ultrastructure, developmental cycles, life styles and habitats. Patterns of buccal (oral) and somatic (body) ciliation have been retained by many workers as user-friendly characters, although they may not reflect true phylogenetic relationships.Lower holotrichs exhibit little distinction between body and oral cilia. Several groups (gymnostomes, trichostomes and hypostomes) have been associated with skin, gill and internal lesions in freshwater fishes but relatively few species appear to be parasitic in marine fishes. Higher holotrichs have specialised oral cilia, usually comprising a paroral membrane adjacent to three membranelles. Several hymenostome species are notorious parasites of freshwater and marine fishes, causing white spot diseases. Large histophagous trophonts (Fig. 2.1Q) feed on epithelial tissues and form encysted stages (tomonts) in the external environment which produce hundreds of infective swarmers (tomites or theronts). Increasing numbers of scuticociliate species, with a non-ciliated scuticum or scutico-vestige (Fig. 2.1R), are being found to cause invasive systemic diseases in marine fishes and decapod crustaceans. Peritrichous ciliates have a conspicuous left-hand spiral of oral cilia and an antapical holdfast organelle. Many species are sessile for most of their life-cycles and they attach to substrates by means of a scopula or stalk (Fig. 2.1S). Many sessile species foul the external surfaces of fish and several have been implicated in hypoxic gill diseases. Other peritrichs are mobile and only attach temporarily to substrates using a concave adhesive disc reinforced with denticles (Fig. 2.1T). Most of these trichodinid species are ectoparasitic on fish and many cause skin lesions.

Myxozoa

Myxozoa form complex valved spores with polar capsules containing extrudible filaments (which are used for attachment to host cells and not to inject the infective sporoplasm). Their development involves multicellular differentiation of valvogenic, capsulogenic and sporoplasmic cells, which does not conform with the unicellular definition of Protista. Recent molecular studies suggest they are bilaterian metazoans but they continue to be documented with Protista

for historical reasons. Over 2770 species have been described, most as coelozoic or histozoic parasites in the organ cavities and tissues of fish although some are found in amphibia, reptiles and various invertebrates. Many infections are asymptomatic provoking little inflammation but some may cause tissue hyperplasia, unsightly cysts, erosive and necrotic lesions, myoliquefaction and deformities. Myxozoa are differentiated on the basis of spore morphology into bivalved (Figs 2.1U and V) and multivalved (Fig. 2.1W) species. The life cycles of several species (mainly from freshwater fishes) have been found recently to involve cyclic development between myxosporean stages in fishes and actinosporean stages in invertebrates, notably oligochaetes. Actinospores appear different and many have elongate protrusions to aid in flotation and anchorage (Fig. 2.1X).

Conclusion

The diversity of protistan organisms is well appreciated both in terms of their structural heterogeneity as well as their species richness, distribution and abundance. They are widespread in most environments and many species affect animal, water and soil health. Studies on marine protistan parasites must concentrate not only on the parasites themselves but also on host interactions culminating in disease. Detailed information is required on parasite morphology, development and virulence as well as host range, susceptibility and pathology in order to develop appropriate treatment, prevention or control strategies.

Important references

Comprehensive and well-illustrated texts on all the groups discussed here can be found in Margulis *et al.* (1990), Harrison and Corliss (1991), Lee *et al.* (2000), and Mehlhorn (2001). Lom and Dyková (1992) provided a detailed account of protistan parasites of fishes.

'Sarcomastigophora' (amoebae and flagellates)

Barbara F Nowak

Introduction

The 'phylum' Sarcomastigophora consists of a diverse group of protozoans. Historically, three 'subphyla' were included in it, the Mastigophora, Opalinata (now included as a class in the Heterokonta, phylum Chromista) and Sarcodina. Mastigophora are flagellates, their trophozoites use one or more flagella for locomotion. Members of the Subphylum Opalinata have numerous short flagella, which make them superficially similar to slowly swimming ciliates. The Subphylum Sarcodina includes the amoebae, characterised mainly by the use of pseudopodia for movement; flagella are uncommon and if they are present it is only in some developmental or sexual stages.

A single genus of the Opalinata contains marine species, whereas many species of flagellates and amoebas infect marine hosts. However, flagellates and amoebae have been little studied and estimates of species numbers are therefore impossible.

Morphology and diversity

The Subphylum Mastigophora includes the blood parasitic trypanosomatids, ectoparasitic bodonids and dinoflagellates, and the diplomonads, which are most commonly endocommensals of the intestine but can also colonise the gall bladder and other internal organs affecting fish health. Few flagellates are intracellular parasites. In the Subphylum Opalinata only the genus *Protoopalina* parasitises marine fish. Some species from this genus are symbiotic or commensals, living in the intestine or rectum of their hosts. Subphylum Sarcodina includes the amoebae,

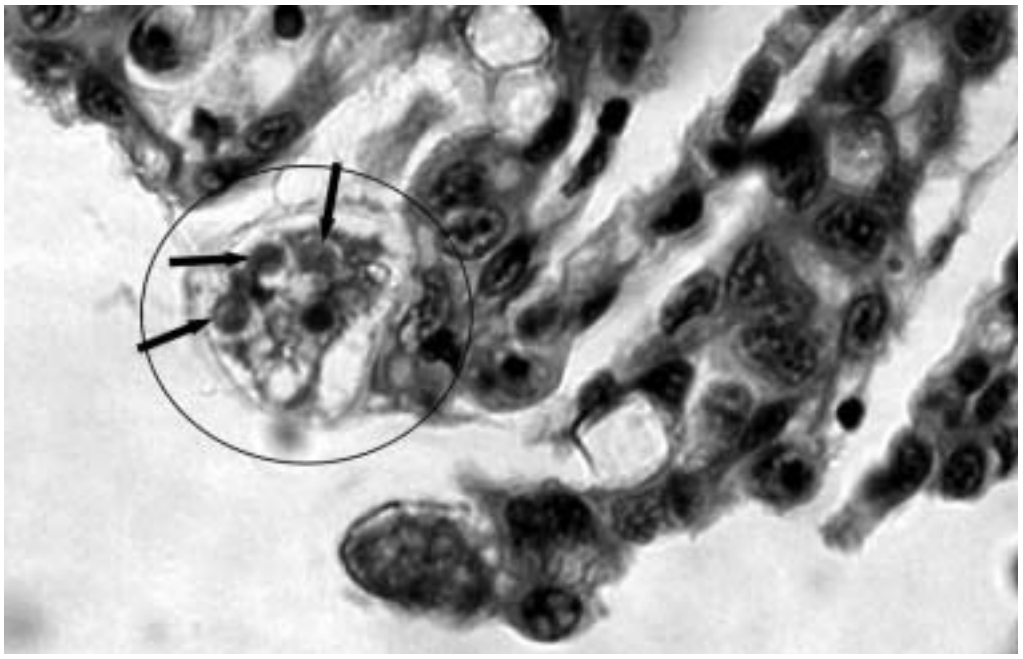

Figure 2.2 Histological section showing paramoeba (circled) on the gills of Atlantic salmon, *Salmo salar*. Note the presence of three parasomes (arrows).

most of which are amphizoic organisms, free living but able to colonise fish and cause significant pathology. A few species (e.g. *Entamoeba* or *Schizoamoeba*) are endocommensals, but can become parasitic.

Amoebae are usually difficult to identify and only now through the combination of molecular taxonomy, observation of living specimens and transmission electron microscopy can they be well characterised. Members of the Family Paramoebidae contain eukaryotic endosymbionts, known as parasomes (Fig. 2.2). Ultrastructural morphology of parasomes was first described from parasitic amoebae of chaetognaths, *Janickina pigmentifera* and *J. chaetognathi* (Hollande 1980). The endosymbiont was considered to be a kinetoplast, related to kinetoplastid flagellates and named *Perkinsiella amoebae* (Hollande 1980). A single, giant kinetoplast-mitochondrion is present in the usually binuclear symbiont (Dyková *et al*. 2003). The parasomes are usually associated with the amoeba nucleus. The number of parasomes may vary and in culture conditions it is usually reduced to one in each amoeba. The relationship between the amoeba and endosymbiont is stable and hereditary and neither of them can exist without the other. Recent phylogenetic analysis of the Small Subunit (SSU) rRNA gene sequence from different *Neoparamoeba* strains indicated a close relationship of the endosymbiont with the flagellate *Ichthyobodo necator* (see Dyková *et al*. 2003). The exact relationship between the endosymbiont and amoeba and the evolutionary origin of the endosymbiont are not known; however, Dyková *et al*. (2003) suggested that the symbiotic association was established in the early phase of kinetoplastid evolution.

Life cycles

Most parasitic flagellates have a simple, one-host life cycle. They reproduce by longitudinal binary fission. Some species can form resistant cysts. The life cycle of parasitic dinoflagellates includes a feeding stage (trophont), living on the host, a stage off the host which undergoes a

series of divisions (tomont) and a free-swimming infectious stage, called the dinospore or gymnospore. Kinetoplastids include the genus *Trypanosoma*, members of which live in the blood of hagfish, elasmobranchs and teleosts. These parasites have a complex life cycle, with several developmental stages within an intermediate host (the leech) including amastigote (stage without flagella), sphaeromastigote, epimastigote and trypomastigote. Infective parasites can be present in a leech for more than two years. In fish, trypanosomes undergo morphological changes, involving small, intermediate and large forms. Life cycles of amoebae are direct. Usually, they do not have distinct life stages; however, some species have flagellated stages or form protective cysts. Only intrusive species (Acanthoamoebidae) of marine Gymnoamoebae produce cysts (Page 1983). Amoebae reproduce by binary or multiple fission.

Effects on hosts and ecological importance

Most Sarcomastigophora are facultative ectoparasites. Some are commensals, for example intestinal amoebae and flagellates; however, these species include potential pathogens. Finally, trypanosomes are obligatory parasites. Most sarcomastigophorans do not seem to be host specific but susceptibility to infection can be species specific. Most of the parasitic species result in a significant host response, in some cases severe enough to affect the host even when the parasite is removed. Severity and rate of infection is temperature dependent. In the case of ectoparasites it can be also related to salinity, and changes in salinity have been used to control disease outbreaks. Whereas salinity usually affects the parasite, temperature has an effect not only on the parasite, but mostly on the host, with immunosuppression common in temperatures lower than the host optimum and stress in temperatures above the host optimum.

Species from the Family Paramoebidae have been shown to be parasitic to crustaceans, echinoderms and fish. *Neoparamoeba pemaquidensis* has been implicated as a cause of outbreaks of Amoebic Gill Disease (AGD) in salmonids cultured in the marine environment and other cultured marine fish species. Interestingly, AGD could not be found in wild fish species, even in wild fish cohabiting with infected Atlantic salmon in their cages (Douglas-Helders *et al*. 2002). This disease has a significant economic effect on salmonid aquaculture in Tasmania, Australia. AGD is defined as the presence of amoebae with parasomes that are in association with characteristic histological changes in gill tissue, including severe hyperplasia of lamellar epithelium and inflammatory response. *Neoparamoeba pemaquidensis* is widely distributed in temperate marine environments and has been isolated from water, sediments and biofouling invertebrates. Its presence is not confined to mariculture areas. No ultrastructural differences have been found so far between different isolates from fish and environmental samples (Dyková *et al*. 2000). Recently, another species of *Neoparamoeba*, isolated from fish gills and associated with AGD infections, has been described (Dyková *et al*. 2005). Further research is required to determine the role of *Neoparamoeba* species in AGD outbreaks.

Flagellates are mostly free living and can be autotrophic or heterotrophic. Autotrophic flagellates rarely become parasitic. Exceptions include *Amyloodinium pilularis* and *A. ocellatum*, both ectoparasitic dinoflagellates affecting marine fish in tropical aquaria. *Amyloodinium ocellatum* is unique, parasitising both elasmobranchs and teleosts (Lawler 1980). It is an obligate parasite causing one of the most significant diseases of temperate and warm-water marine fish over a wide range of temperatures and salinities. Dinoflagellates are considered to be algae by botanists; however, zoologists classify them as protozoans. Molecular studies suggest that *Pfiesteria*-like dinoflagellates and *Pfiesteria piscicida* are closely related to the parasitic *A. ocellatum* (see Litaker *et al*. 1999). The nature of interaction between *Pfiesteria* spp. and fish has been disputed in recent years. Initially, *Pfiesteria* spp. was reported to produce ichthyotoxin (Burkholder *et al*. 1992); however, attachment of the dinoflagellate to fish epithelium and its damage is the only proven cause of fish mortality (Vogelbein *et al*. 2002). *Ichthyobodo necator*,

typically an ectoparasite of freshwater fish, has been reported from marine fish and salmonids after transfer to marine farms (Lom and Dyková 1992). *Ichthyobodo* isolates from the gills of Atlantic salmon reared in fresh, brackish and sea water were nearly 100% identical; however *Ichthyobodo* isolates from the gills of Atlantic cod (*Gadus morhua*) were not closely related to the species affecting salmonids (Todal *et al.* 2004), suggesting that this genus may be more diverse than suggested by their morphology.

From an ecological point of view, sarcomastigophorans form an interesting group, with examples of mixotrophic dinoflagellates, which derive some of their nutrition from autotrophy and some from their hosts. Many species are free living and become parasitic only under particular, as yet not fully understood conditions. The prevalence of some species of trypanosomes is stock specific and has been used as a parasite tag to differentiate between fish stocks for management. For example, *Trypanosoma murmanensis* in Atlantic cod from Newfoundland varied from 4% to 94% in different stocks (Khan *et al.* 1980). Overall, the ecological significance of sarcomastigophorans increases in artificial systems where they can cause substantial losses.

Important references

Lom and Dyková (1992) provide the most detailed description of morphology, taxonomy and life cycles of sarcomastigophorans. A detailed discussion on species descriptions of diplomonad flagellates from fish, using ultrastructural features and culture is covered by Poynton and Sterud (2002).

Labyrinthomorpha (labyrinthomorphs)

Susan M Bower

Introduction

The Labyrinthomorpha (a phylum included in the subkingdom Protozoa by Levine *et al.* 1980) that are pathogenic to shellfish are all thraustochytrids which have been grouped with the lower fungi (slime moulds in the phylum Labyrinthulomycota) and were included in the heterotrophic stramenophiles group by Patterson (2000). The most characteristic feature of thraustochytrids is a unique organelle called the sagenogenetosome (bothrosome or sagenogen).

Morphology and diversity

Although most thraustochytrids are free living and usually associated with organic detrital materials, a few species have been associated with disease in molluscs. In three cases, unidentified thraustochytrids were involved in surface lesions on captive octopus, nudibranchs and squid in the northern hemisphere (Bower 1987a). In addition, *Labyrinthuloides haliotidis* (Fig. 2.3A) was a pathogen of cultured small juvenile abalone (*Haliotis kamtschatkana* and *Haliotis rufescens*) in British Columbia, Canada (Bower 1987a) and an unnamed species, commonly called Quahog Parasite Unknown (QPX), has been associated with mortalities and lesions in hard clams (*Mercenaria mercenaria*) on the eastern seaboard of North America (Whyte *et al.* 1994, Ragone Calvo *et al.* 1998).

In the mollusc host, the vegetative stage (single nucleated organism also called thallus or trophozoite) of parasitic thraustochytrids are usually spheroid and about 2 μm to 10 μm in diameter. In abalone, *L. haliotidis* multiplies by simple binary fission (Bower 1987a). However, in hard clams, QPX develops sporangia from enlarged vegetative cells (10–15 μm in diameter) which undergo endosporulation. Mature sporangia (18–25 μm in diameter) of QPX contain 20 to 40 endospores (immature vegetative cells, 1.5–2 μm in diameter) each with a basophilic cell wall (Smolowitz *et al.* 1998, Ragone Calvo *et al.* 1998). Both pathogens produce biflagellated

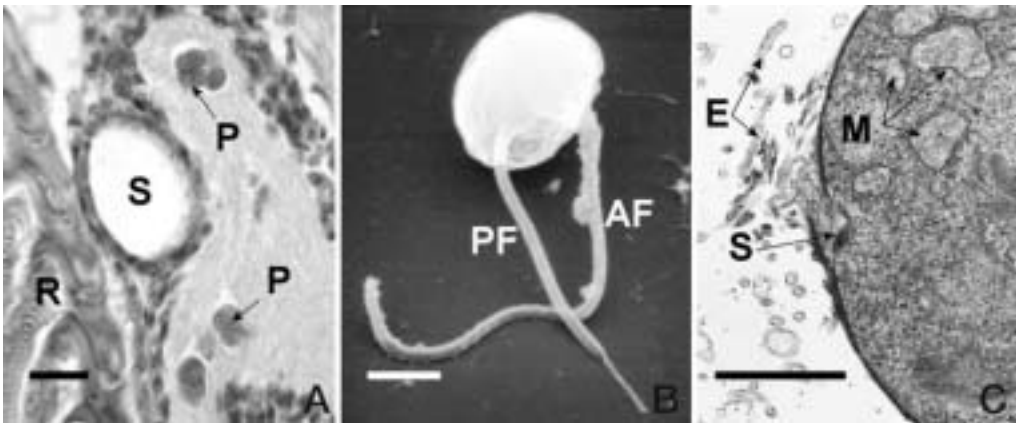

Figure 2.3 *Labyrinthuloides haliotidis*. **A**. Histological section of *L. haliotidis* (P) in the nerve ganglion of *Haliotis kamtschatkana* adjacent to the statocyst (S) and radula (R). Scale bar = 1.5 μm. **B**. Scanning electron micrograph of a zoospore of *L. haliotidis* from sea water. Note the subapical attachment site of the two flagella, the coarse texture of the longer anterior flagellum (AF) where debris has attached to the mastigonemes and the tapered tip of the short glabrous posterior flagellum (PF). Scale bar = 2.5 μm. **C**. Transmission electron micrograph of a *L. haliotidis* from liquid culture media showing the sagenogenetosome (S) on the periphery of a cell that was actively producing an ectoplasm net (E), which consists of a unit membrane with no evidence of cell organelles internally. Mitochondrial profiles (M) containing tubular cristae are evident adjacent to the sagenogenetosome. Uranyl acetate and lead citrate stain. Scale bar = 1 μm.

zoospores (Fig. 2.3B) when transferred to sterile sea water. The zoospores are uninucleate and slightly oval (about 5 μm long and 3.5 μm wide) with two laterally attached flagella. The anterior flagellum (about 12 μm in length) has a brush of mastigonemes along one side and the posterior flagellum (5–10 μm in length) is glabrous and has a tapered tip (Bower 1987a).

The most characteristic feature of thraustochytrids is a unique organelle called the sagenogenetosome (bothrosome or sagenogen) on the cell surface from which arises the ectoplasm net consisting of a unit membrane tube containing no cell organelles (Fig. 2.3C). In the sagenogenetosome an electron-dense plug separates the cell cytoplasm from the ectoplasmic network. Although typical sagenogenetosomes and ectoplasmic nets were absent or very rare in QPX in the clam host and in nutrient culture media where the parasite was usually embedded in a gelatinous matrix or mucofilamentous net (Whyte et al. 1994), Kleinschuster et al. (1998) reported the development of an ectoplasmic net in cultured QPX that had been transferred to sterile sea water. In addition to sagenogenetosomes and ectoplasmic nets, thraustochytrids have scale-like laminated cell walls.

Life cycles

All known parasitic thraustochytrids have direct life cycles. QPX may be an opportunistic facultative parasite not dependent on a parasitic way of life because it appears to be a ubiquitous member of the normal marine and bivalve flora on the east coast of North America. Possibly, *M. mercenaria* disadvantaged in some way (e.g. unfavourable environmental interactions including high planting densities and poor husbandry) may be more susceptible to infection with increased risk of disease development. The same may be true for *L. haliotidis* but nothing is known about its occurrence in the marine environment. Nevertheless, the life cycle of *L. haliotidis* in captive abalone and *in vitro* has been described (Bower 1987a, c) and is similar to that of QPX.

The vegetative cell of *L. haliotidis* removed from a source of nutrients (i.e. placement in sterile sea water), develops by synchronous multiple fission to form a zoosporoblast (6–10 μm in diameter) containing about 10 biflagellate zoospores which escape through a rupture in the zoosporoblast wall. The flagella were shed when the zoospore contacted a hard surface or after about 24 hr of active swimming in sea water. The resulting cell was morphologically similar but slightly smaller than the vegetative stage and survived in sterile sea water at about 5°C for at least two years. Vegetative stages that developed from zoospores were infective to small abalone. Within 4 hr of contacting the host, sagenogenetosomes produced extracellular lytic activity that disrupted the plasmalemma layer of the host epithelial cells adjacent to the parasite, eventually lysing the host cell. By 24 hr post exposure, the ectoplasmic net was well developed, allowing the parasite to move into and within the head and foot tissues of the abalone and dividing forms of the parasite were observed (Bower *et al.* 1989). Division was rapid and tiny abalone were quickly overrun by *L. haliotidis*. As dead abalone decomposed, vegetative stages released from the tissues developed into zoosporoblasts that produced zoospores within about 24 hr to 72 hr. Parasites released from infected abalone were infective to other small abalone on contact.Although alternate hosts have not been described for QPX and *L. haliotidis*, these thraustochytrids can utilise diverse sources of nutrients *in vitro*. Small juvenile Japanese scallops (*Patinopecten yessoensis*) and juvenile Pacific oysters (*Crassostrea gigas*) both less than eight months of age were resistant to infection with *L. haliotidis*. However, juvenile oysters with badly cracked shells became infected suggesting that *L. haliotidis* was capable of utilising oyster tissue as nutrients for growth and multiplication if it was able to gain access to the soft tissues of the oyster (Bower 1987b).

Effects on hosts and ecological importance

Labyrinthuloides haliotidis quickly multiplies in the tissue of it host. Within 10 days after exposure to about 10^4 parasites in 20 mL of sea water, about 90% of the abalone (<4.0 mm shell length and 140 days of age) died with numerous parasites throughout the head and foot (Bower 1987b). Tissues of heavily infected abalone were slightly swollen with a loss of integrity. The prevalence and intensity of infection decreased, and time to death increased as the abalone increased in age and size. Abalone, greater than 1.5 cm in shell length, could not be infected even when about 1.5×10^4 *L. haliotidis* were injected intramuscularly. The mechanism of defence against this parasite is not known.

Although *L. haliotidis* has only been observed in small abalone (<0.5 cm shell length), the high mortalities caused by infection were devastating to the abalone culture facility and this parasite was involved in the demise of an early attempt at abalone culture in British Columbia, Canada (Bower 1987a). Within two weeks of first being detected in a raceway, over 90% of the 100 000 small abalone in that raceway succumbed to infection and the disease quickly spread between raceways. The impact of *L. haliotidis* on wild abalone stocks and its geographical range are unknown because abalone, of the size that are susceptible to infection, are too tiny to be found in the wild.

Quahog Parasite Unknown (QPX) has been associated with mortalities and lesions (swellings and round yellow-tan nodules, 15 mm in diameter) in the mantle, often at the mantle edge, adjacent to the siphon or adductor muscle and gills of hard clams (Whyte *et al.* 1994, Ragone Calvo *et al.* 1998). The mucoid material produced by QPX may prevent phagocytosis by clam haemocytes and thus act as a pathogenic mechanism. However, phagocytic multinucleate giant cells of various sizes containing up to 25 nuclei and haemocyte encapsulation of QPX occur as part of the clam's response to infection (Smolowitz *et al.* 1998). Also, the haemocytic response was often associated with moribund looking QPX (Ragone Calvo *et al.* 1998). Nevertheless, QPX-infected clams grew more slowly and had a lower condition index than uninfected *M. mercenaria* (Smolowitz *et al.* 1998). Observations to date suggest that genetic variability in the host and/or in the QPX pathogen could be responsible for differences in susceptibility toward the

infection and in the presentation of the disease.In 1959 QPX was suggested to be the primary cause of significant wild *M. mercenaria* (quahog or hard clam) stock mortalities in New Brunswick and more recently was associated with 80% to 90% mortalities in juvenile *M. mercenaria* (up to 30 mm in shell length) in a nursery and up to 100% in hatchery broodstock on Prince Edward Island (Whyte *et al.* 1994). In addition, QPX has caused severe mortality (80–95% in some instances) in aquacultured and wild stocks of *M. mercenaria* along the north-eastern coast of the United States to at least Virginia (Ford *et al.* 2002). Mortality is usually most severe in the spring and summer months in *M. mercenaria* that are at least one year old. The dynamics of infection and pathogenicity under different holding and handling conditions will require more investigation if QPX proliferation in cultured *M. mercenaria* is to be circumvented (MacCallum and McGladdery 2000).

Important references

Further details, coloured illustrations and a complete list of references are available on the websites of Fisheries and Oceans Canada (2003, 2004).

Haplosporidia (haplosporidians)

Eugene Burreson

Introduction

The Haplosporidia is a small group of parasitic protists consisting of four genera and about 36 species. Molecular phylogenetic analyses support the Haplosporidia as a monophyletic phylum closely related to the phylum Cercozoa. Most species in the phylum have two life history stages: multinucleate plasmodia, and a resistant spore with an orifice at one end that is covered either by an external lid of spore wall material or an internal flange of spore wall material. Most species of Haplosporidia are histozoic in a wide variety of marine invertebrates, although one species occurs in freshwater invertebrates. Species in the genus *Urosporidium* are often hyperparasites.

Morphology and diversity

Historically, the group has been characterised by two life history stages—multinucleate plasmodia, and a resistant spore with an orifice covered either by an external lid of spore wall material (genera *Minchinia* and *Haplosporidium*) or an internal flange of spore wall material (genus *Urosporidium*). Multinucleate plasmodia (Fig. 2.4A) are generally from 5 μm to 20 μm in diameter, but can reach 50 μm or more with over 100 nuclei. Plasmodia of all species are similar and cannot be used to distinguish species. Spores range in length from 4 μm to 12μm depending on the species. The spore stage (Fig. 2.4B) in *Haplosporidium* spp. has ornamentation consisting of tails or wrappings composed of spore wall material. The spore stage of *Minchinia* spp. has ornamentation composed of epispore cytoplasm. Spore ornamentation is an important taxonomic character, although it is usually visible only with scanning electron microscopy. Recent molecular phylogenetic studies have shown that the enigmatic genus *Bonamia* also is a haplosporidian (Carnegie *et al.* 2000, Reece *et al.* 2004). This genus consists of only three species, all of which infect haemocytes of oysters. The most commonly observed cell type in *Bonamia* spp. is a uninucleate 'microcell' from 2 μm to 3 μm in diameter (Fig. 2.4C), although multinucleate plasmodia also have been reported. No spore stage has ever been observed for any *Bonamia* species, although it is possible that the spore stage occurs in some host other than oysters and has not been discovered. If *Bonamia* spp. truly lack spores, then the definition of the Haplosporidia must be modified to include species that have a spore with an orifice as well as those microcell species that infect oyster haemocytes.

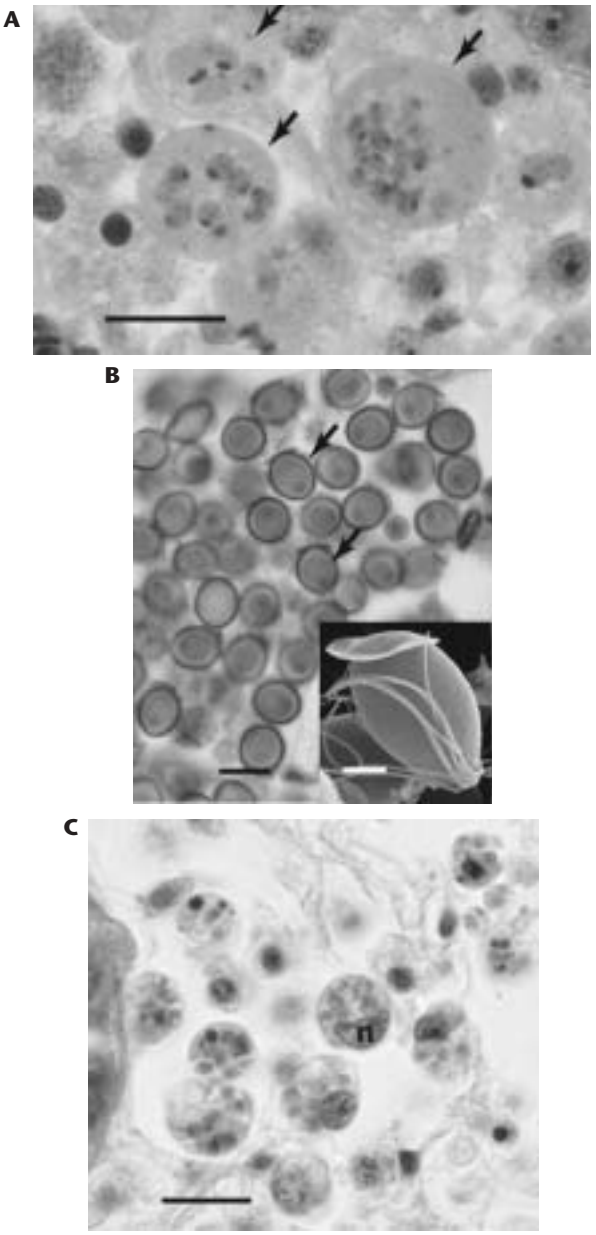

Figure 2.4 **A**. Multinucleate plasmodia (arrows) of *Haplosporidium nelsoni* in the eastern oyster, *Crassostrea virginica*, illustrating the eccentric nucleolus. Plastic section cut at 1 µm and stained with toluidine blue. Scale bar = 10 µm. **B**. Spores of *Haplosporidium louisiana* from the mudcrab, *Panopeus herbstii*. Arrows point to spores demonstrating the typical external lid covering the spore orifice that is present in *Haplosporidium* and *Minchinia*. Scale bar = 10 µm. Inset: Scanning electron micrograph of spore of *H. pickfordi* illustrating the spore lid and spore ornamentation originating from the aboral end. Scale bar = 2 µm. Reproduced from Burreson, EM, Spore ornamentation of *Haplosporidium pickfordi* Barrow, 1961 (Haplosporidia), a parasite of freshwater snails in Michigan, USA, *Journal of Eukaryotic Microbiology* 48(6): 622–626, with permission of the *Journal of Eukaryotic Microbiology*. **C**. Microcells of *Bonamia ostreae* infecting haemocytes of the flat oyster, *Ostrea edulis*; n = nucleus of one infected haemocyte. Nuclei of many other infected haemocytes are condensed and necrotic. Scale bar = 8 µm.

Life cycles

In the traditional spore-forming Haplosporidia, multinucleate plasmodia divide by plasmotomy and eventually undergo synchronous sporulation. Resistant spores are released into the environment, usually upon death of the host. The fate of spores is unknown, but they do not seem to be infective to the host in which they are produced. Repeated attempts by many investigators to transmit *Haplosporidium nelsoni* directly from oyster to oyster by cohabitation or injection of plasmodia or spores have been unsuccessful, and most believe that spores are infective to an intermediate host that is a necessary component of the life cycle (Haskin and Andrews 1988). There is one report of direct transmission of *Haplosporidium pickfordi* to freshwater snails using spores (Barrow 1961), but that study has been viewed with skepticism because of infected controls and needs to be repeated. *Bonamia* spp. can be directly transmitted by cohabitation (Elston *et al.* 1986) or injection of microcells and this is evidence that spores are not necessary for transmission and may have been lost in the *Bonamia* lineage (Reece *et al.* 2004).

Effects on hosts and ecological importance

The end result of infections by species of *Urosporidium*, *Minchinia* or *Haplosporidium* is the presence of large numbers of spores, often completely displacing the target tissue. These infections are often fatal; however, most species of spore-forming haplosporidians are rare and are not important pathogens because of their very low prevalence. Some species cause high mortality in commercially important oysters, however. The best studied haplosporidian is *Haplosporidium nelsoni*, causative agent of Multinucleate Sphere X (MSX) disease in oysters along the east coast of the United States of America (USA) and Canada. This parasite causes a general wasting disease resulting from increasing intensity of plasmodia. Epizootic mortality from *H. nelsoni* began in Delaware Bay in 1957 and in Chesapeake Bay in 1959. Over one million bushels of oysters (>35 million L) were killed by the parasite in each bay within a few years (Andrews 1968, Ford and Haskin 1982). Spread of the parasite along the entire east coast of the USA and into Atlantic Canada, and continuing annual mortality has severely impacted the oyster resource and industry in these areas. *Haplosporidium nelsoni* is a natural parasite of the Pacific oyster (*Crassostrea gigas*) in Japan and Korea, and there is strong evidence that the parasite was introduced to the east coast of the USA from the Pacific Ocean (Burreson *et al.* 2001). After nearly 50 years, some natural resistance to the parasite has developed in Delaware Bay oysters.

All species of *Bonamia* are pathogenic to their oyster hosts. *Bonamia ostreae* has decimated populations of the flat oyster (*Ostrea edulis*) in France (Grizel *et al.* 1988), *B. exitiosa* has caused extensive mortality in *Ostrea chilensis* in southern New Zealand (Hine 1996), and *B. roughleyi* is the cause of winter mortality disease in the Sydney rock oyster (*Saccostrea glomerata*), in southeastern Australia (Farley *et al.* 1988). Microcells of *Bonamia* spp. stimulate phagocytosis by host haemocytes and are not killed by the cellular defence mechanisms. Microcells proliferate and eventually lyse the haemocyte, releasing the microcells. The phagocytosis/proliferation/lysis cycle repeats resulting in massive infections of microcells and destruction of host haemocytes leading to death of the oyster.

Important references

The phylum Haplosporidia has been reviewed by Perkins (1990, 2000) and Burreson and Ford (2004). The most recent molecular phylogenetic analysis of the group is by Reece *et al.* (2004). Carnegie and Cochennec-Laureau (2004) reviewed the genus *Bonamia*.

Apicomplexa (sporozoans)

Kálmán Molnár

Introduction

The phylum Apicomplexa is a huge group including rather different protozoan parasites which have a special cell organelle, the apical complex, which facilitates invasion of the host cell. Apicomplexans have three developmental stages during their life cycles: merogony, gamogony and sporogony. Fish apicomplexans are divided into two major groups. Coccidia proper are primarily intestinal parasites and produce resistant oocysts in the host. Adeleid blood parasites (Coccidia *sensu lato*) have the merogonic and some gamogonic stages in fish, while spore formation takes place in parasitic annelids or gnathiid isopods.

Morphology, diversity and development of coccidian apicomplexans

Coccidia proper belonging to the suborder Eimeriorina comprise two families Eimeriidae (including genera *Eimeria*, *Goussia*, *Calyptospora*, *Crystallospora*) and Cryptosporidiidae (with a single genus *Cryptosporidium*). Most of the known coccidians develop in the gut but there are species developing in inner organs (i.e. the spleen, liver, kidney and swimbladder).

Systematics of eimeriid apicomplexans is based on the morphology of the spore, the oocyst. The oocysts of the Eimeriidae (Fig. 2.5) contain four sporocysts and, occasionally, one to two polar granules. Each sporocyst contains two sporozoites and a residual body. The only important difference between genera is in the structure of the sporocyst. Whereas the oocysts of terrestrial animals have resistant and thick oocyst walls, fish coccidia have a thin, sensitive oocyst wall without micropyle. The thickness of the one- or three-layered wall varies between 3 nm and 200 nm. Most fish coccidians have round, less frequently ellipsoidal oocysts. Only a few fish coccidia (e.g. *Eimeria isabellae*) possess a typical *Eimeria* sporocyst (i.e. having a Stieda body). In most species there is only a thickening, plug or cap at one end of the sporocyst. This is where the sporocyst opens and the sporozoites are released in the host or intermediate host. Sporocysts are elliptical, oval or dodecahedral in shape. The sporocyst wall is thin but usually composed of two layers.

Goussia-type sporocysts are composed of two equal-sized, round, elliptical or coffin-shaped valves united by a suture. This suture is hardly discernible using light microscopy but can be seen under an electron microscope. Sometimes the sporocyst is surrounded also by a membraneous veil, which is attached to the sporocyst by special membranes.The sporocysts of *Calyptospora* are characterised by a thickening or projection at the caudal end, by sporopodia arising from the spore surface or from the caudal projection, and a sporocyst veil supported by sporopodia and surrounding the sporocyst. The opening of the sporocyst is a longitudinal suture which extends only to the anterior one-third of the sporocyst.The sporocyst of *Crystallospora crystalloides* is bipyramidal and opens at a suture situated at the foot of the pyramids. *Cryptosporidium* oocysts contain four naked sporozoites and a residual body. There are reports both about thick-walled and thin-walled oocysts.The sporozoites of fish coccidia are banana-, sausage- or comma-shaped. They usually lie in the sporocyst in a head-to-tail presentation. In the Cryptosporidia the sporozoites are side by side and lie in the same direction. The sporozoite has a nucleus easily discernible by light microscopy and is situated in the middle of the body. The conoid apparatus lies at the anterior end, and the posterior end may sometimes be striated. An oocyst residuum exists only in a few species; all sporocysts, however, have a sporocyst residuum. This residuum is granular in the young oocysts and may be compact in the older ones.

Merogonic stages of fish coccidia are intracellular. They have two or three merogonic stages. The meronts develop in the cytoplasm, or occasionally in the nucleus. Usually 8–16 banana-

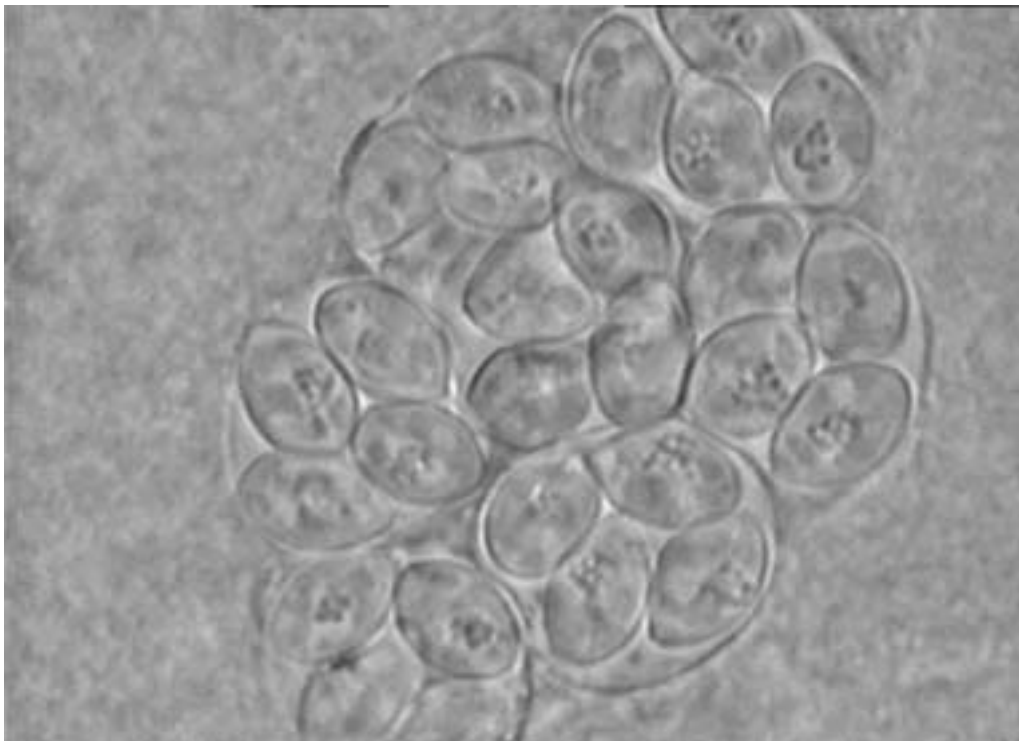

Figure 2.5 Sporulated oocysts of *Eimeria daviesae* from *Gobius kessleri,* a euhaline water fish. The oocysts contain four sporulated sporocysts with sporozoites and sporocyst residuum. × 2000.

shaped merozoites of 8–16 μm in length are formed in the meronts, but in *Goussia cichlidarum*, Landsberg and Paperna (1985) reported meronts containing large numbers of merozoites.

All coccidians develop in a parasitophorous vacuole. In most cases this vacuole is located inside the cytoplasm of the host cells but in case of epicellular development, the parasitophorous vacuole is intracellular but extracytoplasmal and it is covered only by a single unit membrane of the host cell. The merozoites of fish coccidia have a well-discernible nucleus, conoid apparatus, and trimembranous pellicle covering the merozoite. *Cryptosporidium* has incomplete vacuoles. The parasitophorous vacuole formed by enterocyte microvilli surrounds the meronts or gamonts only on the side facing the gut lumen. Between the parasite and the host cell cytoplasm there is a special adhesive zone. All stages of cryptosporidia develop epicellularly.

Gamogonic stages of coccidia comprise male and female developmental stages. Both microgamonts (male) and macrogamont (female) develop intracellularly in a parasitophorous vacuole. Depending on the parasite species the development can be intracytoplasmal, intranuclear or epicellular. Inside the round or ellipsoidal, 10 μm to 20 μm sized microgamonts a large number of microgametes develop after multiple divisions. The macrogamont which develops from the last merozoite generation is always surrounded by a parasitophorous vacuole. Its cytoplasm contains lipid granules and two distinct types of 'wall-forming body'. After being fertilised the macrogamont becomes a young oocyst.

It had been generally accepted that coccidia (among them fish coccidia) develop directly without intermediate or paratenic hosts. Observations made by Landau et al. (1975), however, suggested that vectors might transmit the infection. Experiments made by Molnár (1979), Paterson and Desser (1982) and Steinhagen and Körting (1990) showed that besides direct

transmissions, tubifex paratenic hosts served as vectors in infections of cyprinid fishes with enteric coccidia. However, Solangi and Overstreet (1980) and Fournie and Overstreet (1983) reported that *Calyptospora funduli* required a true intermediate host, the grass shrimp (*Palaemonetes pugio*) for its development.

Morphology, diversity and development of adeleid apicomplexans (*Coccidia* sensu lato)

Fish-parasitic Coccidia *sensu lato* have heteroxenous life cycles which involve two hosts, one being the fish and the other a parasitic leech or insect (gnathiid isopods). Levine (1988) classified adeleid apicomplexans into the suborder Adeleiorina, which has two families, Haemogregarinidae and Dactylosomatidae. Three genera, *Haemogregarina*, *Cyrilia* and *Desseria* belong to Haemogregarinidae and two genera, *Dactylosoma* and *Babesiosoma*, belong to Dactylosomatidae.

The development of coccidia *sensu lato* is divided into merogony, gamogony and sporogony. There is also a special association of the two gamonts prior to encystment (syzygy) and this takes place before sporogony.

Davies and Johnston (2000) believe that in the case of the haemogregarinids *Haemogregarina* and *Desseria*, merozoites are transmitted by the invertebrate host, while in that of *Cyrilia* sporozoites are transmitted. In the case of the dactylosomatid *Babesiosoma* merozoites are transmitted, while for *Dactylosoma* the type of transmission has not been demonstrated yet.

Khan (1980) described that sporozoites of *Cyrilia uncinata* were injected into the blood of fish by leeches which entered lymphocytes, monocytes, neutrophils or blast cells and they developed into meronts and formed merozoites. The merogony of *Haemogregarina* and *Cyrilia* spp. occurs in blood cells while in *Desseria* spp. it takes place mainly in the inner organs. Negm-Eldin (1999) described that *Cyrilia nili* had two successive types of merogonic stages in infected fish and the meronts of the second merogonic cycle were destined to form gamonts. The vermiform merozoites enter erythrocytes or leucocytes to form macrogamonts and microgamonts. In some species there is some division of gamonts in the erythrocytes (Fig. 2.6), while in others they directly change into microgamonts and macrogamonts.

Gamonts are taken in by a leech during a blood meal. In the intestine of the leech they are released from blood cells, and the microgamonts and macrogamonts unite in syzygy. During this process they are surrounded by a thin membrane. In *Haemogregarina* and *Cyrilia* the microgamont produces four microgametes, and one of the resulting microgametes fertilises the macrogamont. Oocyst formation takes place, and depending on the parasite species it may occur either within an enterocyte or on the surface in a parasitophorous vacuole. The sporozoites produced migrate toward the salivary gland and the proboscis of the leech. Intraleucocytic meronts of *Haemogregarina* spp. usually harbour two to eight merozoites and do not significantly enlarge the size of the host cell. The length of the banana- or crescent-shaped merozoites and gamonts varies between 4 μm and 5 μm; intraleukocytic meronts, however, may reach 26 μm × 23 μm.

Effects on hosts and ecological importance

Most fish coccidians have relatively low pathogenicity. No mortality was observed even when 85% to 90% of the liver was infected with *Calyptospora funduli* (Solangi and Overstreet 1980). Lethal infections occur primarily in farm ponds, but severe cases have been reported from natural waters as well. MacKenzie (1978) found a species of *Eimeria* sp. which caused 6% to 10% reduction in body mass in blue whiting, *Micromesistius poutassou*. Fiebiger (1913) as well as Odense and Logan (1976) reported mortality in the haddock caused by *Goussia gadi*. Grabda (1983), who investigated *Eimeria jadvigae* infection in *Coryphaenoides holotrachys*, reported thickening of the swimbladder wall. In more severe cases the inner surface of the bladder was

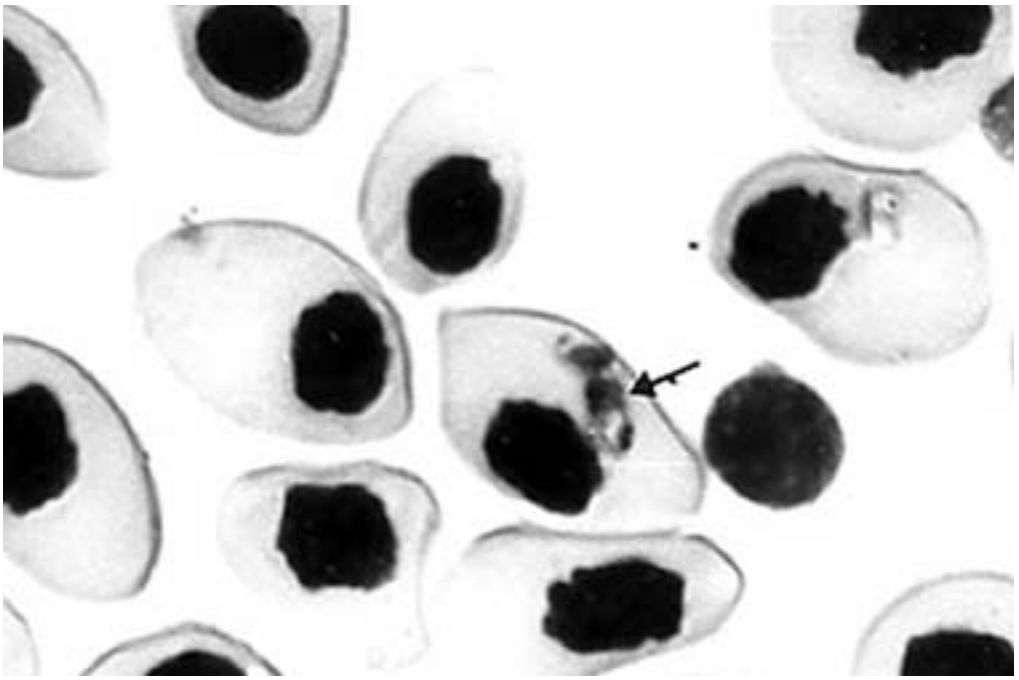

Figure 2.6 Gamont of a *Haemogregarina* sp. (arrow) in one of the Giemsa-stained erythrocytes of a Caspian sturgeon, (*Acipenser persicus*). × 3000. Courtesy of Dr Mahmoud Massoumian.

covered with a white substance, the wall had a spongy texture and the lumen was filled by a mucous exudate containing large numbers of oocysts. Pinto (1956) reported parasitic castration as a result of *G. sardinae* infection. Upton *et al.* (2000) supposed that a heavy infection of the gut with *E. phyllopterici* caused significant morbidity and mortality of the aquarium-cultured sea dragon, but the role of a joint infection with bacterial pathogens could not be excluded.

There is little information on the pathogenic effect of adeleid parasites. In cultured turbot Ferguson and Roberts (1975) reported a proliferative disease of the haematopoietic tissues; Kirmse (1980) found that up to 60% of some populations of *Haemogregarina sachai*-infected turbots were affected with gross tumours in the musculature and viscera. The lesions consisted of necrotic tissue with a caseous centre. Histologically, there was an accumulation of parasitised reticuloendothelial cells, cell debris and pycnotic nuclei. Parasitaemias of up to 36% of all blood cells were observed; most of the infected cells were neutrophils and monocytes. In mackerel caught in certain areas of the Atlantic, 4% of the leucocytes were infected with meronts, and meronts were demonstrated in 100% of impression smears from the spleen. Lesions in the spleen and kidneys contained *Haemogregarina*-like organisms and were often surrounded by a connective tissue capsule (MacLean and Davies 1990).

Important references

Systematics of apicomplexan parasites in this book is mostly based on Levine's (1988) classification, but additional data are available in Duszynski *et al.* (1999) on Coccidia proper. Biology of fish coccidians has been detailed by Davies and Ball (1993). Data on systematics, morphology and development of adeleid apicomplexans are found in Davies (1995) and Davies and Johnston (2000). Useful data on fish apicomplexans in general are summarised by Lom and Dyková (1992) and Molnár (1995).

Microsporidia (microsporans)

Elizabeth Moodie

Introduction

The Microsporidia, also known as Microspora, are a monophyletic phylum of tiny eukaryotic parasites. Growth and reproduction can occur only within host cells. Early research on the ribosomal genes of Microsporidia placed them at the base of the eukaryote evolutionary tree with other 'primitive' amitochondriate protozoans (Keeling and Fast 2002). Subsequent analyses of a variety of genes using more sophisticated methods have indicated that Microsporidia are close to the fungi. Molecular evidence for the relationship between Microsporidia and fungi is supported by biochemical and developmental features of the group (Keeling 2003). Studies on mitochondrial proteins (including Hsp70) have indicated that typical mitochondria have been secondarily lost, although remnants in the form of small membrane-bound organelles have been detected (Williams *et al.* 2002). Parsimonious features of the Microsporidia, including small genome size, prokaryote-like ribosomal genes and the loss of typical eukaryotic organelles are thought to be associated with the highly specialised lifestyle of these parasites (Mathis 2000, Weiss 2000).

Morphology and diversity

The parasites are transmitted between hosts by unicellular spores ranging in length from less than 1 μm to over 30 μm (Larsson 1999). Spores are able to infect new host cells by extruding their contents through the everted polar filament in a manner reminiscent of injection by a hypodermic syringe. In ungerminated spores, the polar filament lies coiled inside the cell (Fig. 2.7). Although the polar filaments of Microsporidia and Myxozoa are superficially similar, they have different origins. Microsporidia, which bear only one polar filament, are single-celled parasites believed to be closely related to Fungi. Myxozoa are of multicellular origin and are thought to be closely related to the Cnidaria. They bear one to four polar filaments, each derived from a different cell.

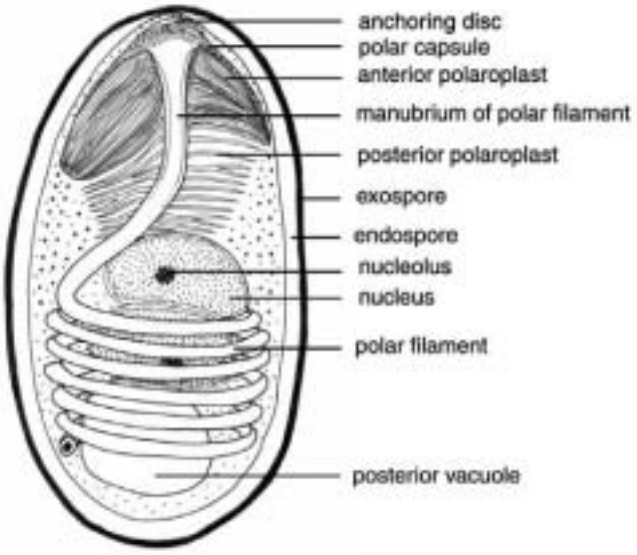

Figure 2.7 Schematic diagram of the ultrastructural features of a binucleate spore.

Table 2.1 Microsporidia genera reported in fish and crustaceans from marine ([M]) and freshwater ([FW]) environments

Genera in fish	Genera in crustaceans	
Amazonspora [M]	*Abelspora* [M]	*Mrazekia* [FW]
Glugea [M]	*Agmasoma* [M]	*Nelliemelba* [FW]
Heterosporis [FW]	*Alfvenia* [FW]	*Norlevinea* [FW]
Ichthyosporidium [M]	*Amblyospora* [FW]	*Nosema* [M]
Kabatana [M]	*Ameson* [M]	*Octosporea* [FW]
Loma [M]	*Baculea* [FW]	*Ormieresia* [M]
Microfilum [M]	*Berwaldia* [FW]	*Orthothelohania* [M]
Microgemma [M]	*Chapmanium* [FW]	*Parathelohania* [FW]
Neonosemoides [FW]	*Courgourdella* [FW]	*Pleistophora* [M]
Nosemoides [FW]	*Duboscqia* [FW]	*Pyrotheca* [FW]
Nucleospora [M]	*Glugea* [M]	*Stempellia* [FW]
Ovipleistophora [M]	*Gurleya* [FW]	*Thelohania* [M]
Pleistophora [M]	*Gurleyides* [FW]	*Tuzetia* [FW]
Pseudoloma [FW]	*Holobispora* [FW]	*Vairimorpha* [FW]
Spraguea [M]	*Inodosporus* [M]	*Vavraia* [FW]
Tetramicra [M]	*Lanatospora* [FW]	
Microsporidium [M]	*Marssoniella* [FW]	

Microsporidia infect most invertebrate phyla and all classes of vertebrates, and to date, more than 1300 species in about 150 genera have been described; the greatest number from fish and arthropods, including marine species (Canning and Vavra 2000). Undoubtedly, many more species exist. Over 158 species in 17 genera are known to infect fish and 34 genera infect crustaceans, as listed in Table 2.1 (Sindermann 1990, Langdon 1991, Undeen 1997, Mathews *et al.* 2001, Azevedo and Matos 2003, Lom and Nilsen 2003, Moodie *et al.* 2003). Other aquatic taxa in which Microsporidia have been found include cnidarians, annelids, molluscs and freshwater bryozoans (Nilsen 1999, Clausen 2000, Canning *et al.* 2002). Microsporidia hyperparasites have been found in gregarines, trematodes and mesozoans that are parasitic in marine invertebrate hosts, and in myxozoan parasites of fish (Canning and Vavra 2000, Lom and Nilsen 2003).

Life cycles

Microsporidia life cycles may be simple or complex. Unfortunately, complete life cycles for most species remain unknown. In those species for which information is available, reviewed most recently by Wittner and Weiss (1999), Petry (2000) and Dunn *et al.* (2001), transmission may be horizontal or vertical, or a combination of the two. Horizontal transmission by the oral route is common, usually by ingestion of infective spores from the environment. Direct transmission from one definitive host to another or indirect transmission via an intermediate host may occur. Many fish Microsporidia are transmitted directly by ingestion (e.g. *Nucleospora salmonis*, *Glugea* spp. and *Loma* spp.). Small crustaceans may function as paratenic hosts or as intermediate hosts for some species, e.g. *Pleistophora* and *Thelohania* spp., although further studies are required to confirm this (Iversen and Kelly 1976, Shaw and Kent 1999, Lom and Nilsen 2003). Infective spores may be dispersed in the environment or localised in faeces, oral secretions or tissues of infected hosts. Transmission by cannibalism is not unusual (Becnel and Andreadis 1999, Cali

and Takvorian 1999). Where vertical transmission occurs, the transovarial route is most commonly used, although venereal transfer has been recorded (Dunn *et al.* 2001).

Relatively little is known about factors influencing persistence of microsporidian spores in the environment. The subject has been reviewed by Becnel and Andreadis (1999) and Cali and Takvorian (1999) who concluded that survival times are highly variable depending on the parasite species involved, moisture conditions, temperature, exposure to solar radiation, the materials in which spores are deposited and the presence of other microorganisms. Spores of some species stored in sterile refrigerated water can remain viable for several years. Spores utilised for vertical transmission or internal transmission between tissues within a host tend to have thinner walls and shorter polar filaments than spores released to the environment (Dunn *et al.* 2001).

After infection of a new host, Microsporidia generally undergo a proliferative phase involving binary or multiple fission of vegetative cells called meronts (merogony) that subsequently transform into sporonts. The sporonts undergo a series of divisions by mitosis or meiosis to form sporoblasts (sporogony), each of which matures into an infective spore (sporulation). Meiosis in Microsporidia has been reviewed by Canning (1988) and Flegel and Pasharawipas (1995).

Meronts, sporonts and spores may be uninucleate or binucleate. Sporulation may occur within sporophorous vesicles (SPVs), also known as pansporoblasts in the older literature, or alternatively spores are formed in direct contact with host cell cytoplasm. Sporophorous vesicles (SPVs) are usually spherical or ovoid, and contain a characteristic number of spores depending on species (e.g. 2, 4, 8, 16, 32, 48 or 64). Patterns of cell division, the stage at which the formation of the SPV is initiated, the number of spores within, and ultrastructural features of spores and SPVs are important taxonomic features (Lom and Dyková 1992, Sprague *et al.* 1992, Larsson 1999, Vavra and Larsson 1999, Canning and Vavra 2000, Lom and Nilsen 2003). Several sporogony pathways may occur in a single species and multiple spore types may be produced.

Effects on hosts

Most Microsporidia that infect fish and many of those that infect crustaceans are detrimental to their hosts, causing either morbidity or mortality. Mass mortalities in wild fisheries and cultured stocks have been reported, for example *Loma salmonae* in salmonid fish, *Agmasoma penaei* in shrimp (Sindermann 1990, Lom and Dyková 1992, Shaw and Kent 1999). Pathogenic effects induced by Microsporidia in host cells include physical disruption of cells due to occupation of intracellular space, host cell hypertrophy, changes to host cell metabolism with destruction, synthesis, or reorganisation of host cell components. Infected host cells often degenerate and die, and if sufficient numbers of cells are affected, tissue function is impaired (Wittner and Weiss 1999, Petry 2000). Different species of Microsporidia vary in their ability to induce severe pathology, depending on the organs invaded and the extent and manner in which parasite proliferation occurs. Direct effects of microsporidiosis include increased mortality and reduced market value of economically important species. Indirect effects include reduced growth, reduced reproductive potential and behavioural changes (e.g. decreased predator avoidance and altered migratory patterns).

Enlarged tumour-like host cells filled with spores (xenomas) are common in fish infected by the genera *Glugea*, *Ichthyosporidium*, *Jirovecia*, *Loma*, *Microfilum*, *Microgemma*, *Nosemoides*, *Spraguea* and *Tetramica*. Diffuse infections without xenoma formation are more common in crustaceans and in fish infected by the genera *Nucleospora*, *Heterosporis*, *Kabatana*, *Pleistophora* and *Thelohania* (Sindermann 1990, Lom and Dyková 1992, Shaw and Kent 1999, Lom and Nilsen 2003). Immune responses are often weak or non-existent where Microsporidia are protected within host cells or encapsulated in xenomas; however, the parasites may be attacked by the host immune system during initial infection or if a host cell or xenoma ruptures.

Suppression of the host inflammatory response and impairment of humoral or cellular responses in association with Microsporidia infection have been reported. Fish that have recovered from infection by *Loma salmonae* show some resistance to reinfection, although the mechanism is unclear. Antibody responses are generally not thought to be protective (Speare *et al.* 1998, Ramsay *et al.* 2002). As yet, no effective vaccine against microsporidiosis has been developed. The ability of a host to resist or contain infection varies between individuals and host taxa, and may be influenced by interactions between the host immune system and environmental factors. Host ranges vary between species. Moderate host specificity is shown by most Microsporidia. Relationships between immune competence and susceptibility to infection by Microsporidia in crustaceans and other marine invertebrates have not yet been explored to any extent.

Detection and treatment of infection

Traditionally, detection of microsporidiosis has depended on examination of tissues for spores under the light microscope or the electron microscope. The sensitivity of microscopic methods is limited by the small size of the parasites and difficulty in detecting early pre-spore stages. polymerase chain reaction (PCR)-based methods are increasingly being used to detect infection and identify the species responsible. They are more sensitive and specific than microscopic methods. Most PCR tests are based on the ribosomal RNA genes and internal transcribed spacer region (Weiss and Vossbrinck 1999).

Antimicrosporidial drugs are usually not cost effective for use in cultured populations of marine organisms, or have limited efficacy. Those that have been used in fish are reviewed by Shaw and Kent (1999). Prevention is a better option than cure; however, preventative measures are hampered by a paucity of information on the complete life cycles of many species (see above).

Ecological importance

Microsporidia may exert a variety of direct effects on their host populations as well as indirect effects on other species and the wider environment. Infection by Microsporidia may result in alteration of sex ratios in host populations, changes to host population demography, changes in host behaviour and alterations to trophic dynamics in communities that include infected hosts.

Vertically transmitted Microsporidia parasites have the potential to alter sex ratios in host populations by male killing or feminisation of the host, as described in the review by Dunn *et al.* (2001). Late male killing, due to the development of large numbers of spores in males but not females, is a strategy which effectively increases horizontal transmission rates. It has been reported in several species that infect mosquito hosts (e.g. *Amblyospora californica*). Feminisation of hosts is a strategy likely to increase transmission success via the transovarial route, and is exhibited by species from at least three genera that infect the marine amphipod *Gammarus duebeni*, including *Thelohania*, *Nosema* and *Octosporea*.

Juvenile hosts are more likely to suffer mortality than adults as a result of infection by some Microsporidia (e.g. *Loma salmonis*) (Shaw and Kent 1999). Where many spores develop in host musculature (e.g. *Thelohania* spp. in shrimp and *Glugea* spp. in fish), there is a greater chance that infected individuals will be caught by predators. Infected hosts may also be unable to engage in normal behaviours such as migratory movements. As yet, no models have been developed that predict the effects of Microsporidia infection on the ecology of wild host populations or their communities, in the marine environment.

Relatively little information is available on the extent to which environmental factors influence host susceptiblity to Microsporidia infection, or whether Microsporidia infection increases the likelihood of coinfection of hosts by other pathogens or vice versa. Chronic exposure to

stressors such as pollutants (Barker *et al.* 1994) and high stocking densities in aquaculture are thought to increase prevalence and intensity of infection (Overstreet 1973, Sindermann 1990, Shaw and Kent 1999).

Although relatively little is known about the virulence dynamics of microsporidian infections in marine hosts, elegant experiments by Ebert and his coresearchers on microsporidian parasites of the freshwater crustacean *Daphnia* have contributed substantially to the body of knowledge on factors influencing virulence of microparasites. Increased virulence is associated with the production of larger numbers of spores (Ebert 1994) and may also be a consequence of within-host competition where multiple strains infect the same host (Ebert and Mangin 1997). Manipulative experiments involving infection of clonal populations of *Daphnia* by Microsporidia have shown that parasites may influence microevolution in the host during both asexual and sexual reproduction (Capaul 2003).

Relatively few studies have specifically addressed coevolution of Microsporidia and their marine hosts, although in a recent analysis of fish-infecting species, Lom and Nilsen (2003) found that all but one of the 15 genera they analysed were grouped together on the same branch of the phylogenetic tree, suggesting either coevolution or cospeciation. Further studies on coevolution are warranted.

Important references

Excellent reviews of the Microsporidia have been produced by Wittner and Weiss (1999), Canning and Vavra (2000) and Petry (2000). Important features used in taxonomy prior to the widespread use of molecular techniques are described by Sprague *et al.* (1992) and Larsson (1999). Keeling (2003) reviewed the biology and evolution of the group, including data from recent molecular studies. Life cycles, with an emphasis on transovarial pathways, are reviewed by Dunn *et al.* (2001).

Mikrocytos mackini (microcell)

Susan M Bower

Introduction

Mikrocytos mackini is a protist of unknown taxonomic affiliations, commonly referred to as a microcell. It is characterised by the apparent lack of mitochondria and haplosporosomes.

Morphology and diversity

The tiny size of *M. mackini* (2–4 μm in diameter) and non-descript spheroid shape (Fig. 2.8A) necessitates the examination of specimens by electron microscopy for the observation of relevant features. The three morphological forms identified by Hine *et al.* (2001) include:

1 Quiescent Cells (QC) with a central round to ovoid nucleus, less than seven cisternae of inactive nuclear membrane-bound Golgi, few vesicles and lysosome-like bodies
2 Vesicular Cells (VC) containing many small coated and uncoated vesicles, lacking nuclear membrane-bound Golgi-like arrays and with the nuclear membrane sometimes dilated to form a cisternal chamber
3 Endosomal Cells (EC, Fig. 2.8B) with a dilated nuclear membrane, a well-developed anastomosing endoplasmic reticulum connected the nuclear and plasma membranes and endosomes in the cytoplasm.

There was an overlap in features between QC and VC, between VC and EC and between EC and QC. Few organelles including the apparent lack of mitochondria occurred in all forms of *M. mackini.*

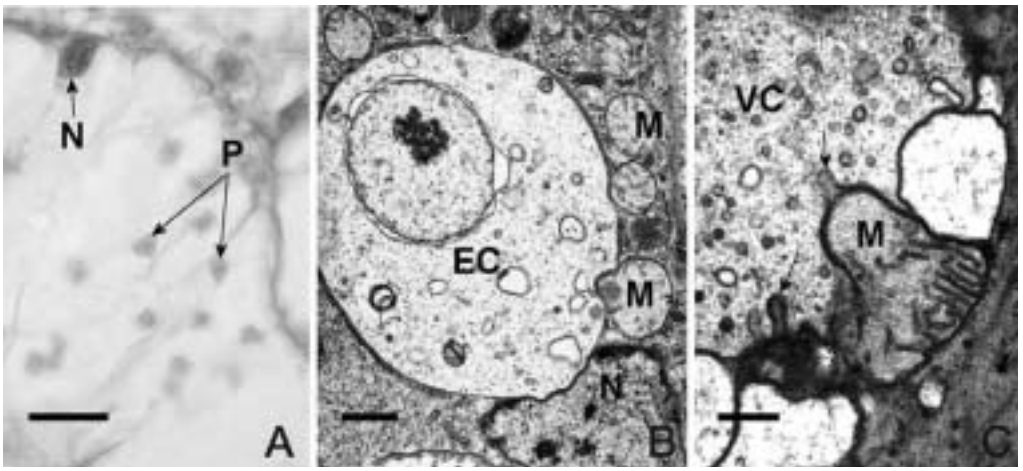

Figure 2.8 *Mikrocytos mackini*. **A**. Histological section of several *M. mackini* (P) in the cytoplasm of vesicular connective tissue cells (N = nuclei of host cells) of the labial palps of *Ostrea edulis*. Scale bar = 10 µm. **B**. Transmission electron micrograph of a *M. mackini* endosomal cell (EC) in close association with mitochondria (M) and the nucleus (N) of a haemocyte of *Crassostrea gigas*. The anastomosing endoplasmic reticulum is not evident in this specimen. Scale bar = 0.5 µm. **C**. Transmission electron micrograph of a *Crassostrea gigas* myocyte mitochondrium (M) with tube-like structures (arrows) extending into the cytoplasm of *M. mackini* vesicular cell (VC). Scale bar = 0.25 µm.

Mikrocytos mackini can de differentiated from other microcells (*Bonamia* spp.) by its location in vesicular connective tissue cells, adductor muscle myocytes and less frequently in haemocytes, and by the apparent lack of mitochondria and haplosporosomes. Also, *M. mackini* seems to have a unique way of obtaining energy from its host cell. Hine *et al.* (2001) depicted tube-like structures extending into the cytoplasm of *M. mackini* from the mitochondria of its host cell (Fig. 2.8C). Thus, the contents of the host cell mitochondria appeared to pass through a tubular extension into the cytoplasm of the parasite.

The parasite is infective to at least four species of oysters (Pacific oysters; eastern oysters, *Crassostrea virginica*; flat oysters and Olympia oysters, *Ostrea conchaphila*) (Bower *et al.* 1997). The only other described species of *Mikrocytos*, *M. roughleyi* from the Sydney rock oyster, *Saccostrea glomerata* (=*S. commercialis*) in New South Wales, Australia (Farley *et al.* 1988), is now believed to be a species of *Bonamia* (Cochennec-Laureau *et al.* 2003). Although *Bonamia* spp. are also known as microcells that parasitise various species of oysters, they are not related to *M. mackini*. The inability to assign *M. mackini* to a phylum and lack of knowledge on close relatives (Hine *et al.* 2001, Carnegie *et al.* 2003), makes it impossible to compensate for information gaps on various biological parameters by extrapolation.

To date, *M. mackini* has been reported only from oysters in the southern part of British Columbia, Canada, and the adjacent state of Washington, USA. However, disease caused by *M. mackini* appears to be restricted to older oysters (over two years) in some locations in British Columbia and mortalities occur in the spring (April and May) after three to four months when temperatures are less than 10°C. The requirement for cool temperatures and the long prepatent period may explain why the disease only occurs during the spring and seems to be confined to oysters cultured in more northerly locations.

Life cycles

The three different morphological forms of *M. mackini* appear to be preferentially located in different tissues and host cells of the oyster. Tissue locations and overlap in features between the

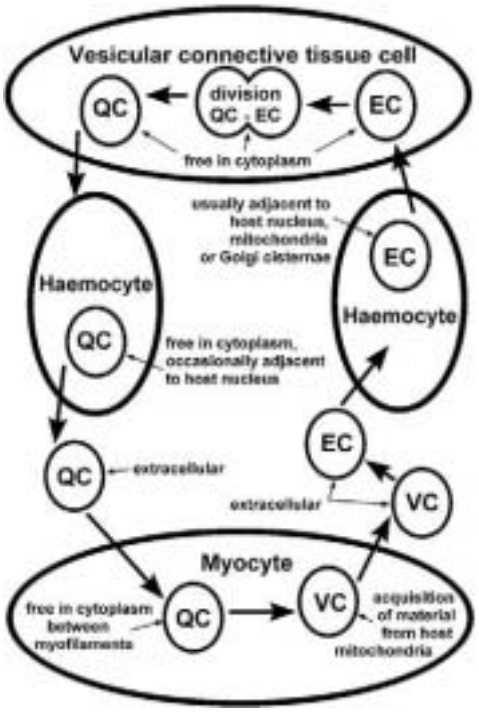

Figure 2.9 Proposed developmental cycle of *Mikrocytos mackini* indicating host cell type and host organelle affiliations for the three recognised morphological forms consisting of quiescent cell (QC), vesicular cell (VC) and endosomal cell (EC). Reprinted with permission from Hine, PM, Bower, SM, Meyer, GR, Cochennec-Laureau, N and Berthe, FCJ 2001. Ultrastructure of *Mikrocytos mackini*, the cause of Denman Island disease in oysters *Crassostrea* spp. and *Ostrea* spp. in British Columbia, Canada. *Diseases of Aquatic Organisms* 45: 215–227.

different morphological forms suggested a developmental cycle of the parasite in its host (Fig. 2.9). Apparently QC, which appear to lack energy reserves and cytoplasmic organelles usually associated with energy production in eukaryotic cells, travel from the vesicular connective tissue cells in haemocytes to the adductor or heart muscles. Once inside the myocytes, the QC changed to VC which appeared to endocytose the contents of myocyte mitochondria, acquiring ATP and other proteins. The VC then changed to EC in the process of leaving the muscle and entering haemocytes. The EC maintain close contact with haemocyte mitochondria and appear to obtain ribonucleoproteins from the haemocyte nucleolus. While travelling to the vesicular connective tissue cells in haemocytes or extracellular, the EC began mitosis by binary fission which was completed in the vesicular connective tissue cells where the daughter generation changed to QC and reinitiated the cycle.

Alternately, *M. mackini* may acquire the morphological form suited to its energy acquisition opportunities available in the host cell. The lack of organelles in *M. mackini*, including mitochondria or their equivalents that are found in most eukaryotic cells, may be due to its obligate parasitism. The utilisation of host cell organelles could have reduced the need for parasite organelles. Possibly, *M. mackini* is a primitive eukaryote that is able to survive because of a parasitic existence or is a highly evolved parasite that has secondarily lost most energy-producing organelles.

In addition to being very cryptic, experimental evidence suggests that most infections of *M. mackini* in oysters are subclinical. Exposed oysters, held at 18°C, can retain *M. mackini* at

subclinical levels for at least six months. *Mikrocytos mackini* is only capable of causing disease in oysters held at less than 10°C for at least three months (Hervio *et al.* 1996). This apparent requirement for long periods at low temperatures for pathogenic expression and the prolonged prepatent period at warm temperatures may explain why *M. mackini* is only detectable in the field during the spring (March–May). Possibly, subclinical infections occur in oysters from enzootic areas throughout the year. *Mikrocytos mackini* can be directly transmitted between oysters but transmission seems to be limited to periods when the infection is active in diseased oysters during the spring. To date, no evident mechanisms of protection for existence outside the oyster host (i.e. no spore-like stage) and no alternate hosts have been detected (Bower 2001).

Effects on hosts and ecological importance

The disease (Denman Island disease or mikrocytosis) associated with *M. mackini* was first detected in the early 1960s among beach cultured oysters on Henry Bay, Denman Island, when it caused high mortalities (about 30%) of oysters at low tide levels (Quayle 1982). From 1960 to 1994, prevalence of infection in Pacific oysters from Henry Bay fluctuated from 11% (1967) to 48% (1988), in mid-March to mid-May (Hervio *et al.* 1996). In addition to mortalities, active infections of *M. mackini* induce the development of focal green abscess-like lesions (pustules) up to 5 mm in diameter usually within the body wall and adductor muscle or on the surfaces of the labial palps or mantle. The pustules usually contain a central area of tissue necrosis surrounded by haemocyte infiltration. Often, a brown scar occurs on the shell adjacent to a pustule on the mantle surface. Although the lesions caused by infection can persist throughout the summer, *M. mackini* is usually no longer detectable by early summer using routine histological techniques. Experimental evidence suggests that oysters become infected when the disease is active in the early spring but the infection remains cryptic until the following spring. Juvenile oysters (seed) held on affected beaches during the spring will develop the disease the following spring in British Columbia, regardless of subsequent culture techniques. About 10% of infected *C. gigas* appear to recover. *Crassostrea gigas* seems to be more resistant to the disease than the other species of oysters challenged experimentally under laboratory and field conditions (Bower *et al.* 1997).

Important references

Further details, coloured illustrations and a complete list of references are available on the website (Fisheries and Oceans Canada 2004).

Ciliophora (ciliates)

Jiří Lom

Introduction

Among the protozoans, the monophyletic assemblage of ciliates is perhaps the most numerous with more than 8000 species ranging from about 10 μm to 4.5 mm in size. Most species have a pellicle covered with cilia, although ciliature may be reduced or even absent. They have the most complicated cell structure among protozoans: free-living species inhabit a range of aquatic and terrestrial environments, and other species live in various symbiotic relations with aquatic animals. There is hardly an aquatic animal, be it a fish, invertebrate or even a mammal, without some epibiotic, ectoparasitic or endozoic ciliate; there are also free-living ciliates often behaving like facultative parasites. Although the ciliates in most cases do not inflict any harm, there are some severely pathogenic species (e.g. in fish).

Morphology and diversity

The ciliate cell is covered with a pellicle consisting of an outer cell membrane subtended by a layer of flat pellicular alveoli. The pellicle is covered by cilia, either uniformly, arranged longitudinally in rows or kineties, or some or all cilia grouped together to form compound ciliary organelles like cirri or membranelles. In some groups the ciliature may be reduced or even completely absent. The basal bodies or kinetosomes of cilia are associated with a complex fibrillar network, the infraciliature, composed of microfibrillar ribbons and microtubules. Ciliates exhibit nuclear dualism, there are one to several small, diploid, mitotically dividing micronuclei with a complete genome and one to several large, amitotically dividing ampliploid ('polyploid') macronuclei for vegetative functions of the cell. Ciliates divide essentially by transverse binary fission, rarely by budding or multiple fission. The sexual process is conjugation, in which two individuals undergo a partial and transient fusion and reciprocally fertilise themselves by products of meiotic division of their micronuclei. Feeding takes place by elaborate buccal structures involving either simple somatic ciliature or special ciliary organelles like membranelles and paroral membrane, all these serving for driving the food into the cytostome. In some, suctorial tubes serve for sucking in the prey cytoplasm, and some are secondarily astome. Ciliates overcome unfavourable conditions inside protective cysts; a special kind of cyst is used as shelter for proliferation. Generally, the life cycle is simple, continuous division of trophic stages with insertion of cyst stages.

Evolution of ciliates resulted in diversification that lead to formation of special morphological structures (stalks, loricae and cysts). Essential pressure for evolution of various special morphological and life cycle adaptations is, however, a symbiotic life in different ecological niches. The resulting diversification has led to groups of ciliates that are so different that it is rather difficult to treat them here as a single assemblage.

Free-living ciliates are an important constituent of the food web in marine environments and the same can be said of the symbiotic ones. Symphorionts utilise bacteria, algae and organic particles from the water around them. Commensals feed on substances offered by the hosts (abundant populations of several families of endocommensal scuticociliates (e.g. Entodiscidae, Entorhipidiidae and Cryptochilidiidae live in the intestine of sea urchins). Some of the ciliates, which act as true parasites, feeding at the expense of their hosts, may deplete the host's viability or even reduce the population. This applies to ciliate infection in stressed, cultured fish.

Until the second half of the 20th century, ciliates were classified essentially according to the type of the buccal and also somatic ciliature. This made it possible to determine the taxa using light microscopy. Now, electron microscopy yields characters at a higher level. In the current classification (Lynn 2003), the phylum is divided into two subphyla: Postciliodesmatophora with characteristic microtubular ribbons linking all kinetosomes in a kinety, comprising two classes; and Intramacronucleata, in which macronuclear division involves microtubules that lie inside it. This subphylum comprises nine classes with a total of 19 subclasses. The genera of symbiotic ciliates from the marine environment belong mostly to two classes of this subphylum: Phyllopharyngea with subclasses Phyllopharyngia (*Brooklynella*) and Rhynchodia (*Ancistrocoma*); and Oligohymenophorea with subclasses Scuticociliatia (with holotrichous ciliation, e.g. *Mesanophrys*), Apostomatia (with a few spiralling ciliary rows, primitive buccal structure and a complex life cycle, e.g. *Ascophrys* and *Vampyrophrya*) and Peritrichia (*Trichodina*).

Symbiotic adaptations and effects on hosts

Ciliates display a wide range of adaptations to a symbiotic way of life, ranging from facultative parasites to innocuous symphorionts to true parasites. Species of the genus *Lagenophrys*, peritrichous ciliates like all in this subclass devoid of somatic cilia and only equipped with a buccal ciliary spiral, are essentially innocuous symphorionts. However, on blue crab gills they can grow so exuberantly that its host, if confined in a tank, dies for lack of oxygen (see Hausmann and Bradbury 1996).

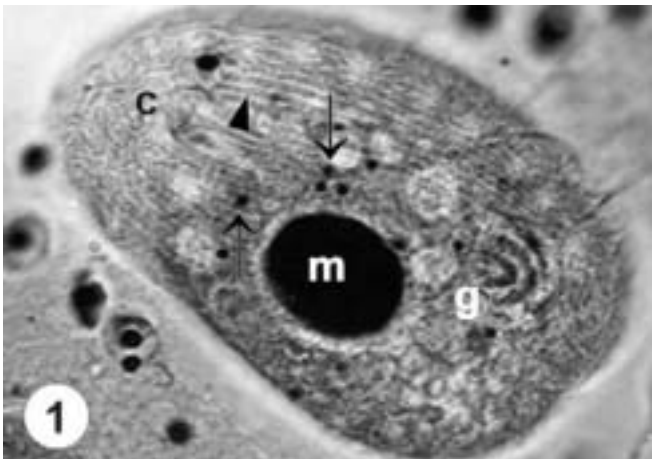

Figure 2.10 *Brooklynella hostilis*. Haematoxylin-stain reveals macronucleus (m) with dot-like micronuclei (arrows) above it, faintly visible ciliary rows on the ventral surface (arrowhead), cytostome (c) and glandular organelle (g). × 850. Reprinted from 'Protozoan Parasites of Fishes'. Developments in Aquaculture and Fisheries Science, Vol. 26, Lom and Dyková (1992), with permission from Elsevier.

The scuticociliate genus *Paralembus* has species which browse innocuously through the surface mucus of sea anemones and feed on trapped bacteria and cellular debris from surface injuries. Free-living ciliates of the genera *Helicostoma* or *Euplotes* may sometimes prove to be pathogenic for debilitated cultured fry of commercial fish, causing lethal skin lesions. Scuticociliates *Miamiensis avidus* or *Uronema marinum* are known as facultative parasites. Especially in fish cultures, they attack the skin and gills first, hampering respiration, and then make their way into the internal body organs, disintegrating them and causing mortalities. In these and other infections, the fish need to be predisposed by stress, environmental or other, for the ciliates to fully develop their pathogenic potential (Lom and Dyková 1992). Like the following *Brooklynella*, they have no host preferences and attack all fish available.

The phylopharyngiid ciliate *Brooklynella hostilis* (Figs 2.10 and 2.11) with flattened cell, only ventrally equipped with rows of cilia, is a scavenger gliding over the surface of fish gills. It feeds on desquamated cells. In stressed captive fish (in cultures, aquaria) it may multiply massively

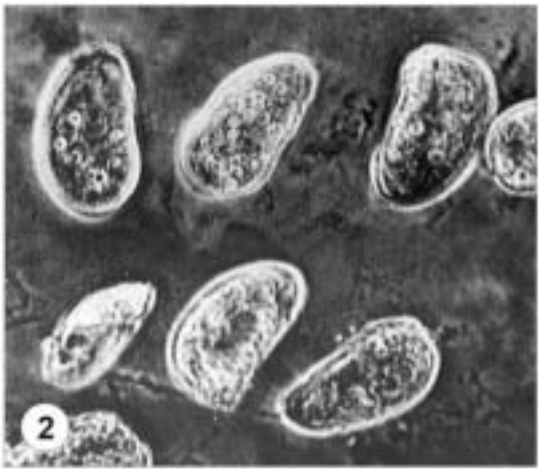

Figure 2.11 *Brooklynella hostilis*, live ciliates. × 330.

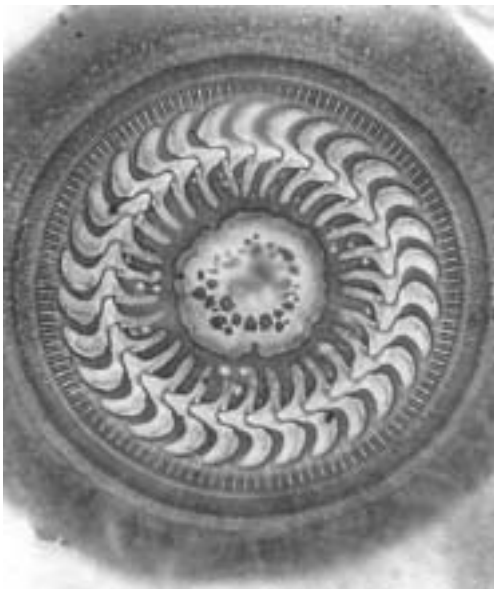

Figure 2.12 Silver impregnated adhesive disc of *Trichodina murmanica,* serving for attachment to fish surface, reveals fine details of the proteinaceous cytoskeleton. × 1000.

and turn into a pathogen. In the lack of cell debris, the extensible cytopharyngeal armature destroys the gill cells and the ciliate feeds on them. Severe gill lesions – the secondary lamellae are sometimes completely denuded of the epithelium – inflict heavy losses in fish stocks.

Species of the peritrichous genus *Trichodina* are common inhabitants of the surface of some freshwater and marine invertebrates and especially of fish (Lom 1995). Their disc-shaped cell has a ciliated spiral on the upper side and a sucker-like, reinforced adhesive disc (Fig. 2.12) on the lower side, by means of which they can attach temporarily to the substrate. In unstressed feral fish populations, they behave like harmless ectocommensals feeding on cell debris and micro-organisms. In cultured fish, they occur in large numbers and damage the surface tissue by the action of the disc and feed on the detached cells.

Species of the scuticociliate genus *Mesanophrys* live as scavengers on the exoskeleton of crabs and lobsters. When by chance they find an animal with a break in its shell, they invade the haemocoel, multiply, destroy the amoebocytes and, feeding on the tissue, they may kill the crustacean. In crustaceans kept in holding tanks or cages where the injuries may be commonplace they may cause many die-offs.

Ciliates of the subclass Apostomatia mostly do their host no harm, feeding on exuvial fluid or secretions of cuticular hairs. Some are, however, pathogenic. Mortalities in cultured *Palaemon serratus* (Deroux *et al.* 1975) are also due to the apostome ciliate *Ascophrys rodor* causing cuticular lesions. *Vampyrophrya parasitica* has stages (tomites) encysted on the shell of marine copepods. If the host's exoskeleton is breached, the tomites excyst, get through the wound into the body, where they start feeding on the tissues. Eventually, when only an empty shell is left, the grown ciliates encyst and divide into small tomites again. Then they leave the dead host, find another host on the surface of which they encyst again and wait. In other apostomes, like *Synophrya hypertrophica*, the tomites actively bore through the exoskeleton of the gills and feed inside on the blood by osmotrophy.

A severe pathogen of practically all marine teleosts, rare in feral fish but often abounding in piscicultures and sea aquaria, is *Cryptocaryon irritans* of the class Prostomatea. It has a life cycle

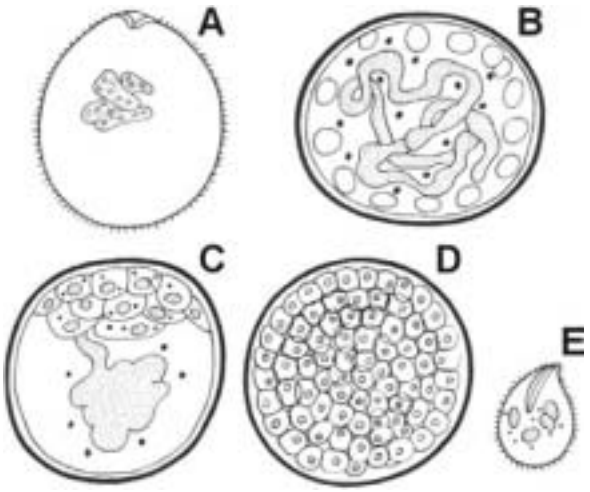

Figure 2.13 *Cryptocaryon irritans*, diagram of the life cycle. **A**. Mature trophont with four macronuclei at the surface of fish. **B**. Large encysted ciliate with macronuclei fused into one ribbon prepares to divide. **C**. Palintomic division within the cyst. **D**. Many new tomites within the cyst. **E**. A tomite transformed into a theront, again with four macronuclei, in search of a new host. Fig. 2.13 modified from 'Protozoan Parasites of Fishes'. Developments in Aquaculture and Fisheries Science, Vol. 26, Lom and Dyková (1992), with permission from Elsevier.

(Fig. 2.13) very similar to its freshwater counterpart *Ichthyophthirius* but is morphologically different (e.g. has quadripartite macronucleus). Small individuals (theronts) burrow into the fish surface, grow there into a trophont up to 450 μm long, which then leaves the host, drops to the substrate as a tomont and encysts. Within the cyst, up to about 200 tomites are produced within six to nine days. Then they emerge from the cyst as theronts and swim in search of another host. The surface tissue may be severely disintegrated and serious epizootics in affected stocks are common.

Marine ciliates offer an almost unfathomable range for studies of adaptations to symbiotic life.

Important references

Comprehensive treatment of ciliates can be found in Corliss (1979), Grassé (1984), Hausmann and Bradbury (1996), Lynn and Small (2000) and de Puytorac (1994).

Myxozoa (myxozoans)

Jiří Lom

Introduction

Myxozoa attract growing attention. Being predominantly parasites of fish, they are a significant and increasing impediment to modern piscicultures, which have to cope with steadily emerging new myxozoan pathogens. Presently, more than 2770 species assigned to 61 genera have been described from freshwater and marine fish and many of them have a disease potential. Myxozoa have multicellular spores consisting of specialised cells. Previously assigned to Protozoa, they have now been transferred to the Metazoa. The debate still continues as to what assemblage of metazoans they belong, either to the Radialia or to Bilateria. Thus, they are a group interesting from both practical and theoretical standpoints.

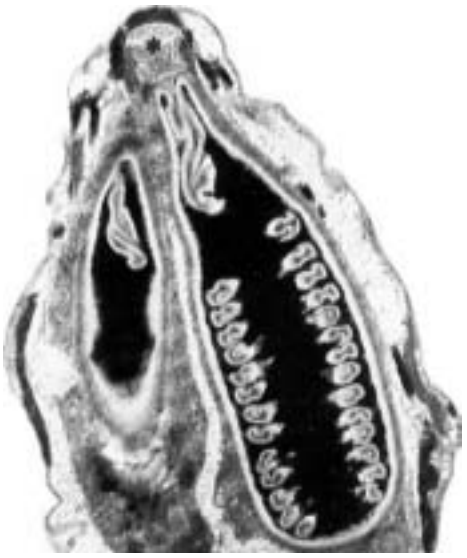

Figure 2.14 The main myxozoan feature, polar capsule with spirally wound ejectible polar filament in the spore of the genus *Henneguya*, as seen in electron microscopical section. × 9.400.

Morphology and diversity

Myxozoa have multicellular spores consisting of specialised cells – valvogenic cells forming the spore shell valves, amoeboid infective germ or sporoplasm (there may be one to many) and capsulogenic cells forming polar capsules with evertible polar filaments (Fig. 2.14). They are virtually identical with cnidarian nematocysts. Although nematocysts are instrumental in food capture, polar capsules serve for attachment to new hosts. The structure of myxozoan spores reflects what is also found in trophic stages (i.e. multicellularity and morphological and functional specialisation of cells). It is now generally accepted that the myxozoan life cycle requires an alternation of vertebrate and invertebrate host.

Spores are produced in different trophic structures. In the class Myxosporea, they originate in sporogonic plasmodia (SP) in the vertebrate phase of their life cycle and in the invertebrate phase of the cycle within a simple envelope of two to several cells, the pansporocyst. In the vertebrate host, coelozoic SP occur in organ cavities (associated with gall and urine secretion, rarely blood vessels or pericardium), histozoic SP occur intercellularly or intracellullarly in almost all tissues. Spores of this phase (myxospores) have mostly two polar capsules, less frequently one, three, four, five, rarely more, and are bilaterally or radially symmetric.

The most common vertebrate hosts are bony fishes, a few species live in elasmobranchs, amphibians and chelonid reptiles, three in agnaths. Most common hosts of the invertebrate phase are oligochaetes, also a few polychaetes and sipunculids; the infection affects mostly intestinal tissue. Spores of this phase (actinospores) are as a rule triradial with three capsules.

In the class Malacosporea, spores originate in a worm-like or sac-like stage living in the body cavity of bryozoans. This happens by differentiation from cells derived from the inner cell sheet or from agglomeration of cells inside the parasite.

Life cycle

Myxosporea: As late as in 1984, Wolf and Markiw discovered that transmission requires a developmental phase in an invertebrate host; since then, about 32 cases of fish–invertebrate life cycles have been discovered. Only one, that of *Ellipsomyxa gobii*, was observed to take place in the marine environment. The universal validity of the two-host cycle, especially for myxosporeans

from marine fish, has not yet been safely confirmed; rare cases of direct fish to fish transmission have been reported (Redondo *et al*. 2004).

Myxospores released from the fish (Figures 2.15A–G) can survive in the aquatic environment for more than one year. When ingested by an oligochaete (in freshwater) or by a polychaete

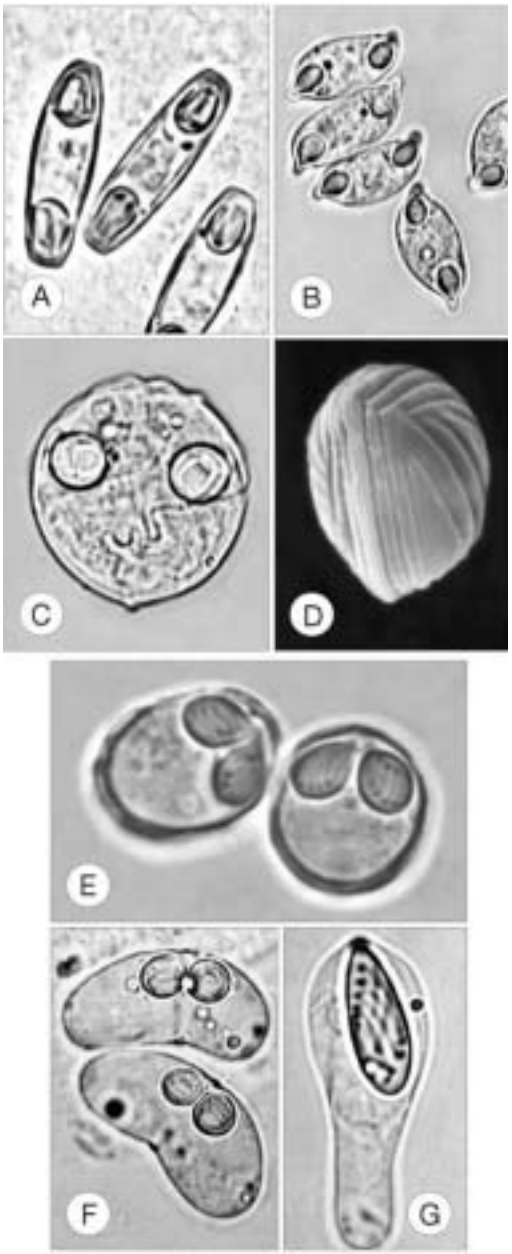

Figure 2.15 Spores of myxosporeans that infect marine fishes. **A**. *Sphaeromyxa magna*, × 2000. **B**. *Myxidium gadi*, × 1900. **C**. *Sinuolinea* sp., × 1300. **D**. *Ortholinea australis* (seen in scanning electron microscope), × 2200. **E**. *Myxobolus spinacurvatura*, × 2400. **F**. *Ceratomyxa macrospora*, × 1300. **G**. *Auerbachia pulchra*, × 2200. Figs A, C, F, G reprinted from 'Protozoan Parasites of Fishes'. Developments in Aquaculture and Fisheries Science, Vol. 26, Lom and Dyková (1992), with permission from Elsevier.

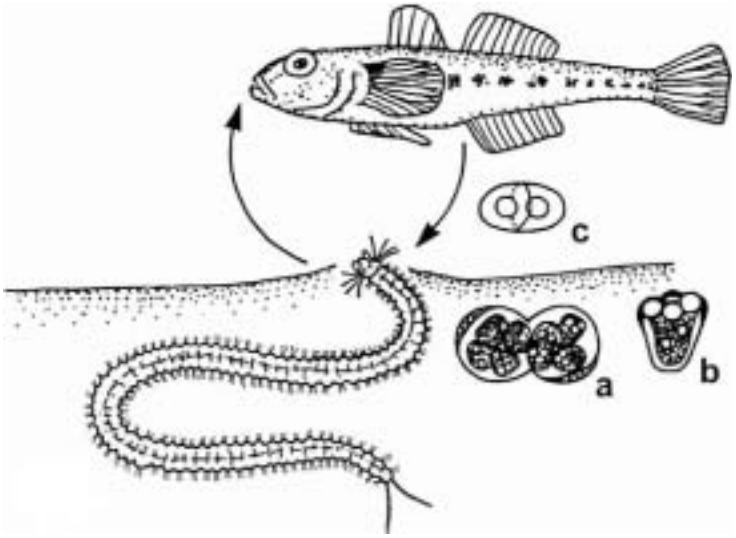

Figure 2.16 Life cycle of *Ellipsomyxa gobii* involves two hosts: it lives as a myxosporean stage (with the spore c) in the gall bladder of the common goby (*Pomatoschistus microps*), and (a) as an actinosporean stage in the polychate *Nereis* sp., producing pansporocysts with (b) spores. From Køie *et al*. (2004); reproduced with permission from the author, Dr Marianne Køie and the Editor-in-Chief, *Folia Parasitologia*.

(Fig. 2.16) (in the sea; this has been little studied until now – see Køie *et al*. 2004), they extrude their polar filaments, attach to the intestinal wall, the shell valves open, the sporoplasm escapes and invades the worm's intestinal tissue. After a period of proliferation by merogony (El-Matbouli and Hoffmann 1998), there appears a complex of four cells, the outer two will become the envelope cells of the future pansporocysts, the structure in which the spores develop. The inner two cells will divide to produce 16 gametic cells, which fuse to form eight zygotes. This is the only well-proven sexual process of Myxosporea and thus invertebrates must be regarded as definitive hosts. The zygotes develop into triradiate actinospores. Most of them have long, folded caudal projections, which as soon as the spore is released into water extend telescopically to a great length to assure a longer buoyancy in water. The damage done to the annelid hosts has not yet been sufficiently studied; infection of annelids can persist for two years.

When a floating actinospore – which is rather short lived – randomly contacts a fish, the extruded polar filaments attach to the skin or gills, the spore opens, and the sporoplasm penetrates into the surface tissue. From there on, the parasite wanders in a very complicated way, dividing on the way to produce a large number of cells. They may use blood circulation (*Sphaerospora*) or they may make their way as intracellular and intercellular stages through different body organs and tissues (*Myxobolus cerebralis*) before they eventually reach the final site where the sporogonic plasmodium develops (El-Matbouli *et al*. 1995). The stages produced, often in large numbers, in blood or various tissues are called presporogonic stages. They consist of a large mother (primary) cell in which are produced by inner cleavage two to many inner (secondary, and even tertiary) cells. These stages reveal another typical myxosporean feature, the cell-in-cell organisation. Eventually, the mother cell disintegrates and inner cells start the cycle all over again. Ultimately, the final site of infection is reached and the established sporogonic plasmodium starts to produce myxospores.

Sporogonic stages range from simple, small pseudoplasmodia, actually a uninucleate mother cell (about 10 μm) containing generative cells developing into one or two myxospores up to

large multinucleate plasmodia (2 cm in *Sphaeromyxa maiyai*) in which a large number of myxospores are produced. Spores originate by division of a single generative cell into the number of specialised cells necessary to constitute the spore, or they arise by union of two cells: a pericyte which envelops a generative cell, which inside this envelope divides to produce the cells necessary to constitute two spores. Mature spores may be released from a living host or are set free after its death when the tissues disintegrate.

Malacosporea are freshwater organisms living as worm-like or sac-like creatures in the body cavity of freshwater bryozoans (Canning and Okamura 2004). Some (*Tetracapsuloides bryosalmonae*) produce spores infective for anadromous salmonids, causing the dangerous Proliferative Kidney Disease (PKD). In kidney tubules they may produce spores of slightly different appearance (Hedrick *et al*. 2004).

Classification and evolution

Phylum Myxozoa has 61 genera in two classes:

1 Malacosporea (one family and two genera – *Buddenbrockia*, *Tetracapsuloides*).
2 Myxosporea, with two orders:
   a Bivalvulida, with three suborders:
      – Sphaeromyxina (one family and the single genus *Sphaeromyxa*)
      – Variisporina (10 families and 38 genera e.g. *Myxidium*, *Ceratomyxa*, *Sphaerospora*)
      – Platysporina (one family and 13 genera e.g. *Myxobolus*, *Henneguya*)
   b Multivalvulida (six families and seven genera e.g. *Unicapsula*, *Kudoa*)

The worm- or sac-like malacosporeans are supposed to be the early steps in the evolution of Myxozoa (Okamura *et al*. 2002); consequently, the simple structure of myxosporean trophic stages (plasmodia) would reflect an extreme simplification due to parasitism. Invertebrates are viewed as the primary and definitive hosts wheras vertebrates were only secondarily acquired to serve for asexual population increase (Canning and Okamura 2004). A series of adaptations has evolved, such as alternation of hosts, mechanisms of invasion, modes of proliferation, migration through body organs and intracellular parasitism.

Effects on hosts and ecological importance

The ecological role of marine myxosporeans lies in their effect on fish populations. Most species live in a balanced state with their hosts in feral fish; striking epizootics have been rather exceptional. However, in high sea fisheries, heavy infections with muscle-invading multivalvulid species (see pp. 378–391) can make a large part of the catch unmarketable because the flesh is unsightly or degraded to mushy substance. In fish in captivity, the pathogenic potential of some species can be fully unravelled. Depending on parasite species and body organs, the pathogenic action may be extremely varied; for example pressure atrophy in tissues exerted by parasite masses (plasmodia of *Myxobolus* species), irritation of epithelia of organ cavities (*Sphaerospora*, *Ceratomyxa*), enzymatic lysis of muscle tissue (species of the order Multivalvulida, like the genera *Hexacapsula*, *Kudoa* or *Unicapsula*), destruction of tissues (*Ceratomyxa shasta* or *Enteromyxum* species) or anaemia (*Tetracapsuloides*). Pathogenicity has been reviewed in Lom and Dyková (1995).

Among the recently emerged myxosporean pathogens in maricultures are the species *Kudoa thyrsites* (Kent *et al*. 2001), a cosmopolitan parasite infecting muscle tissue of many species of fish. It is the cause of 'soft flesh', muscle tissue degradation in *Merlucius productus*. It produces unmarketable flesh quality in pen-reared *Salmo salar*. Species of the genus *Enteromyxum* develop as small sporogonic pseudoplasmodia in the intestinal tissue causing severe enteritis

with high rate of mortalities. *Enteromyxum leei* not only infects cultured *Sparus aurata* and some other commercial fish species, it was also found to infect 25 host species from different orders and genera (Padrós *et al*. 2001). *Enteromyxum scophthalmi* is the cause of acute enteritis, starvation and death in cultured *Scopthalmus maximus*. The high virulence and rare development of mature spores in this host suggest that turbot is an accidental host for the parasite (Redondo *et al*. 2004). Cultured *Sparus aurata* also suffers from infections with *Ceratomyxa sparusaurati*. The parasite lives in the gall bladder and causes its swelling, sloughing of the epithelial cells and trickling mortalities (Palenzuela *et al*. 1997).

The challenge for future research of marine myxosporeans is to assess pathogenicity of new intruders into maricultures and especially to discover to what extent marine Myxosporea follow in their life cycle the pattern of alternation of fish and invertebrate host.

Important references

Monographic treatment of Myxozoa can be found in Canning and Okamura (2004), Kent *et al*. (2000, 2001), Kudo (1919), Lom (1990), Lom and Dyková (1992, 1995) and Shulman (1966).