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Haplosporidium sp. (Alveolata: Haplosporidia) associated with mortalities among rock oysters *Saccostrea cucullata* in north Western Australia

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ABSTRACT: *Haplosporidium* sp. is described from rock oysters *Saccostrea cucullata* Born, 1778 experiencing epizootics on the northwestern coast of Western Australia. All stages were observed as focal infections in the connective tissue of the gills, or as disseminated infections in the mantle and around digestive diverticulae. *Haplosporidium* sp. occurred between epithelial cells of the gut, in focal lesions in the gills, but not in the epithelium of the digestive diverticulae, and sporulation was confined to the connective tissue. Plasmodia developed into sporonts and sporocysts in a loose syncytium that gave rise to binucleate and uninucleate sporoblasts from which spores developed. Spores were flask-shaped, 5.6–6.7 × 3.3–4.0 µm, with a characteristic operculum, a few filamentous wrappings and rod-like structures in the posterior sporoplasm. Mature spores had a wall comprising inner (90 nm wide), middle (30 nm wide) and outer (130 nm wide) layers, and a surface coat of microtubules giving them a furry appearance. Oysters with empty gonad follicles were most heavily infected, and oyster condition and mortality appeared to be related to degree of infection.

KEY WORDS: Rock oysters · *Saccostrea cucullata* · Haplosporidian · *Haplosporidium* · *Minchinia* · Epizootics · Histopathology · Ultrastructure

INTRODUCTION

Protozoans currently recognized as haplosporidians are a small phylum of obligate parasites that infect several invertebrate groups. They have received considerable attention because some species are pathogenic in commercially important shellfish. *Haplosporidium nelsoni* and *H. costale*, along with another alveolate protistan, *Perkinsus marinus*, have severely affected oyster (*Crassostrea virginica*) fisheries in the eastern United States. *H. nelsoni* was first reported in association with *C. virginica* mortalities in Delaware Bay in 1957 (Haskin et al. 1966), and in Chesapeake Bay in 1959. Molecular evidence shows that *H. nelsoni* is conspecific with a similar *Haplosporidium* sp. in *C. gigas* from Japan (Friedman et al. 1991, Friedman 1996) and Korea (Kern 1976), and that it was introduced into California (Friedman 1996) in *C. gigas* from those countries (Burreson et al. 2000). *H. nelsoni*, or a very similar parasite, has also been introduced into France in *C. gigas* (Renault et al. 2000). A dissimilar *Haplosporidium* sp. has also been reported from *C. gigas* in France (Comps & Pichot 1991). Unidentified stages of haplosporidians in flat oysters *Ostrea edulis* in Europe (Pichot et al. 1979, Bachère & Grizel 1983, Bonami et al. 1985) may be those of *H. armoricanum*, which infects *O. edulis* in France (Cahour et al. 1980), the Netherlands (van Banning 1977), Spain (Azevedo et al. 1999), and *O. angasi* introduced into France (Bachère et al. 1987).

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Spores of the haplosporidian genera *Minchinia* and *Haplosporidium* are characterised by a distinctive operculum. The criteria for distinguishing *Minchinia* from *Haplosporidium* have altered many times (Marchand & Sprague 1979, Azevedo 1984, La Haye et al. 1984, McGovern & Burreson 1990, Azevedo et al. 1999), but are here taken to be the possession of tail-like epispore cytoplasm extensions (ECE) in *Minchinia* spp. (McGovern & Burreson 1990, Comps & Tigé 1997, Azevedo et al. 1999, Azevedo 2001), and the possession of filaments or ribbon-like ornaments in the epispore cytoplasm that are wrapped around the spore in *Haplosporidium* spp., and which derive from the spore wall (Azevedo 1984, Azevedo et al. 1999).

Unquantified mortalities among rock oysters were first recognised by energy companies on the Northwest Gas Shelf of Western Australia, in the early 1990s. The companies subsequently submitted samples for diagnosis and research. This paper reports a previously undescribed haplosporidian associated with these mortalities, compares the haplosporidian with similar haplosporidians identified in other oyster species, and discusses their inter-relationships.

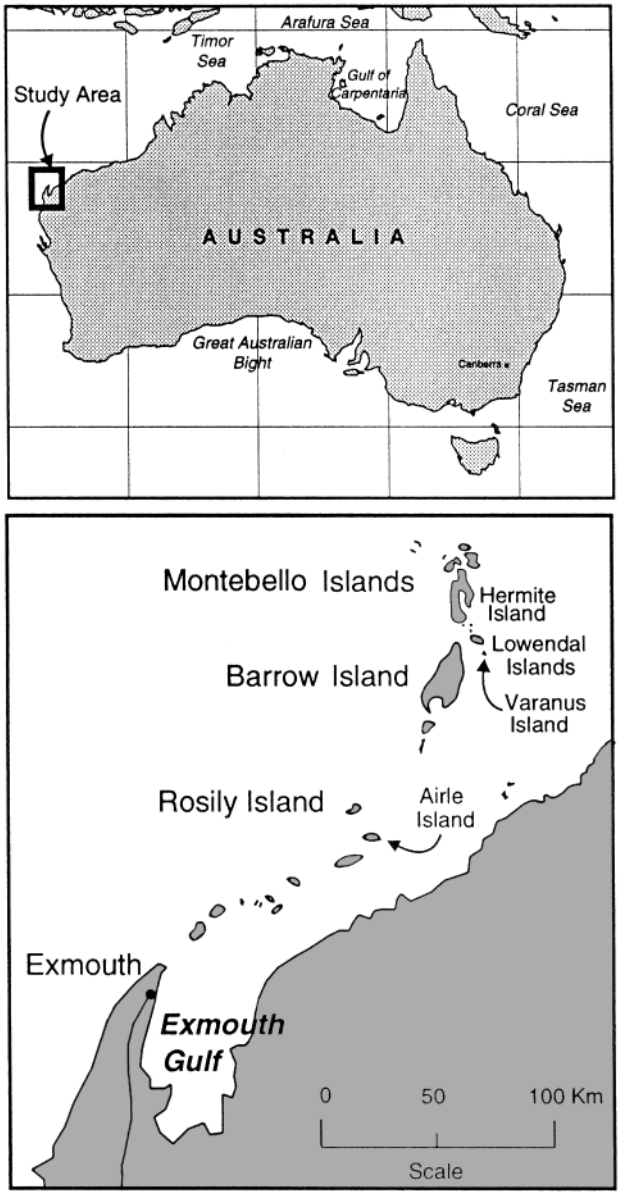


Fig. 1. Locations of sampling sites on the northwest coast of Western Australia

MATERIALS AND METHODS

During 1993 and 1994, 791 blacklip rock oysters *Saccostrea cucullata*, 808 Sydney rock oysters *S. glomerata* (see Table 1 & Fig. 1) and 106 tropical rock oysters *S. echinata* were sampled along the north coast of Western Australia. The oysters were opened and wet smears were prepared from the digestive gland. A standard section through the digestive gland, gills, labial palps and mantle, was fixed in Davidson's fixative, and stained with haematoxylin and eosin, or Ziehl-Neelsen's acid-fast stain with Loeffler's methylene blue as counterstain, or Ziehl's carbol fuchsin (Farley 1965). Small pieces of digestive gland from heavily infected oysters were fixed in 2.5% glutaraldehyde in 0.22 µm-filtered seawater for transmission electron microscopy (TEM). Samples for TEM were post-fixed for 1.0 to 1.5 h in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.2. Semi-thin sections were stained with 1% toluidine blue and 1% sodium tetraborate in distilled water for 20 s at 60°C. Ultrathin sections were stained with 2% uranyl acetate for 10 min, 5% lead citrate for 5 to 6 min, and examined on a Philips CM10 transmission electron microscope.

Attempts were made to sample more oysters, but the area concerned is harsh and remote, requiring access by helicopter, and the attempts were unsuccessful. Following the development of a sensitive and specific probe for *Haplosporidium nelsoni* by Burreson et al. (2000), further attempts were made to sample oysters, but infected oysters could not be found (Dr Brian Jones pers. comm).

RESULTS

Prevalence, gross signs and infection pattern

A haplosporidian parasite occurred in the connective tissue of *Saccostrea cucullata*, but not of *S. glomerata*, around the islands off the northwest of Australia (Table 1, Fig. 1). Further east, around the Dampier Archipel-

ago, 4 of 430 (0.9%) of *S. cuccullata* and 0 of 117 (0.0%) *S. glomerata* were infected, but 33 *S. cuccullata*, 8 *S. glomerata* and 12 *S. echinata* from King Sound, and 94 *S. echinata* from Darwin Harbour and Bynoe Harbour were not infected (see Hine & Thorne 2000 for locations). It must be noted that *S. glomerata* were only sampled from sites where *S. cuccullata* were also uninfected. The irregular frequency of sampling did not allow discrimination of seasonal patterns of infection by the haplosporidian.

Infected oysters varied in appearance, from oysters that appeared normal to oysters with meat of one-third normal size. The digestive gland was light brown and watery due to the absence of gonadal tissue overlying the digestive tissue. Overall 27% of male oysters, 23% of female oysters and 45% of oysters with empty gonad follicles were infected. Spores were present in 4% of infected male oysters, 13% of infected female oysters, and 59% of infected oysters with empty gonad follicles. Of 26 *Saccostrea cuccullata* from the Montebello Islands, 1 was infected with *Marteilia lengehi*, and of 44 *S. cuccullata* from East Lewis Island, Dampier Archipelago, 1 was systemically infected with *Perkinsus* sp., otherwise no other potentially serious infections were present.

Histopathology

Parasite stages usually occurred in focal lesions in connective tissue of the gills (Fig. 2), or as disseminated infections in connective tissue of the mantle and around digestive diverticulae (Fig. 3). Binucleate or multinucleate plasmodia, containing 2 to 25 nuclei in section, occurred in connective tissue (Fig. 4) and between (Fig. 5), but not in, epithelial cells of the main digestive tract, and not in the epithelium of digestive diverticulae. Some multinucleate stages were shaped like round or ovoid rings (Fig. 6). Assuming that binucleate and tetranucleate forms precede multinucleate stages, early infection occurred in the connective tissue of the gills, mantle and around the main digestive tract, from which infection became disseminated. Hyalinocytes were abundant in tissues heavily infected with plasmodia, but phagocytosis of plasmodia was rarely observed. Brown cells were numerous among plasmodia in connective tissue, but did not appear to attach to or phagocytose the parasite.

Sporulation was confined to connective tissue (Fig. 7), in which cells containing indistinct nuclei appeared to develop into a loose syncytium comprising cytoplasm with 1 or 2 nuclei of unequal size (Table 2). Flask-shaped spores developed asynchronously in these syncytia, forming spore clusters (Fig. 7). In heavily infected oysters, plasmodia, syncytia, sporocysts and spore clusters occupied >50% of the connective tissue in section. Even in these infections, there was little if any haemocytic infiltration in response to sporulation, but brown cells were abundant at these sites.

Table 1. Prevalence of *Haplosporidium* sp. in *Saccostrea cuccullata* and *Saccostrea glomerata* along the north coast of Western Australia. ne: not examined; na: not applicable

Location	*Saccostrea cuccullata*		*Saccostrea glomerata*	
	No. infected	% infected	No. infected	% infected
Carnarvon	0/22	0.0	0/758	0.0
Exmouth Gulf	0/76	0.0	0/50	0.0
Barrow Island	0/103	0.0	ne	na
Airlie Island	26/211	12.3	ne	na
Rosily Island	1/31	3.2	ne	na
Varanus Island	90/322	28.0	ne	na
Montebello Islands	4/26	15.4	ne	na
Total	121/791	15.3	0/808	0.0

Electron microscopy

Development appeared to occur by enlargement of plasmodia to multinucleate sporonts, which changed to sporocysts with cytoplasmic cleavage around individual nuclei, forming a syncytium containing sporoblasts and spores.

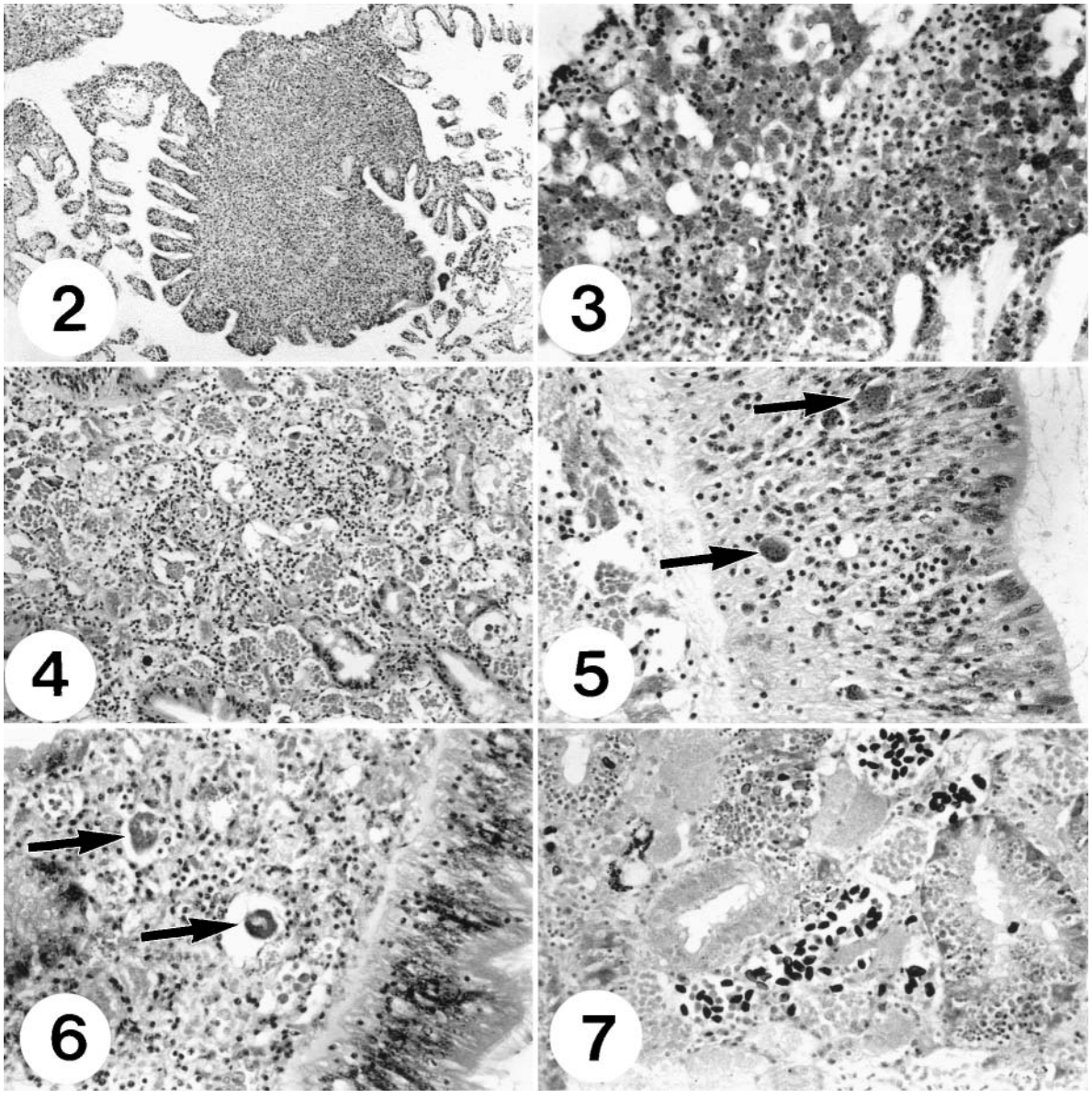
Plasmodia were distinguished by the presence of haplosporosomes and unmodified plasmalemma, and

Table 2. *Haplosporidium* sp. infecting *Saccostrea cuccullata*. Dimensions of the cells and their nuclei, each based on 50 measurements except TEM of plasmodia (n = 12). LM: light microscopy; TEM: transmission electron microscopy

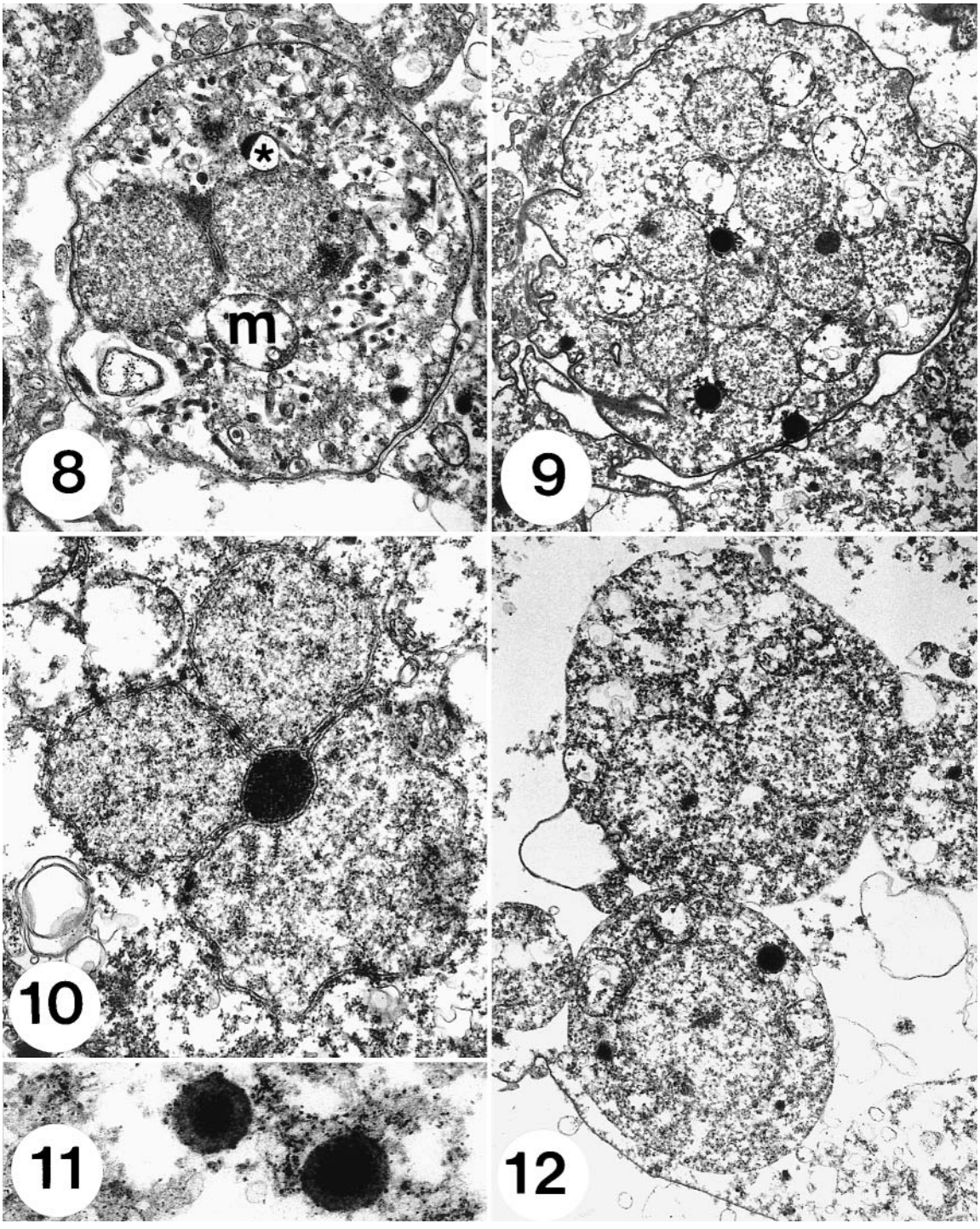
Stage/organelle	Method	Dimensions
Plasmodium	LM	6.3–15.4 µm
Plasmodium	TEM	4.0–4.5 × 4.0–5.0 µm
Plasmodial nuclei	TEM	1.3–1.5 µm
Sporonts	LM	17.0–22.5 µm
Sporonts	TEM	7.0–29.7 × 4.6–21.8 µm
Sporont syncytium	LM	20.0–31.0 µm
Spore clusters	LM	28.0–31.8 µm
Sporont nuclei	TEM	1.7–2.4 µm
Uninucleate sporoblasts	TEM	4.5–5.0 × 3.6 4.3 µm
Uninucleate sporoblast nuclei	TEM	2.3–3.0 µm
Binucleate sporoblasts	TEM	6.0–6.2 × 3.7–5.8 µm
Early spores (without operculum)	TEM	6.3–6.7 × 3.7–4.1 µm
Early spore nuclei	TEM	2.2–2.4 µm
Mature spores	TEM	5.6–6.7 × 3.3–4.0 µm
Mature spore nuclei	TEM	1.8–2.3 µm

were rarely found in TEM preparations. They were irregular in outline and diplokaryotic, with the 2 nuclei separated by dark material, 50 nm across (Fig. 8, Table 2). Each nucleus had a single round eccentric nucleolus. Mitochondria with oval cristae and sparse smooth endoplasmic reticulum (sER) occurred in the cytoplasm. Haplosporosomes were pleomorphic, being rod-like, 50–67 × 330 nm long and round in cross-section, ovoid to spherical 100–150 nm across, or small spherical and dense 60–70 nm, with a poorly defined bipartite sub-structure.

Sporonts and sporocysts developed from plasmodia, and were distinguished by having few or no haplosporosomes and a layer of dark material, 27–36 nm



Figs. 2 to 7. Occurrence of *Haplosporidium* sp. in *Saccostrea cucullata*. Light microscope histopathology. Fig. 2. Section through the gills showing a focal lesion, in which the connective tissue is packed with plasmodia (×75). Fig. 3. Occurrence of several developmental stages in the connective tissue of the digestive gland, around digestive diverticulae and ducts (×210). Fig. 4. Multinucleate plasmodia and haemocytes in the digestive gland (×190). Fig. 5. Multinucleate plasmodia (arrows) between the epithelial cells of the digestive tract (×270). Fig. 6. Two Multinucleate plasmodia lying in clears spaces (arrows), showing ring-like configuration (×300). Fig. 7. Ziehl-Nielsen stained section in which the spores stain strongly, showing their occurrence around, but not within, digestive tubules (×340)



Figs. 8 to 12. *Haplosporidium* sp. infecting *Saccostrea cucullata*: TEM of plasmodia, sporonts, sporocysts and sporoblasts. Fig. 8. Diplokaryotic plasmodium showing the dark material between the nuclei, elongated haplosporosomes, mitochondria (m) and lipid droplets (★) (×16 475). Fig. 9. Sporont with 7 nuclei showing the dense plasma membrane, lipid droplets, and lack of haplosporosomes (×7440). Fig. 10. Sporont nuclei showing the dense material in shallow indentations of the nuclear membrane (×21 635). Fig. 11. Sporont haplosporosomes lacking a distinct internal membrane (×63 700). Fig. 12. Uninucleate and binucleate sporoblasts lacking a dense plasma membrane, and haplosporosomes (×9210)

wide, on the plasma membrane, which bore short pseudopodia containing actin-like filaments. Sporonts contained 2 to 13 spherical nuclei/section, with a single nucleolus, which were often grouped as diplo-, tetra-, and poly-karya (Fig. 9, Table 2) enclosing dense granular masses that occupied shallow indentations on the nuclear surface (Fig. 10). Dense material, 50 nm across, also occurred between closely apposed surfaces of these tightly grouped nuclei. Nuclear divisions and mitotic spindles were not observed. The cytoplasm comprised ragged mitochondria, a little sER and several lipid droplets. In multinucleate sporonts, nuclei were separate and distributed throughout the cytoplasm. The few haplosporosomes observed were spherical and dense (<120 nm across; Fig. 11).

Uninucleate and larger binucleate sporoblasts were distinguished from sections in which sporonts contained 1 or 2 nuclei by the lack of a thickened plasma membrane around the sporoblasts (Fig. 12, Table 2). Nuclear divisions were not observed, nor were the early stages of sporulation.

The earliest observed spore stages, characterised by presence of a spore wall (Fig. 13) and operculum developing in the episporoplasm, were ovoid, with a single nucleus, a wall 76–95 nm wide, and a few spherical dense vesicles (DVs) 140–178 nm in diameter (Table 2). Maturing spores were ovoid, with a wall 160–202 nm across composed of very fine concentric laminations in 3 layers: inner dense, 25–40 nm; middle lucent, 19–33 nm; outer dense, 110–133 nm across. The wall was sometimes thickened posteriorly. The spore wall appeared to develop at nodes which became joined to complete the wall. Microtubule-like structures, 22–25 nm across, with a periodicity of 20 nm, occurred on the outside of the spore wall at the interface with episporoplasm. Sections through surface filaments, which may arise from a thickened portion of the posterior spore wall (Fig. 14), were only observed as 1 to 2 partial filaments around <2% of spores.

An anterior spherule appeared to produce DVs (Fig. 15) that were numerous throughout the sporoplasm, spherical, 159–228 nm in diameter, and which sometimes developed an internal membrane to form spherical haplosporosomes 153–180 nm in diameter (Fig. 15). The nucleus was spherical to irregular in shape, situated equatorially, and near it were spherical aggregations of dense granular material around which DVs were clustered (Fig. 16, Table 2). Bundles of microfilaments were seen in some sporoplasms (Fig. 16), and lipid droplets were commonly observed (Fig. 15), often bordered by 1 to 2 cisternae of sER. A lipid body was frequently observed in the episporoplasm adjacent to the middle of the spore (Figs. 15 & 16).

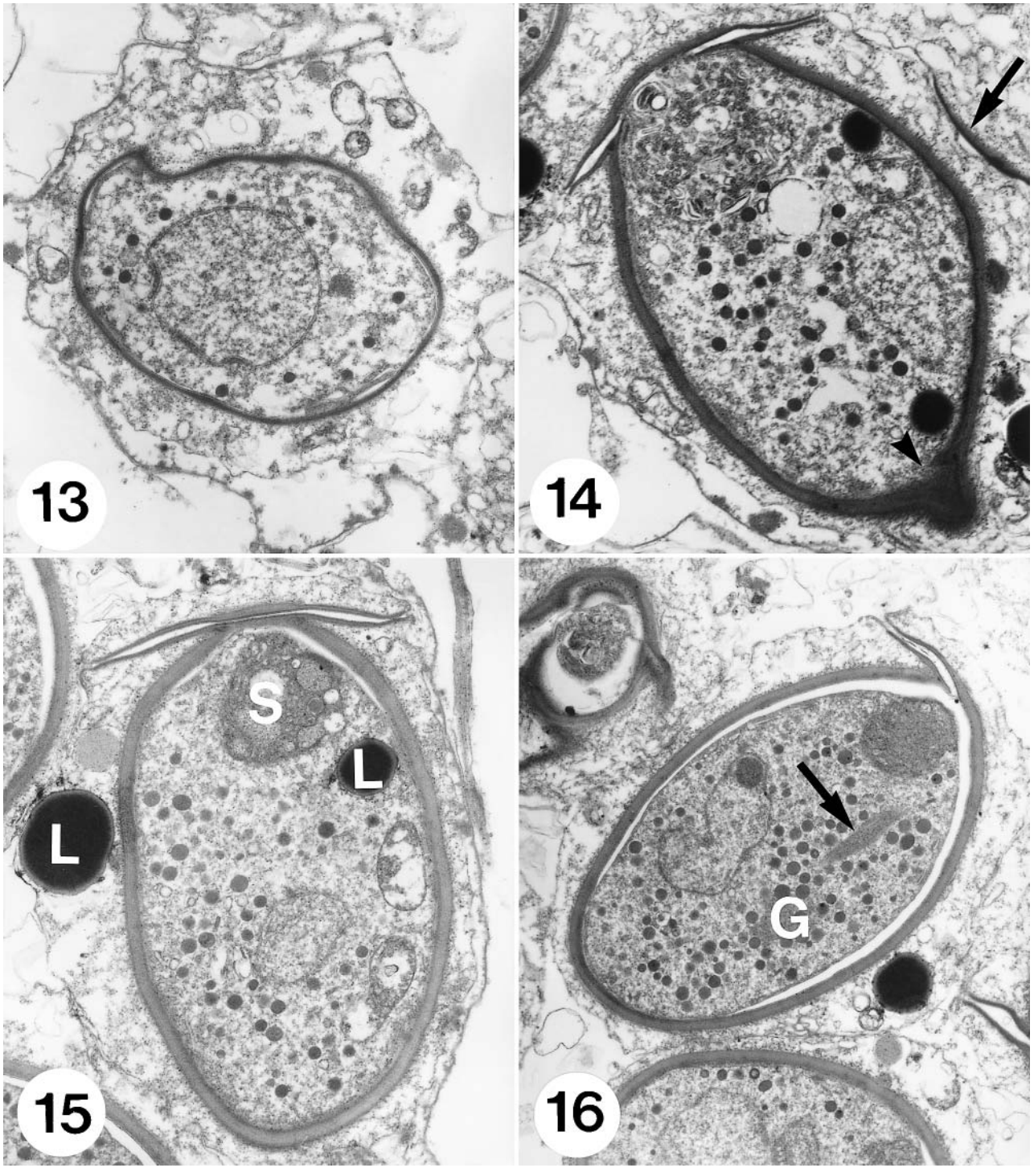
The operculum appeared to initially develop in the episporoplasm as 2 thin plates, 3.0–3.9 µm long, bearing a hinge at one side. Later the lower plate became attached to the spore wall at the anterior orifice. The inner dense layer of the 3 layers comprising the wall was reduced in the operculum, being 8–10 nm, with a middle lucent layer of 20–25 nm, and the outer dense layer reducing from 120–125 nm to 45 nm in the operculum (Fig. 17).

Mature spores had a wall 250 nm thick divided into 3 layers: inner dense, 90 nm; middle lucent, 30 nm; and outer dense, 130 nm across. After loss of the episporoplasm, the outer spore wall bore many microtubules 25 nm in diameter that showed no orientation or coalescence and were at least 1.25 µm long (Fig. 17). The operculum had a similar structure to the spore wall and was also coated with unorientated microtubules. Rod-like structures (695 × 50 nm) developed posteriorly in maturing spores, and were grouped near inversions of the spore wall or at the posterior pole of mature spores (Fig. 18). Similar rod-like structures (950 × 100 nm) remained inside empty spores, which appeared to have exsporulated, as a uninucleate stage resembling the sporoplasm was often adjacent to the empty spore.

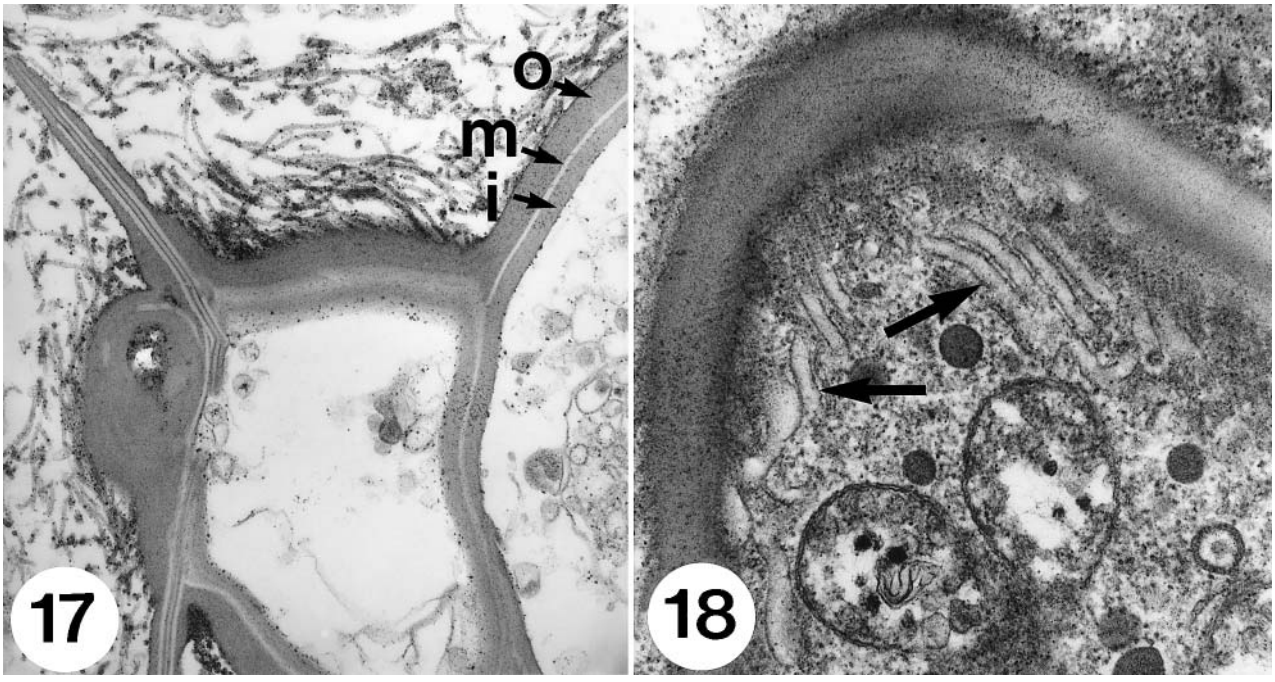
Ultrastructural observations on the inflammatory exudate showed it to be composed of >80% hyalinocytes, <5% granulocytes, and unidentified immature haemocytes.

DISCUSSION

Prevalence of infected oysters is not a good indicator of the severity of disease unless the temporal aspects of disease progression and number of freshly dead oysters can be determined. Despite this, the data suggest that infection was more prevalent in the west (Airlie, Varanus, Montebello and Lowendell Islands, than in the east (Dampier Archipelago, King Sound). Few oysters were lightly infected, and disease appeared to be progressive and fatal. The area concerned is remote, harsh, and sparsely populated. *Saccostrea cucullata* is not considered commercially important, and there have been no known movements of oysters into or out of the area. The mortalities therefore appear to be a natural event, unassociated with human activity. No other infections were observed that might account for the mortalities. No other dead and dying aquatic organisms were observed, suggesting mortalities were not due to environmental changes. The higher prevalence of infection and more common sporogenesis in oysters with empty gonad follicles may have been due to loss of condition after spawning increasing the susceptibility to infection. Alternatively, infection may reduce the host energy budget causing reduction in, or cessation of, gametogenesis, similar to that reported in



Figs. 13 to 16. *Haplosporidium* sp. infecting *Saccostrea cucullata*: spore formation. Fig. 13. Developing spore showing spore wall, central round nucleus and a few sperical dense vesicles, DVs (×12 870). Fig. 14. Spore showing posterior thickening of the spore wall (arrowhead) and a filament in the episporoplasm (arrow) (×13 125). Fig. 15. Spore with an anterior spherule (S) containing bodies resembling DVs. Prominent lipid droplets (L) are present (×16 360). Fig. 16. Spore containing a bundle of microfilaments (arrow) and granular material (G) near the nucleus (×12 890)



Figs 17 to 18. *Haplosporidium* sp. infecting *Saccostrea cucullata*: spores. Fig. 17. Anterior of a mature spore showing the inner (i), middle (m) and outer (o) layers of the operculum and wall, and surface microtubules (×28 815). Fig. 18. Posterior of spore showing rodlike structures (arrows) (×42 890)

Haplosporidium nelsoni infections of *Crassostrea virginica* (Barber et al. 1988, Ford & Figueras 1988, Ford et al. 1990).

Comparison with other haplosporidian studies in the literature is difficult because of inconsistencies in terminology (particularly the use of 'plasmodia' and 'sporocysts') for multinucleate sporonts, and because spore dimensions vary between fresh smears, sections in light and electron microscopy and re-embedding of tissues for TEM from paraffin wax blocks. Also, as in this study, most observations have been made on maturing (but immature) spores, as the contents of mature spores were often lost or, when present, were often very dense. Despite this, differences in spore size and tissue tropism can be discerned (Table 3).

The species described here was difficult to place in either of the genera *Haplosporidium* or *Minchinia* with any certainty. The surface microtubules that

Table 3. *Haplosporidium* spp. reported from oysters, connective tissue, CT and digestive tubule epithelium, DTE. (+: present; –: absent); (f): fresh spores, (s): in section; ng: not given

Species	Host	CT	DTE	Spore dimensions (µm)	Source
H. nelsoni	*Crassostrea virginica*	+	+	8.1 × 5.5	Perkins (1968)
Haplosporidium sp.	*C. gigas*	+	+	6.7 × 5.1 6–8 × 5–6	Friedman et al. (1991)
Haplosporidium sp.	*C. gigas*	+	+	6.3 × 4.6 5.0–8.0 × 4.0–6.0	Kern (1976)
H. costale	*C. virginica*	+	–	4.3 × 3.3	Perkins (1969)
Haplosporidium sp.	*C. gigas*	+	–	4.8 × 3.4	Comps & Pichot (1991)
Haplosporidium sp.	*C. gigas*	+	–	ng	Katkansky & Warner (1970)
H. armoricanum	*Ostrea edulis*	+	–	5.0–5.5 × 4.0–4.5^{f} 4.0–4.5 × 3.0–4.0^{s}	van Banning (1977)
H. armoricanum	*O. edulis*	ng	ng	4.5–5.0 × 3.5–4.5^{f} 3.0–4.0 × 2.5–3.5^{s}	Pichot et al. (1979)
H. armoricanum	*O. edulis*	+	–	4.0–6.0 × 2.5–4.0	Bachère & Grizel (1983)
H. armoricanum	*O. angasi*	+	–	4.0–5.0 × 2.5–4.0	Bachère et al. (1987)
Haplosporidium sp.	*Pinctada maxima*	+	–	6.7–7.7 × 3.8–4.3	Hine & Thorne (1998)
Haplosporidium sp.	*Saccostrea cucullata*	+	–	5.6–6.7 × 3.3–4.0	This study

appeared to derive from the episporoplasm resemble similar microtubules in epispore cytoplasmic vacuoles of *Minchinia* in crabs (Rosenfield et al. 1969, Perkins 1975). These may be seen aligned under the epispore plasma membrane in *M. chitonis* (Ball 1980), appear to enter the ECE as bundles of microtubules in *M. teredinis* (McGovern & Burreson 1990), and form the support of ECE in *Minchinia* sp. from mussels (*Mytilus galloprovincialis*) (Comps & Tigé 1997). However, in these latter cases the microtubules are aligned, and it is only in *Haplosporidium ascidiarum* from tunicates that similar unaligned microtubules have been reported (Ormières & de Puytorac 1968). A recent study on *H. ascidiarum* suggests that the spore wall is assembled from similar filaments (Ciancio et al. 1999). Filaments have not been observed around *H. ascidiarum* (Azevedo et al. 1999, Ciancio et al. 1999), and it may therefore be a species of *Minchinia*. However, on the basis of rare observations suggesting that spore filaments may occur in this study, and that they derive from the spore wall (Fig. 14), the present species is tentatively placed in the genus *Haplosporidium.*

Haplosporidians are usually host genus-specific, and the other species described from oysters need to be considered (Table 3). Although the oyster genera *Crassostrea* and *Saccostrea* are closely related and sometimes used interchangeably, they are distinct genera (Brock 1990). Restriction of plasmodial and sporulation stages to connective tissue has been reported from all *Haplosporidium* spp. of ostreids (Table 3), except *H. nelsoni* (Perkins 1968, Kern 1976, Friedman et al. 1991, Friedman 1996, Renault et al. 2000), which infects epithelia as well as connective tissue.

The *Haplosporidium* sp. in *Saccostrea cucullata* has spores similar in size to haplosporidians of *Ostrea* spp., including *H. armoricanum* (van Banning 1977, Cahour et al. 1980) (Table 3). The spores are smaller than those of *H. nelsoni* (Perkins 1968) from *Crassostrea virginica*, and from *C. gigas* in Japan (Friedman et al. 1991) and Korea (Kern 1976), but larger than spores of *H. costale* (Perkins 1969) in *C. virginica*, and *Haplosporidium* sp. in *C. gigas* from the French Mediterranean (Comps & Pichot 1991) (Table 3). The species reported here also differs from *H. nelsoni* in its lack of plasmodial multivesicular bodies. There is also a dissimilarity between the rod-like and small dense haplosporosomes of *Haplosporidium* sp. plasmodia, and those of *H. nelsoni* (Perkins 1968). A specific probe for *H. nelsoni* (Stokes & Burreson 1995) did not react with the *Haplosporidium* sp. described herein (Dr E. M. Burreson, Virginia Institute of Marine Science, pers. comm.). It has a closer similarity with *H. costale* in that both contain granular material in nuclear membrane indentations and free at the surface of plasmodial and sporont nuclei (Perkins 1969), but the rod-like haplosporosomes of *Haplosporidium* sp. from *S. cucullata* are unlike the pyriform haplosporosomes of *H. costale* (Perkins 1969). The species reported herein differs from *H. armoricanum* in the appearance of haplosporosomes and large sporocysts (30 to 50 µm) with 100 to 150 nuclei in the latter species. It also differs from the *Haplosporidium* sp. infecting pearl oysters (*Pinctada maxima*: Pteriidae) on the same coast, in size (spores in *P. maxima* 6.7–7.7 × 3.8–4.3 µm, position of the nucleus in the spores, the presence of lipid droplets in the spore and epispore, the presence of rod-like structures in the posterior sporoplasm, and surface microtubules (Hine & Thorne 1998).

The developmental stages observed here are similar in general detail to those reported from other *Haplosporidium* and *Minchinia* species (Perkins 1968, 1969, Marchand & Sprague 1979, Ball 1980, Desportes & Nashed 1983, La Haye et al. 1984, Hillman et al. 1990), except that the persistent mitotic spindle, division, and the nuclear fusion followed by enclosure of the sporoplasm by the episporoplasm (Desportes & Nashed 1983) were not observed. However, the greater nuclear size of the earliest uninucleate sporoblasts (2.3 to 3.0 µm) than preceding (2.0 to 2.4 µm) or succeeding (2.2 to 2.4 µm) nuclei, may reflect fusion of the nuclei of a preceding binucleate stage, as in *M. dentale* (Desportes & Nashed 1983). The rod-like structures at the periphery and posterior of the sporoplasm have not been previously reported. If the rod-like bodies in empty spores derive from them, they may have a function in expulsion of the sporoplasm.

Just as the haplosporidians of xanthid crabs may be a single species or closely related species (Perkins & van Banning 1981), similarities may be seen between the *Haplosporidium* and *Minchinia* species of ostreids. Molecular evidence suggests that discrimination of *Haplosporidium* and *Minchinia* on the basis of the presence of tails, filaments, wrappings, ribbons, or other ornaments (Azevedo 1984) may be invalid (Flores et al. 1996). Even such phenotypic traits as spore formation itself may be unreliable. Recent studies suggest that, phylogenetically, *Bonamia* spp., for which spores are unknown, may lie between *Haplosporidium* and *Minchinia* (Carnegie et al. 2000, Hine et al. 2001), and it has even been suggested that *B. ostreae* may be placed in the genus *Haplosporidium* (Cochennec et al. 2000). Formal description of the species reported here must await clarification of the phylogeny of haplosporidian genera. Formal descriptions should be based on both molecular phylogeny, and the ultrastructure of phenotypes.

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