

# Haplosporosomes, sporoplasmosomes and their putative taxonomic relationships in rhizarians and myxozoans

## Review

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## Abstract

This paper reviews current knowledge of the structure, genesis, cytochemistry and putative functions of the haplosporosomes of haplosporidians (*Urosporidium*, *Haplosporidium*, *Bonamia*, *Minchinia*) and paramyxids (*Paramyxa*, *Paramyxoides*, *Marteilia*, *Marteilioides*, *Paramarteilia*), and the sporoplasmosomes of myxozoans (*Myxozoa* – *Malacospora*, *Myxosporea*). In all 3 groups, these bodies occur in plasmodial trophic stages, disappear at the onset of sporogony, and reappear in the spore. Some haplosporidian haplosporosomes lack the internal membrane regarded as characteristic of these bodies and that phylum. Haplosporidian haplosporogenesis is through the Golgi (spherulosome in the spore), either to form haplosporosomes at the *trans*-Golgi network, or for the Golgi to produce formative bodies from which membranous vesicles bud, thus acquiring the external membrane. The former method also forms sporoplasmosomes in malacosporeans, while the latter is the common method of haplosporogenesis in paramyxids. Sporoplasmodogenesis in myxosporeans is largely unknown. The haplosporosomes of *Haplosporidium nelsoni* and sporoplasmosomes of malacosporeans are similar in arraying themselves beneath the plasmodial plasma membrane with their internal membranes pointing to the exterior, possibly to secrete their contents to lyse host cells or repel haemocytes. It is concluded that these bodies are probably multifunctional within and between groups, their internal membranes separating different functional compartments, and their origin may be from common ancestors in the Neoproterozoic.

## Introduction

The term ‘haplosporosome’ was coined for cytoplasmic bodies defined as, ‘membrane-bound, osmiophilic structures which are oval, spherical, pyriform or short rods in the haplosporidian *Urosporidium crescens*’ (Perkins, 1971). This term was then applied to morphologically similar organelles in the paramyxid *Marteilia refringens* (Perkins, 1976, 1979) because they form in plasmodia, disappear during sporogenesis and reform in spores, as in haplosporidians. Paramyxids were therefore placed with haplosporidians in the class Haplosporea (Perkins, 1976). Similar cytoplasmic organelles in the sporoplasm of a myxosporean myxozoan, *Hoferellus gilsoni*, that were reminiscent of haplosporosomes, were given the name ‘sporoplasmosomes’ (Lom *et al.*, 1986). Other myxozoan parasites that infect fish and bryozoans, the malacosporeans, contain cytoplasmic electron dense bodies (EDBs) (Seagrave *et al.*, 1980; Morris *et al.*, 2000), which are also called sporoplasmosomes (Morris and Adams, 2008).

Before electron microscopy and molecular phylogenies haplosporidians and paramyxids were classified together in the phylum Ascetosporea, then separated and given phylum status. Subsequently, the Ascetosporea was resurrected as a class in the phylum Cercozoa (Berthe *et al.*, 2004; Burrenson and Ford, 2004), and currently, they are regarded as endomyxan rhizarians (Cavalier-Smith *et al.*, 2018). The class Ascetosporea comprises the orders: (1) the Haplosporida (Hartikainen *et al.*, 2014a), (2) the Mikrocytida (Hartikainen *et al.*, 2014b), (3) the Paramxyida (Ward *et al.*, 2016), (4) the Claustrosporida, and (5) the Paradinida (Bass *et al.*, 2009; Ward *et al.*, 2018). The Claustrosporida comprises two species, *Claustrosporidium gammari* and *C. aselli* which infect the amphipod *Rivulogammarus pulex* (see Larsson, 1987). They differ from haplosporidians in having spores that lack an orifice, but molecular data are not available and spore form may not be of taxonomic importance (Burrenson and Ford, 2004). While haplosporidians, paramyxidans and claustrosporidians have haplosporosomes, mikrocytidans appear not to (Hine *et al.*, 2001a). The spot prawn parasite (Meyers *et al.*, 1994; Bower and Meyer, 2002) was placed at the base of haplosporidian phylogeny (Reece *et al.*, 2004), but appears to be more closely related to *Paradinium* spp. (Skovgaard and Daugbjerg, 2008) and is not included here. The phylum Haplosporidia comprises 4 described genera (*Urosporidium*, *Haplosporidium*, *Bonamia*, *Minchinia*) and several unclassified species (Hartikainen *et al.*, 2014a). Forms attributed to possible spore-like stages

of *B. exitiosa* (Fig. 6 in Hine, 1991, Figs 12–16 in Hine *et al.*, 2001b) have not been reported from *B. exitiosa* elsewhere, and are probably a different haplosporidian representing an undescribed genus. The features of most ultrastructurally described haplosporidians were reviewed in 2009 (Hine *et al.*, 2009), and the reader is referred to that paper for details.

Myxozoans were earlier thought to be related to paramyxids because both have cells contained within other cells and show early development of somatic elements (Desportes and Lom, 1981; Berthe *et al.*, 2004). However, electron microscopy showed myxozoan spores contained polar capsules similar to cnidarian nematocysts (Lom and de Puytorac, 1965) and now myxozoans are recognized as cnidarians (Siddall *et al.*, 1995; Jiménez-Guri *et al.*, 2007) that form a sister clade to the cnidarian parasite *Polypodium hydriforme* (Chang *et al.*, 2015; Foox and Siddall, 2015). Myxozoans of the class Myxosporea have rich biodiversity and cycle from invertebrate hosts, usually, annelids, in which actinospores are formed, to ectothermic vertebrates, particularly fish, in which myxospores are formed (Lom and Dyková, 2006; Holzer *et al.*, 2018). Proliferative kidney disease (PKD) in salmonids was found to be caused by a myxozoan (Kent and Hedrick, 1985), which based on the stages infecting bryozoan hosts was later named *Tetracapsuloides* (*syn. Tetracapsula*) *bryosalmonae* (Canning *et al.*, 1999). This and several related species that use freshwater bryozoans as hosts (Anderson *et al.*, 1999; Canning *et al.*, 2000, 2007, 2008), have been placed in the class Malacosporea (Canning *et al.*, 2000). They, and their sister clade, Myxosporea, originally infected invertebrate hosts, and subsequently infected fish with which they co-evolved (Holzer *et al.*, 2018). Because of previous uncertainties regarding the taxonomic placement of *T. bryosalmonae*, initial descriptions of sporoplasmosomes in this species referred to them variously as haplosporosomes or EDBs (Seagrave *et al.*, 1980; Morris *et al.*, 2000). These bodies are now considered synonymous with the sporoplasmosomes seen in other myxozoan species (Canning *et al.*, 2000). The malacosporean spores formed in the bryozoan are called malacospores, while those found in the fish can be referred to as fish-malacospores (after Lom and Dyková, 2006).

This paper considers the structure, development, genesis and function of haplosporosomes and sporoplasmosomes of these obligate parasitic groups, how they may inter-relate and the implications for the taxonomy of these disparate protist and metazoan organisms. Ultrastructural studies which contain insufficient information on these bodies are not included.

## Haplosporidia

### Structure and cytochemistry

Haplosporidian haplosporosomes were originally described in detail from *Haplosporidium nelsoni* (Perkins, 1968; Fig. 1B) and other species (Perkins, 1979) as having a delimiting unit membrane and an internal membrane separating the medulla from the cortex. While usually spheroid or ovoid, they may also be vermiform or tubular, club-shaped, pyriform, or resembling an axehead (Table 1; Fig. 1C), that in *Haplosporidium armoricanum* has a hollow core (Hine *et al.*, 2007). Formative bodies (FBs) lacking an internal membrane occur in the spherulosomes of spores (Desportes and Nashed, 1983; La Haye *et al.*, 1984; Ciancio *et al.*, 1999; Hine and Thorne, 2002; Hine *et al.*, 2007; Hine *et al.*, 2009). The internal membrane usually runs parallel to the external membrane, but in *U. crescens* it is C-shaped, sometimes appearing as 3 concentric rings (Perkins, 1971), in *Haplosporidium patagon* there is a cup-like indentation of the membrane (Figs 3B, 3C, 4B and 4E in Ituarte *et al.*, 2014), and in *H. nelsoni*, it is vase-shaped (Perkins, 1968; Scro and Ford, 1990; Renault *et al.*, 2000). There is a lack of an internal

membrane in haplosporosomes of *Haplosporidium comatulae* in the crinoid *Oligometra serripinna* (see La Haye *et al.*, 1984), and in polymorphic forms in *Haplosporidium parisi* from the polychaete *Serpula vermicularis* (see Ormières, 1980). Internal membranes also lack in inclusion bodies in *Haplosporidium ascidiarum* from the ascidian *Ciona intestinalis* (Ciancio *et al.*, 1999). Some haplosporosomes of a New Zealand abalone parasite (NZAP) (Hine *et al.*, 2002) have tails (P.M. Hine *unpub. obs.*).

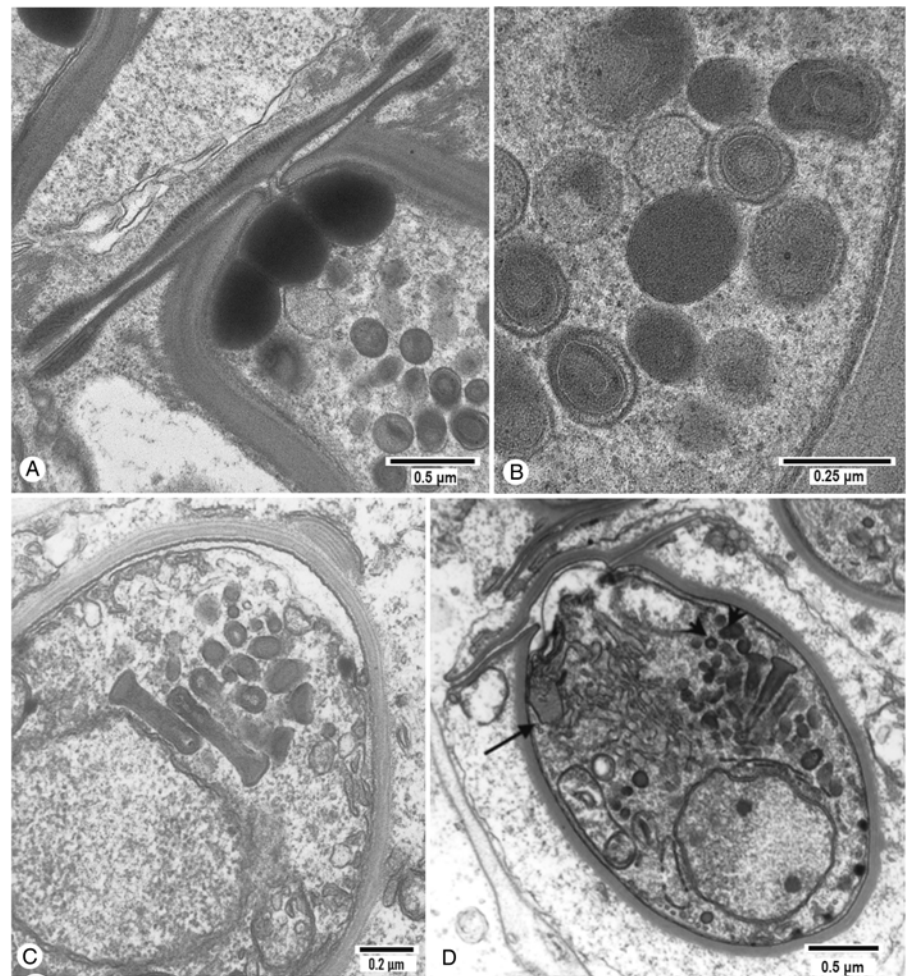
The ultrastructure of crustacean haplosporidians has been reported from amphipods (Winters and Faisal, 2014; Urrutia *et al.*, 2019), prawns (Dyková *et al.*, 1988; Meyers *et al.*, 1994; Bower and Meyer, 2002) and crabs (Perkins, 1975; Newman *et al.*, 1976; Marchand and Sprague, 1979; Stentiford *et al.*, 2004, 2013). *Haplosporidium louisiana* (*syn. Minchinia cadomensis*) has typical haplosporosomes in plasmodia and spores (Perkins, 1975; Marchand and Sprague, 1979), and the plasmodia of two unclassified species (Newman *et al.*, 1976; Dyková *et al.*, 1988) also have typical haplosporosomes. *Haplosporidium littoralis* has haplosporosome-like bodies (H-LBs) which appear to be immature haplosporosomes, and dense vesicles (DVs) with or without an internal membrane, regarded as haplosporosomes. In 3 species (Newman *et al.*, 1976; Dyková *et al.*, 1988; Stentiford *et al.*, 2004, 2013) haplosporosomes cluster around and are in contact with the nucleus. *Haplosporidium* spp. have DVs without an internal membrane, and some, e.g. *H. parisi* (Ormières, 1980), appear to lack haplosporosomes and have very different organelles. However, the genus *Haplosporidium* is paraphyletic, acting as a repository for orphan species, and may contain many genera and families (Hartikainen *et al.*, 2014a; Hine, 2020).

Homogeneous osmiophilic, probably lipid, bodies from the spherule in spores of *Haplosporidium nelsoni* (Fig. 1A), *Haplosporidium pickfordi* (see Burreson, 2001), *Haplosporidium lusitanicum* (Azevedo, 1984; Azevedo and Corral, 1985, 1987), *Haplosporidium armoricanum* (see Hine *et al.*, 2007), *Haplosporidium montforti* (see Azevedo *et al.*, 2006), and *Haplosporidium* sp. (Comps and Tigé, 1997), which lie peripherally in sporoplasm, are probably not related to haplosporosomes.

Cytochemically, the cortex of H-LBs of the NZAP (Hine *et al.*, 2002), is labelled by zinc iodide-osmium tetroxide (ZIO) which labels Golgi and tubulovesicular systems. ZIO also labels the haplosporosomes and multivesicular bodies (MVBs) of *Bonamia exitiosa* (Fig. 2), but imidazole-buffered OsO<sub>4</sub> showed lipid is weak on the haplosporosome surface (Hine and Wesney, 1994a). The haplosporosomes of the NZAP, *Bonamia ostreae* and *B. exitiosa* lack acid phosphatases (Hervio *et al.*, 1991; Hine and Wesney, 1994a; Hine *et al.*, 2002). Haplosporosomes of *H. lusitanicum* react weakly for glycoproteins, the external unit membrane reacting more strongly than the more lipoidal inner membrane (Azevedo and Corral, 1985, 1987). *Haplosporidium lusitanicum* spores have haplosporosomes containing polysaccharides and basal and apical peripheral membrane-bound DVs lying just under the plasma membrane which stain intensely for polysaccharides (Azevedo, 1984) and may be involved in the formation of the spherule (Azevedo and Corral, 1985). FBs and haplosporosomes in *H. nelsoni* are positive with the Feulgen stain for DNA (Perkins 1968, 1979).

### Development and haplosporogenesis

Haplosporidian haplosporosomes occur in presporogonic plasmodial, stages, disappear at the onset of sporogony and are then formed in the sporoplasm. The methods of formation in relation to species are given in Table 2. Haplosporogenesis in some haplosporidian plasmodia and spores begins when granular material, apparently of nuclear origin occurs in an indentation in the nuclear surface (INS) (Fig. 3A). Budding of the nuclear



**Fig. 1.** Ultrastructural aspects of haplosporosomes in haplosporidian species. (A) Longitudinal section through the apical portion of a *H. nelsoni* spore showing spore operculum and internal osmiophilic lipid droplets with numerous cytoplasmic haplosporosomes. (B) Detail of *H. nelsoni* haplosporosomes showing structural pleomorphism with internal membranes. (C) Spore of *H. armoricanum* showing detail of the hollow axehead-shaped haplosporosomes. (D) Spore of *H. armoricanum* showing nuclear membrane-bound Golgi between the nucleus and haplosporosomes, and an MVB (arrowed).

membrane occurs near nuclear membrane-bound Golgi (NM-BG) along which balls of the putative nuclear material are processed. H-LBs bud from a *trans*-Golgi network (TGN) and condense to form haplosporosomes (Perkins, 1979; Hine and Wesney, 1992; Hine et al., 2002). NM-BG may also occur in spores (Fig. 1D) (Hine et al., 2007). In *Haplosporidium pinnae* H-LBs appear to bud from the nuclear membrane (Catanese et al., 2018). The H-LBs mature into haplosporosomes in the TGN (Perkins, 1979; Hine and Wesney, 1992; Hine et al., 2002). The cytoplasmic distribution of haplosporosomes in the NZAP may be related to the occurrence of endoplasmic reticulum (Fig. 3B). In *H. nelsoni* plasmodia, haplosporosomes are formed when membranous vesicles in MVBs bud off to acquire an outer membrane (Perkins, 1968, 1979; Renault et al., 2000). In spores, the putative homolog of Golgi, the spherulosome or spherule, either produces haplosporosomes directly (*Urosporidium* spp.) or produces membrane-bound dense FBs which may be striated. They may remain as FBs (Perkins, 1968; Azevedo, 1984; La Haye et al., 1984) or haplosporosomes may develop from them (Perkins, 1979; Desportes and Nashed, 1983; Ciancio et al., 1999). Haplosporosomes may also form by budding of membranous vesicles from FBs or MVBs in the plasmodia of *H. nelsoni* (Perkins, 1975) and in spores (Table 2). The spore of *H. armoricanum* has both a spherule and NM-BG (Hine et al., 2007) (Fig. 1D). MVBs reported from the NZAP (Fig. 9 in Hine et al., 2002) and *B. exitiosa* (Fig. 22 in Hine and Wesney, 1992), from which tubules extend contain putative haplosporosome cores. They may be autophagic or crinophagic lysosome-like bodies. They are not similar to the MVBs associated with haplosporosomes in paramyxids.

#### Putative functions of haplosporidian haplosporosomes

The large plasmodia of the NZAP and *H. nelsoni* are extracellular and release their contents to destroy surrounding cells, but intracellular *Bonamia* spp. have small plasmodia and retain their haplosporosomes (Hine, 2020). It cannot be assumed that haplosporosomes have the same functions in plasmodia and spores, or that within those stages they have a uniform function. For example, *Minchinia occulta* spores (Bearham et al., 2008a) have concurrent ultrastructurally different haplosporosome populations (Hine and Thorne, 2002). Also, differences in the density of the matrix in compartments such as the cortex and medulla (Figs 2 and 9 in Azevedo and Corral, 1985, Fig. 11 in Hine and Thorne, 2002, Figs 2 and 10 in Perkins, 1979, Fig. 12 in Stentiford et al., 2004), suggest differences in content. The internal membrane, when present, may have the dual functions of separating the content of different compartments and the external membrane of fusion with the plasma membrane before exocytosis of the internal matrix. It has been proposed that plasmodial haplosporosomes participate in thickening the plasma membrane in the early stages of sporogony (Perkins, 1971, 1979), while spore haplosporosomes facilitate the release of the sporoplasm from the spore (Azevedo and Corral, 1989). This suggests that haplosporosomes in plasmodial and spore stages have unique and distinct functions.

Plasmodia of 3 early-diverging haplosporidians (Reece and Stokes, 2003; Reece et al., 2004; Ward et al., 2018), the NZAP (Hine et al., 2002), *U. crescens* (Perkins, 1971) and *H. nelsoni* (Scro and Ford, 1990), release haplosporosomes. While those of the NZAP cause massive damage to surrounding cells (Diggle et al., 2002; Hine et al., 2002), those of *H. nelsoni* and

**Table 1.** The shapes of haplosporidian haplosporosomes in relation to different developmental stages. Numbers in the table refer to the references given below

Species	Ref.	Plasmodia	Spores
Spot prawn parasite (SPP)	1	Spherical, ovoid DVs	
New Zealand abalone parasite (NZAP)	2	Ovoid, spherical, elongated	
<i>Urosporidium cannoni</i>	3		Ovoid, spherical, elongated
<i>Urosporidium crescens</i>	4	Spherical, oblate FBs	Pyriiform
<i>Urosporidium</i> sp.	5		Spherical
<i>Urosporidium jiroveci</i>	6		Spherical
<i>Urosporidium spisuli</i>	7		Spherical, ovoid
<i>Haplosporidium littoralis</i>	8	Ovoid, spherical DVs, uncommon haplosporosomes	
<i>Haplosporidium armoricanum</i>	9	Spherical	Axehead-shaped
<i>Haplosporidium costale</i>	10	Spherical ovoid	Axehead-shaped
<i>Haplosporidium hinei</i>	11, 12	Spherical ovoid	Large, spherical, ovoid, axehead-shaped
<i>Haplosporidium</i> sp.	13		Spherical, ovoid, axehead-shaped
<i>Haplosporidium patagon</i>	14		Spherical
<i>Haplosporidium lusitanicum</i>	15–17		Spherical
<i>Haplosporidium nelsoni</i>	18, 19	Spherical FBs	Ovoid spherical FBs
<i>Haplosporidium louisiana</i>	20, 21	Spherical, fusiform, elongated	Ovoid FBs, spherical haplosporosomes
<i>Haplosporidium parisi</i>	22		Large, polymorphic complex
<i>Haplosporidium ascidiarum</i>	23	Amorphous, ovoid	Amorphous, ovoid
<i>Haplosporidium montforti</i>	24, 25		Spherical, ovoid
<i>Haplosporidium comatulae</i>	26		Ovoid, spherical, elongated FBs
<i>Haplosporidium pinnae</i>	27	Spherical, ovoid	Ovoid, elongated
<i>Bonamia exitiosa</i>	28–30	Ovoid, spherical	
<i>Bonamia ostreae</i>	31, 32	Spherical	
<i>Bonamia perspora</i>	33	Spherical	Spherical
<i>Minchinia teredinis</i>	34, 35		Ovoid FBs
<i>Minchinia chitonis</i>	36		Spherical, ovoid
<i>Minchinia tapetis</i>	37, 38		Ovoid FBs, spherical haplosporosomes
<i>Minchinia occulta</i>	39, 40		Spherical FBs, sometimes haplosporosomes
<i>Minchinia dentali</i>	41	Spherical, ovoid	FBs
<i>Minchinia</i> sp.	42		Ovoid FBs, spherical haplosporosomes
Unclassified	43	Ovoid, spherical	
Unclassified	44	Ovoid, spherical	

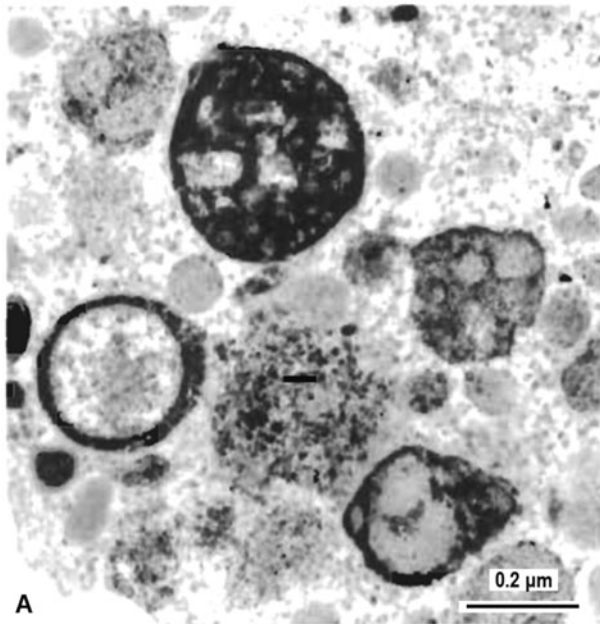
1. Bower and Meyer, 2002; 2. Hine *et al.*, 2002; 3. Anderson and Lester, 1992; 4. Perkins, 1971; 5. Carballal *et al.*, 2005; 6. Ormières *et al.*, 1973; 7. Perkins, 1975; 8. Stentiford *et al.*, 2004; 9. Hine *et al.*, 2007; 10. Perkins, 1969; 11. Bearham *et al.*, 2008b; 12. Hine and Thorne, 1998; 13. Comps and Pichot, 1991; 14. Ituarte *et al.*, 2014; 15. Azevedo, 1984; 16. Azevedo and Corral, 1985; 17. Azevedo and Corral, 1987; 18. Perkins, 1968; 19. Renault *et al.*, 2000; 20. Perkins, 1975; 21. Marchand and Sprague, 1979; 22. Ormières, 1980; 23. Ciancio *et al.*, 1999; 24. Azevedo *et al.*, 2006; 25. Azevedo *et al.*, 2008a; 26. La Haye *et al.*, 1984; 27. Catanese *et al.*, 2018; 28. Hine and Wesney, 1992; 29. Hine and Wesney, 1994a; 30. Hine and Wesney, 1994b; 31. Pichot *et al.*, 1979; 32. Hervio *et al.*, 1991; 33. Carnegie *et al.*, 2006; 34. Hillman *et al.*, 1990; 35. McGovern and Bureson, 1990; 36. Ball, 1980; 37. Azevedo, 2001; 38. Azevedo and Corral, 1989; 39. Hine and Thorne, 2002; 40. Bearham *et al.*, 2008a; 41. Desportes and Nashed, 1983; 42. Comps and Tigé, 1997; 43. Dyková *et al.*, 1988; 44. Newman *et al.*, 1976.

*U. crescens* plasmodia appear not to (Perkins, 1971, 1979). However, at sporulation in *U. crescens*, the release of haplosporosomes causes extensive damage to surrounding cells resulting in the host being 'bags' of spores (Perkins, 1971). Histologically, NZAP plasmodia are surrounded by a halo, due to destruction of surrounding host cells, leaving the host as bags of plasmodia (Diggles *et al.*, 2002; Hine *et al.*, 2002). Many NZAP haplosporosomes appear to accumulate in autophagic or crinophagic vacuoles.

The cores of some NZAP haplosporosomes occur in coated pits or thickenings of the plasma membrane and some lie beneath the plasma membrane. They become elongated and orientate at right angles to the plasma membrane, sometimes piercing it (Hine *et al.*, 2002). Similarly, plasmodia of *H. nelsoni* are closely juxtaposed to host cells, align haplosporosomes (>65 nm) beneath

the plasma membrane with the narrow end of the flask-shaped internal membrane orientated toward the exterior (Fig. 5 and 6 in Scro and Ford, 1990). *In vitro*, when oyster (*Crassostrea virginica*) granular haemocytes come in contact with *H. nelsoni* plasmodia they quickly retreat and agranular haemocytes become inactive, suggesting the plasmodia produce a substance inhibitory to haemocytes (Ford *et al.*, 1993). Such a substance may derive from the haplosporosomes aligned beneath the plasmodial membrane. Treatment of *H. nelsoni* plasmodia with enzymes and metabolic inhibitors increase phagocytosis by haemocytes, particularly the glycolysis inhibitor iodoacetate (Ford and Ashton-Alcox, 1998).

The function or functions of haplosporosomes in spores is/are uncertain. In *U. crescens*, the disappearance of haplosporosomes



**Fig. 2.** Zinc iodide-osmium tetroxide positive MVBs in the cytoplasm of *Bonamia exitiosa*.

at the onset of sporulation coincides with damage to surrounding cells which intensifies as sporulation progresses (Perkins, 1971). This loss of haplosporosomes also occurs widely in spore-forming species, but damage at sporulation has seldom been reported (Hine et al., 2009). Other membrane-bound dense bodies at the periphery of spores of *H. lusitanicum* (Azevedo and Corral, 1985, 1987), *H. armoricanum* (Hine et al., 2007), *H. patagon* (Ituarte et al., 2014), and a haplosporidian resembling *Haplosporidium costale* (Comps and Pichot, 1991) may not be related to haplosporosomes. They are formed by the spherule and are released from the sporoplasm to lie between it and the spore wall. Their function is unknown.

There is some evidence regarding the haplosporosomes of some haplosporidians as viruses or virus-like. In the NZAP (Hine et al., 2002) and haplosporidians infecting ostreid oysters (Hine and Wesney, 1992; Hine and Thorne, 2002; Hine et al., 2007) balls of putative nuclear material pass from indentations in the nuclear membrane (Fig. 3A) to the NM-BG where, at the TGN, H-LBs are formed (Table 2). The haplosporosomes of *H. nelsoni* are Feulgen +ve indicating the presence of DNA (Perkins, 1968, 1979). Occasionally H-LBs of *B. exitiosa* (Hine: unpub. obs.) and *H. littoralis* (Stentiford et al., 2013) are hexagonal in cross-section (Stentiford et al., 2004). These features resemble the ultrastructure of nucleocapsids of some DNA viruses (Hine, 1992). Furthermore, *B. exitiosa* may contain multiple cylindrical confronting cisternae which are associated with underlying viral infection in mammalian cells (Hine and Wesney, 1992). Against a viral interpretation is that haplosporosomes do not invade other cells, do not appear to hijack the nucleus to replicate and do not then exit the cell to infect other cells. Also, incorporation of nuclear material has not been reported from other haplosporidians and paramyxids.

### Paramyxida

#### Haplosporosome structure in relation to developmental stages

In the absence of a uniform nomenclature, the developmental stages of paramyxids will be designated C1–C6, C1 being the plasmodial stage, C2 being equivalent to a sporont, C3 being equivalent to the outer cell of the spore and C4–6 being

equivalent to the cells within the spore. Haplosporosomes occur in C1, C3, C4 and C5 (Table 3), but it is unclear whether the dense inclusions in C1 of *Paramyxa paradoxa* (see Desportes, 1981), in C1 and C3 of *Paramarteilia orchestiae* (Ginsburger-Vogel and Desportes, 1979a, 1979b) and in flattened elongated ‘vesicles’ of *Marteilia octospora* (Ruiz et al., 2016) are haplosporosomes. The C4 cells of *Marteilia cochillia* (Fig. 6D in Carrasco et al., 2013) and *Marteilia sydneyi* (Fig. 10 in Perkins and Wolf, 1976) have tubular haplosporosomes aligned at  $\sim 90^\circ$  to the plasma membrane. The C4 of *M. refringens* (Fig. 9 in Perkins, 1979) and *Marteilia granula* (Fig. 6E in Itoh et al., 2014) have vesicles in the same position, that resemble the vesicles in MVBs of *M. sydneyi* (Fig. 9, stage 2, in Perkins and Wolf, 1976). They are observed during haplosporogenesis and are therefore identified as haplosporosomes. In *Paramarteilia canceri*, C3 contains two haplosporosome populations. One population is comprised of cylindrical and bacilliform forms as in C1. The second is comprised of large ( $>3.5\ \mu\text{m}$  long) forms with one end bulbous to give a tadpole-like body. They have complex membranes giving the appearance of two eyes and an apical sheath behind the ‘head’ running nearly half-way towards the tail (Fig. 4A and B) (Fig. 23 in Feist et al., 2009). However, in the congeneric *P. orchestiae* (Ginsburger-Vogel and Desportes, 1979a), the dense bodies in C3, while being similar in shape and size to those of *P. canceri*, appear to lack internal membranes. They have a heterogeneous glycogen-like content, migrate to the surface of the endospore and eject their filamentous content into the space between endospore and epispore.

#### Haplosporogenesis

In C1, haplosporosomes in some species are formed in MVBs (Table 2) as bilaminar vesicles that probably obtain their outer membrane by budding from the vesicles, as in some haplosporidians (Perkins, 1968). Golgi has not been reported from C1. The haplosporosomes of C3 of *M. sydneyi* also arise from vesicles (Perkins and Wolf, 1976), but their genesis in other species is unknown, their appearance seeming to be rapid. In the C4 of *P. paradoxa*, haplosporosomes derive from Golgi (Figs 9A and 9B in Desportes, 1981), and in the C4 and C5 of *Paramyxoides nephthys*, haplosporosome like bodies arise from vacuoles (Fig. 4C and D) (Fig. 19 in Larsson and Koie, 2005). In *P. orchestiae* it is claimed that the large dense inclusions of C3 arise from Golgi (Figs 15 and 16 in Ginsburger-Vogel and Desportes, 1979a), but an alternative interpretation is that they derive from circular profiles of sER, as would be expected if they contain mucopolysaccharides.

#### Putative function

There are no clues to the function of C1 haplosporosomes, and although they underlie the fibrous ectoplasm in the periphery of C1 in *P. orchestiae* (Ginsburger-Vogel and Desportes, 1979a, 1979b), they do not orientate toward the exterior as in the haplosporidians NZAP (Hine et al., 2002) and *H. nelsoni* (Scro and Ford, 1990). However, in the intermediate spore cell (C4) of *Marteilia christensenii* (Figs 5 and 6 in Comps, 1983 [1985]), *M. sydneyi* (Figs 9–11 in Perkins and Wolf, 1976), and *M. cochillia* (Fig. 6A in Carrasco et al., 2013), the thin vermiform haplosporosomes are orientated at right angles to the C4 plasma membrane. In *M. sydneyi* the distal end appears to contain osmiophilic matter, but it is unlikely to be involved in spore wall formation as this occurs around the previous C3 cell (Fig. 11 in Perkins and Wolf, 1976). In *P. orchestiae* the heterogeneous content of dense bodies in C3, probably haplosporosome homologs, contain glycogen and are thought to be involved in spore wall formation (Ginsburger-Vogel and Desportes, 1979a).

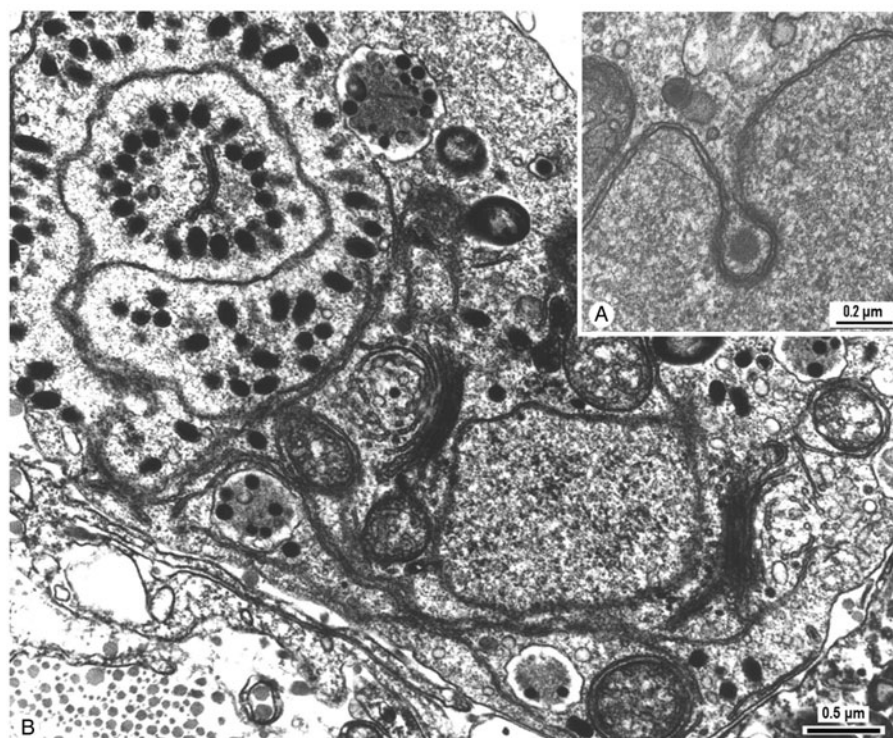
**Table 2.** The process of haplosporosome and sporoplasmosome/electron dense body (EDB) formation in different taxa. Numbers in the table refer to the references given below

Haplosporogenesis/EDBs	Haplosporidians		Paramyxians			Myxozoans
	Plasmodia	Spores	C1	C3	C4	Plasmodia
Golgi → EDB						41–46
Haplosporosomes from FBs by budding	1					
INS → NM-BG → H-LBs → haplosporosomes	4–7	4, 9				
Golgi → FBs	8 <sup>1</sup>	10 <sup>2</sup> , 11, 12				
Diffuse Golgi in spores		13				
Golgi/spherule → haplosporosomes		3, 4, 12, 14–16				
Golgi/spherule → MVBs/FBs → haplosporosomes		1, 2, 17–22		28, 29	28, 29, 39, 40	
Golgi/spherule → MVBs/FBs → haplosporosomes by budding	23	7, 24–27	29, 30 <sup>3</sup> , 31–34, 35 <sup>3</sup> , 36	29 <sup>4</sup> , 31–33, 35, 37, 38	31, 35 <sup>5</sup> , 37 <sup>5</sup> , 38 <sup>5</sup> , 40 <sup>5</sup>	

EDBs, electron dense bodies; FBs, formative bodies; H-LBs, haplosporosome-like bodies; INS, indentations in the nuclear surface; MVBs, multivesicular bodies; NM-BG, nuclear membrane-bound bodies.

Superscript numbers indicate variations in structure. <sup>1</sup>Some DVs with membrane, many without. <sup>2</sup>No internal membrane. <sup>3</sup>Also in C2, but of unknown origin. <sup>4</sup>In outer sporoplasm. <sup>5</sup>Elongated vesicle-like structures.

1. Perkins, 1969; 2. Carnegie *et al.*, 2006; 3. Comps and Pichot, 1991; 4. Hine *et al.*, 2007; 5. Hine and Thorne, 2002; 6. Hine *et al.*, 2002; 7. Hine and Wesney, 1992; 8. Stentiford *et al.*, 2004; 9. Hine *et al.*, 2009; 10. La Haye *et al.*, 1984; 11. Perkins, 1968; 12. Ormières *et al.*, 1973; 13. Anderson *et al.*, 1993; 14. Azevedo, 1984; 15. Azevedo and Corral, 1989; 16. Ituarte *et al.*, 2014; 17. Azevedo *et al.*, 2006; 18. Azevedo *et al.*, 2008a; 19. Bearham *et al.*, 2008a; 20. Bearham *et al.*, 2008b; 21. Comps and Tigé, 1997; 22. Hine and Thorne, 1998; 23. Renault *et al.*, 2000; 24. Ball, 1980; 25. Hillman *et al.*, 1990; 26. Marchand and Sprague 1979; 27. Perkins, 1975; 28. Desportes, 1981; 29. Perkins and Wolf, 1976; 30. Carrasco *et al.*, 2013; 31. Comps, 1983, [1985]; 32. Feist *et al.*, 2009; 33. Ginsburger-Vogel and Desportes, 1979a; 34. Ginsburger-Vogel and Desportes, 1979b; 35. Ruiz *et al.*, 2016; 36. Villalba *et al.*, 2014; 37. Itoh *et al.*, 2014; 38. Perkins, 1979; 39. Desportes and Lom, 1981; 40. Perkins, 1976; 41. Canning *et al.*, 2007; 42. Canning *et al.*, 2008; 43. Morris and Adams, 2006; 44. Morris and Adams, 2007; 45. Morris and Adams, 2008; 46. Morris, 2012.



**Fig. 3.** Haplosporosomes of a New Zealand abalone parasite. (A) Putative nuclear material in a nuclear membrane indentation in the NZAP. (B) Haplosporosomes arrayed around endoplasmic reticulum in the NZAP.

### Myxozoa

The Myxozoa is divided into two main clades; the Malacosporea and the Myxosporea. The Myxosporea is further sub-divided based on phylogeny, development, host and habitat into four further clades; *Sphaerospora*, marine species, *Kudoa* and freshwater species (Morris and Adams, 2008; Holzer *et al.*, 2018). All myxozoan spores are multicellular and usually contain a single sporoplasm which infects the subsequent host in the life cycle.

Exceptions are malacospores which contain two sporoplasms and *Sphaerospora* myxospores which can contain two or more sporoplasms. For freshwater clade actinospores, malacospores and *Kudoa* myxospores, the sporoplasms are multicellular composed of a sporoplasm primary cell within which one or more secondary cells reside. For marine clade actinospores a single sporoplasm is usually described with no secondary cells present, however, in one study secondary cells have been reported (Rangel *et al.*, 2012).

## Malacosporea

### *Sporoplasmosome structure in relation to developmental stages*

H-LBs (not structurally like haplosporidian H-LBs) of malacosporeans were called EDBs (Smith *et al.*, 1984; Morris *et al.*, 2000) but are now recognized as a type of sporoplasmosome (Canning *et al.*, 2000; Morris and Adams, 2006; Canning *et al.*, 2007; Morris and Adams, 2007, 2008). They occur in primary (1°) cells, pre-saccular cells and mural cells of *T. bryosalmonae* (Smith *et al.*, 1984; Morris *et al.*, 2000; Morris and Adams, 2006, 2008), *Buddenbrockia plumatellae* (Canning *et al.*, 2007; Morris and Adams, 2007) and *Buddenbrockia allmani* (Canning *et al.*, 2007), although they are relatively sparse in mural cells (Canning *et al.*, 2008).

To date, two morphologically distinct types of sporoplasmosomes have been described in malacosporeans. These can exist within the life-cycle of a single species and it is possible that this is common within the group. The only malacosporean for which the life cycle has been examined ultrastructurally in both hosts is *T. bryosalmonae* (Ferguson and Needham, 1978; Smith *et al.*, 1984; Canning *et al.*, 2000; Morris and Adams 2006, 2007, 2008). For this species, the sporoplasmosomes observed in the sporoplasm of the fish malacospore and the presaccular cells observed in the bryozoan host appear identical. However, they are distinct from the sporoplasmosomes observed in the 1° cell of the intra-piscine extrasporogonic stage of *T. bryosalmonae* and sporoplasm of the malacospore released from the bryozoan.

Structurally the malacosporean sporoplasmosomes are membrane-bound dense spherical bodies around 200 nm in diameter. Those that exist in the 1° cells of intra-piscine extrasporogonic stages and the malacospore sporoplasm contain a lucent bar extending into a lipid-rich core (Smith *et al.*, 1984; Morris *et al.*, 2000; Morris and Adams, 2007, 2008). The top of the lucent bar is capped by an additional membrane (Morris *et al.*, 2000). While observed in the 1° cell they may be endocytosed by secondary (2°) cells (Morris *et al.*, 2000). In the intra-bryozoan presaccular cells and the sporoplasm of the fish malacospore there is no bar, but an elliptical lucent region at one pole of the dense core (Canning *et al.*, 2007, 2008; Morris and Adams, 2006, 2008). Interestingly *T. bryosalmonae* also has a labyrinthine structure resembling the spherule in haplosporidian spores (Smith *et al.*, 1984), although this appears to have been a unique observation and may represent an unusual plane of section through the TGN (Morris *et al.*, 2000).

Intra-piscine presporogonic stages of other malacosporeans have yet to be described, however, parasites comparable to *T. bryosalmonae* have been noted in carp (Voronin, 1993; Voronin and Chernysheva, 1993; Bartošová-Sojčková *et al.*, 2014). These parasites may represent presporogonic stages of *B. plumatellae*, or related Malacosporea (Grabner and El-Matbouli, 2010a, 2010b).

### *Sporoplasmodgenesis*

Malacosporean sporoplasmosomes form at Golgi, on the transface (Smith *et al.*, 1984, Fig. 1D in Feist and Bucke, 1987; Morris *et al.*, 2000; Morris and Adams, 2006, 2007). This occurs in the presaccular proliferating stages within the invertebrate host and the proliferating extrasporogonic stage of *T. bryosalmonae* in the vertebrate host. The sporoplasmosomes of *T. bryosalmonae* also array along confronting cisternae (CC) of ER (Fig. 14 in Morris *et al.*, 2000) and are also found in cup-like bodies (Fig. 13 in Morris *et al.*, 2000) that lack a thick double membrane (unlike paramyxid MVBs). There is no evidence that either of these represents a site of formation and conversely, the latter is considered evidence of autophagocytosis (Morris *et al.*, 2000).

### *Putative function*

Sporoplasmosomes array under the plasma membrane (Smith *et al.*, 1984; Morris *et al.*, 2000; Morris and Adams, 2006, 2007; Canning *et al.*, 2008). Within the intra-piscine extrasporogonic stages of *T. bryosalmonae* they orientate the lucent bar at right angles to the plasma membrane (Ferguson and Needham, 1978; Morris *et al.*, 2000). For the sporoplasmosomes observed in the proliferative stages in bryozoans hosts the lucent area orients parallel to the plasma membrane (Morris and Adams, 2006, 2007).

The contents of the lucent inclusion may be secreted externally by *T. bryosalmonae* (Smith *et al.*, 1984) or beneath the plasma membrane by *B. plumatellae* (Morris and Adams, 2007). However, it remains unclear whether the sporoplasmosomes (Morris *et al.*, 2000) or their lucent inclusions fuse with the plasma membrane (Ferguson and Needham, 1978; Morris *et al.*, 2000; Morris and Adams 2006, 2007). The sporoplasmosomes of *T. bryosalmonae* may be exocytosed or released when the 1° cell of the extrasporogonic stage disintegrates, but there is no evidence that they are expelled (Smith *et al.*, 1984; Morris *et al.*, 1997, 2000; Morris and Adams, 2006).

There is a change in the distribution and orientation of sporoplasmosomes of intra-piscine *T. bryosalmonae* depending on site within the host. When the parasite is interdigitated with macrophages in the kidney interstitium they lie beneath and orientate to the plasma membrane. However, when the parasite is released into the kidney tubule lumen and not associated with macrophages the sporoplasmosomes have random cytoplasmic distribution. It has been suggested that sporoplasmosomes may be associated with the formation of the plasma membrane (Morris *et al.*, 2000), membrane recycling (Morris *et al.*, 2000), or aid in recognition and adherence/fusion of certain cell types (Morris and Adams, 2006).

## Myxosporea

### *Sporoplasmosome structure in relation to developmental stages*

While myxosporeans are considered to have indirect life cycles, these have only been completed for a relatively few species in two of the four main myxosporean clades. However, given available knowledge, it is considered that freshwater species usually cycle between fish and oligochaetes and marine species usually cycle between fish and polychaetes. The life cycles of any *Kudoa* and *Sphaerospora* species have yet to be elucidated (Holzer *et al.*, 2018). Sporoplasmosomes occur in actinospore developmental stages within invertebrate hosts of invertebrate hosts and in the myxospores of vertebrate hosts (Lom and Dyková, 1997). In actinospores, they are reported from binucleate pre-pansporocyst stages (Morris and Freeman, 2010; Morris, 2012), which contain Golgi (Lom *et al.*, 1989; Morris and Freeman, 2010) and the actinospore cytoplasm. One study observed the sporoplasmosomes orientating to the plasma membrane within the binucleate stages in a similar manner to the sporoplasmosomes observed in the Malacosporea (Morris and Freeman, 2010). The sporoplasmosomes of *Sphaeractinomyxon*, *Aurantactinomyxon*, and *Triactinomyxon* observed in the actinospore sporoplasm are diverse in structure (Lom and Dyková, 1997) and do not resemble typical sporoplasmosomes (Fig. 5D). The sporoplasmosomes in the actinospore sporoplasm appear to be a different structure from those observed in the pre-pansporocyst binucleate cells (Morris and Freeman, 2010; Morris, 2012).

Despite the description of >2596 myxosporean species by 2018 (Okamura *et al.*, 2018), the sporoplasmosomes of myxospores are seldom illustrated or described (but see Azevedo *et al.*, 1989; Casal *et al.*, 2003; Vita *et al.*, 2003; Casal *et al.*, 2006, 2007; Azevedo *et al.*, 2008b, 2011), those that have, show great diversity in

**Table 3.** The shapes of paramyxid haplosporosomes in relation to different developmental stages. C1–C5 indicate developmental stages (see text). Numbers in the table refer to the references given below

Species	Ref.	Plasmodia C1	Spore C3, C4, C5
<i>P. paradoxa</i>	1	Spherical, ovoid, irregular	Irregular
<i>P. nephthys</i>	2	Short, membrane-lined tubules	(C4, C5) spherical
<i>P. canceri</i>	3	Cylindrical or bacilliform *	(C3) complex polymorphic
<i>P. orchestiae</i>	4	Elongated *	(C3) complex polymorphic
<i>M. branchialis</i>	5	Spherical to vermiform *	(C4) elongated
<i>M. christenseni</i>	6	**	(C3) spherical, ovoid *, (C4) vermiform *
<i>M. chungmuensis</i>	7	Spherical, fusiform	Ovoid
<i>M. cochillia</i>	8	Spherical, ovoid *	(C3) spherical, (C4) vermiform *
<i>M. granula</i>	9	**	(C3) Rod-like, ovoid, (C4) elongated vesicles *
<i>M. maurini</i>	10	**	Oblong, spherical *
<i>M. octospora</i>	11	Oblate, vermiform	(C3) spherical, (C4) elongated vesicles
<i>M. refringens</i>	12, 13	Spheroid to oblate *	(C3) spherical, ovoid (C4) elongated vesicles
<i>M. sydneyi</i>	14, 15	Vermiform to spherical *	(C3) spherical, ovoid, (C4) vermiform *

\* typical haplosporosomes as defined by Perkins (1971).

\*\* not reported.

1. Desportes (1981); 2. Larsson and Koie (2005); 3. Feist *et al.* (2009); 4. Ginsburger-Vogel and Desportes (1979a, ); 5. Anderson and Lester (1992); 6. Comps (1983 [1985]); 7. Comps *et al.* (1986); 8. Carrasco *et al.* (2013); 9. Itoh *et al.* (2014); 10. Auffret and Poder (1983 [1985]); 11. Ruiz *et al.* (2016); 12. Longshaw *et al.* (2001); 13. Perkins (1979); 14. Kleeman *et al.* (2002); 15. Perkins and Wolf (1976).

form (Fig. 6A–H). All reports have been from the sporoplasm contained within the binucleate sporoplasm of the maturing spore. In one study (Lom *et al.*, 1989), the polymorphic sporoplasmosomes of 14 myxosporean spores are illustrated. *Kudoa lunata* sporoplasmosomes were described as occurring in a variety of forms (linear, groove-like and ring-like) that were interpreted as cross-sections through dense tubes that were cut at different angles (Lom and Dyková, 1988). No myxosporean sporoplasmosomes have an internal membrane as in the original descriptions of haplosporosomes (Perkins, 1971, 1979). However, nearly all the sporoplasmosomes have a complex substructure and some appear to have compartments delimited by membranes (Figs 6C and 6H) (Vita *et al.*, 2003; Azevedo *et al.*, 2011).

#### Sporoplasmodogenesis and function

The method of sporoplasmosome formation is unknown in myxosporeans. While attributed to Golgi in one study (Morrison *et al.*, 1996), no evidence is presented and it appears to be based on the origin of sporoplasmosomes in malacosporeans. The functions of sporoplasmosomes are also unknown. The structure of sporoplasmosomes in the binucleate stages observed in annelids is proposed to resemble that observed in the corresponding myxospore sporoplasm released from the fish host (Morris and Freeman, 2010). The alignment along the plasma membrane further suggests a functional relationship with sporoplasmosomes observed in malacosporeans.

#### Overview

Haplosporidians and paramyxids are linked by haplosporosome morphology, haplosporidians and malacosporeans by the array of haplosporosomes under the plasma membrane and alignment of their internal structures, and paramyxids and myxozoans by cell within cell development. In the 3 groups, haplosporosomes and sporoplasmosomes occur in feeding plasmodial stages, they disappear afterwards and reappear during sporogony. In all 3 groups (Haplosporidia, Paramyxa and Myxozoa) the plasmodia are trophic, but methods of feeding are different. Haplosporidian

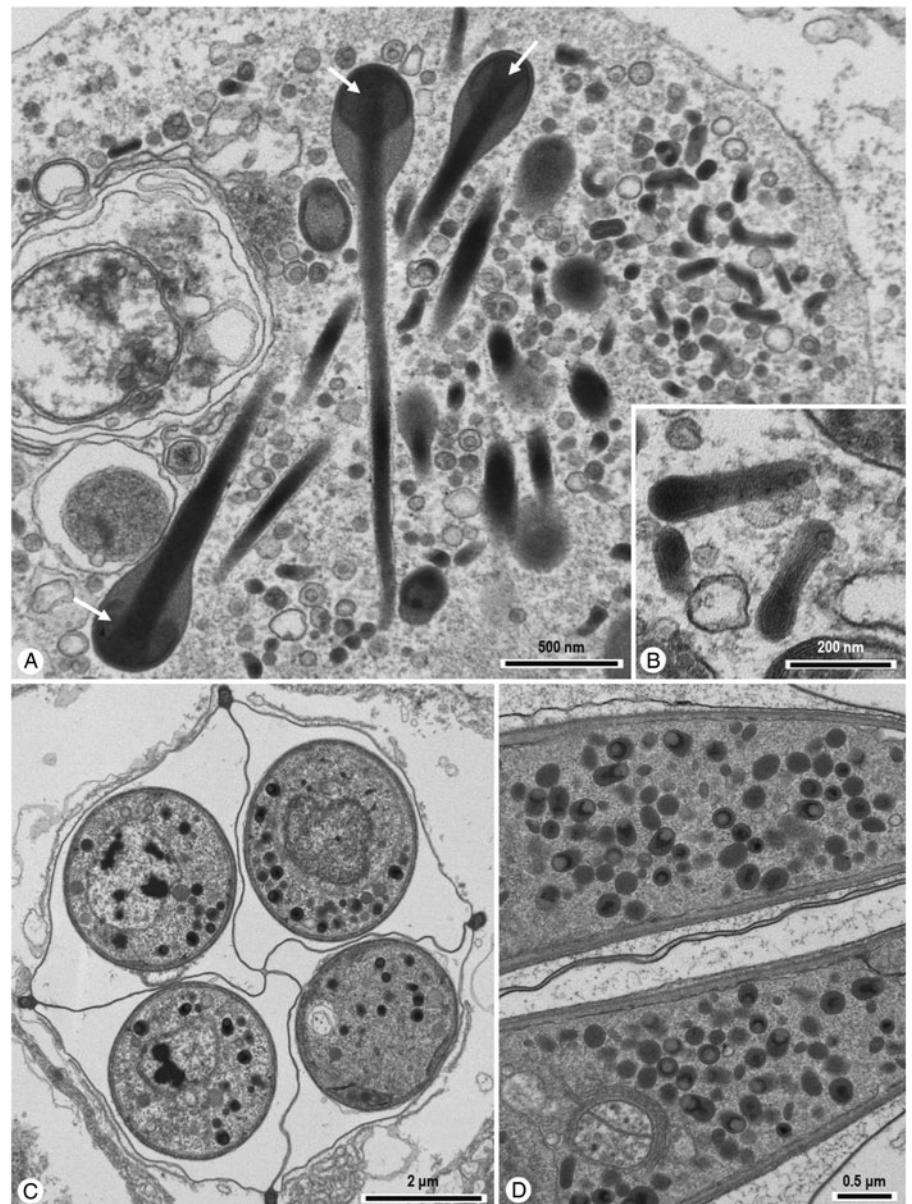
(except *Bonamia* spp.) and paramyxid plasmodia are extracellular, presumably feeding on nutrients gained from lysing surrounding host cells. Myxozoan plasmodia lie in close inter-digitating contact with host cells and feed by pinocytosis (Azevedo *et al.*, 2013).

#### Comparative morphology of haplosporosomes and sporoplasmosomes

Comparison of haplosporosomes and sporoplasmosomes is constrained by the vagueness of their definitions. Haplosporosomes of *U. crescens* were defined as ‘membrane-bound, osmiophilic structures which are oval, spherical, pyriform or short rods’ (Perkins, 1971). Similarities between haplosporosomes of haplosporidians and paramyxids were recognized by Perkins (1979). Subsequently, similar bodies in the sporoplasm of the myxozoan *Hoferellus gilsoni* were described as having ‘an electron dense core and a thin lucent envelope’ that ‘to a slight extent remind of haplosporosomes’, and were named sporoplasmosomes (Lom *et al.*, 1986). However, these bodies differ greatly in form and in different developmental stages of haplosporidians (Table 1), as do haplosporosomes in paramyxids (Table 3) and sporoplasmosomes in myxozoans (Lom *et al.*, 1989). The situation is complicated by the involvement of FBs, DVs and MVBs in haplosporogenesis in haplosporidians and paramyxids (Table 2). It is therefore difficult to identify which of the many forms in the 3 groups are haplosporosomes, which are sporoplasmosomes and which are unrelated.

Understanding patterns in the occurrence and morphology of haplosporosomes in haplosporidians is limited by taxonomic constraints and sampling. The NZAP, *Urosporidium* spp., *Bonamia* spp., *Minchinia* spp. and some *Haplosporidian* spp. (Perkins, 1968, 1969, 1971, 1975, 1979; Marchand and Sprague, 1979; Hine *et al.*, 2007, 2009) have haplosporosomes *sensu* Perkins (1971). However, studies have been mainly on infections in molluscs and crustaceans, and the genus *Haplosporidium* is a paraphyletic repository for orphan species (Hine *et al.*, 2009). It may therefore comprise several genera or even families that vary greatly in haplosporosome morphology. This is supported by the diverse forms of cytoplasmic bodies, but not haplosporosomes





**Fig. 4.** Details of haplosporosomes in paramyxids species. (A) Tertiary cell of *Paramarteilia canceri* showing the presence of numerous small haplosporosome stages ranging from bacilliform to curved and more rounded containing linked electron-dense bodies (arrow). Larger bulb-shaped haplosporosomes with internal membranous internal structure and striations are prominent. (B) Detail of bacilliform haplosporosome also present in the tertiary cell of *P. canceri*. (C) Section through spores of *Paramyxoides nephthys* showing the presence of numerous spherical haplosporosomes. (D) Detail of *P. nephthys* haplosporosomes showing pleomorphism with internal electron-dense structure at one end and elongation of the haplosporosomes.

*sensu* Perkins (1971), observed in *H. parisi* from a polychaete (Ormières, 1980), *H. ascidiarum* infecting an ascidian (Ciancio *et al.*, 1999) and *H. comatulae* reported from a crinoid (La Haye *et al.*, 1984).

The haplosporosomes of paramyxids are polymorphic, may show the classic structure of haplosporidian haplosporosomes (Perkins and Wolf, 1976; Desportes and Lom, 1981; Comps *et al.*, 1986; Anderson and Lester, 1992; Longshaw *et al.*, 2001; Larsson and Koie, 2005; Feist *et al.*, 2009; Carrasco *et al.*, 2013) and in some cases (C1 in Desportes and Lom, 1981, C3 in Feist *et al.*, 2009, C3 in Perkins and Wolf, 1976) an internal membrane is not distinct. The haplosporosomes of the C3s of the two *Paramarteilia* spp. are different from those of other genera, but they also differ markedly. Those of *P. canceri* have a complex substructure (Feist *et al.*, 2009) with membranes dividing the haplosporosome into compartments (Fig. 4A).

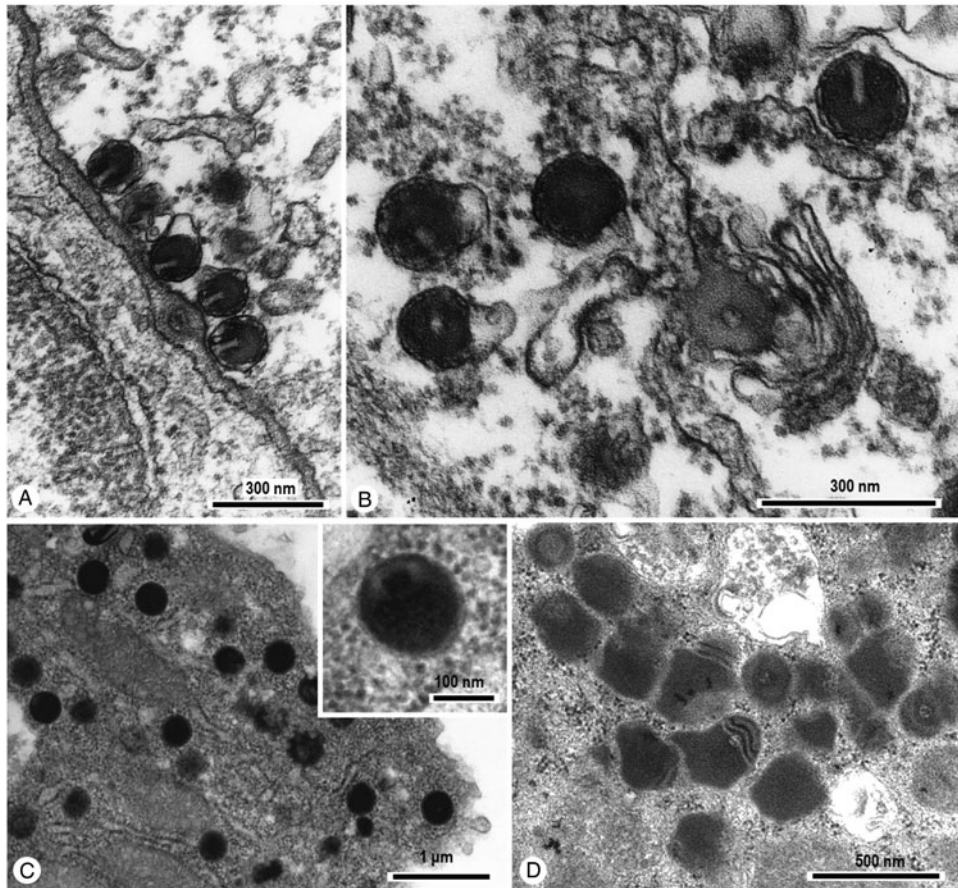
Sporoplasmosomes of myxozoans show great diversity in size and shape (Lom *et al.*, 1989). The internal membranes/inclusions of haplosporosomes/sporoplasmosomes are also very varied from apparently absent (Desportes and Nashed, 1983; Lom *et al.*, 1989; Bower and Meyer, 2002; Vita *et al.*, 2003; Stentiford *et al.*, 2004; Casal *et al.*, 2007) to complex (Auffret and Poder, 1983 [1985]; Lom *et al.*, 1989; Morris *et al.*, 2000; Azevedo *et al.*, 2008b;

Feist *et al.*, 2009; Azevedo *et al.*, 2011). Presumably, when present, they separate functional compartments and therefore have different content/functions.

Myxosporean sporoplasmosomes do not resemble haplosporosomes, except for the sporoplasmosomes (EDBs) of malacosporan 1° cells, presaccular and mural cells of *Tetracapsuloides bryosalmonae* (Feist and Bucke, 1987; Morris *et al.*, 2000). Like the haplosporosomes of *H. nelsoni* plasmodia they are round, electron-dense and arrayed along the plasma membrane with the internal membrane orientated toward the exterior. The internal membrane is like a squat vase in *H. nelsoni* (Scro and Ford, 1990) and like a T-bar in non-osmicated *T. bryosalmonae* (Morris *et al.*, 2000). However, the *T. bryosalmonae* bars have a separate and distinct membrane that caps the bar (Morris *et al.*, 2000). In the C3 and C4 of some paramyxids tubular haplosporosomes are orientated toward the plasma membrane (Perkins and Wolf, 1976; Comps, 1983 [1985]; Carrasco *et al.*, 2013), before spores occur in the C5 and C6 stages.

#### Function of haplosporosomes and sporoplasmosomes

Little is known of the functions of haplosporosomes and sporoplasmosomes. In haplosporidians they appear to act



**Fig. 5.** Ultrastructural details of sporoplasmosomes in malacosporans and actinosporans. (A) Sporoplasmosomes of *Tetracapsuloides bryosalmonae* with internal membranes containing electron-dense material and with an invagination aligned perpendicularly along the plasma membrane of the primary cell of the parasite. (B) Formation of *T. bryosalmonae* sporoplasmosomes via the Golgi in close association with the endoplasmic reticulum. (C) Sporoplasmosomes of presaccular *Buddenbrockia plumatellae* stages forming on Golgi apparatus. Inset: *B. plumatellae* sporoplasmosome within the sporoplasm of a malacospore. There they resemble those of *T. bryosalmonae*. In the presaccular stages they have a different organization. (D) Sporoplasmosomes of *Triactinomyxon A* showing complex structure.

extracellularly in the NZAP and *H. nelsoni*, but remain intracellular in derived groups (Hine, 2020). Their occurrence in plasmodia, disappearance during sporogony and reappearance in spores suggests they may be involved in spore wall formation in haplosporidians (Perkins, 1971, 1979) and paramyxids (Ginsburger-Vogel and Desportes, 1979a). However, there is no evidence for this in myxozoans.

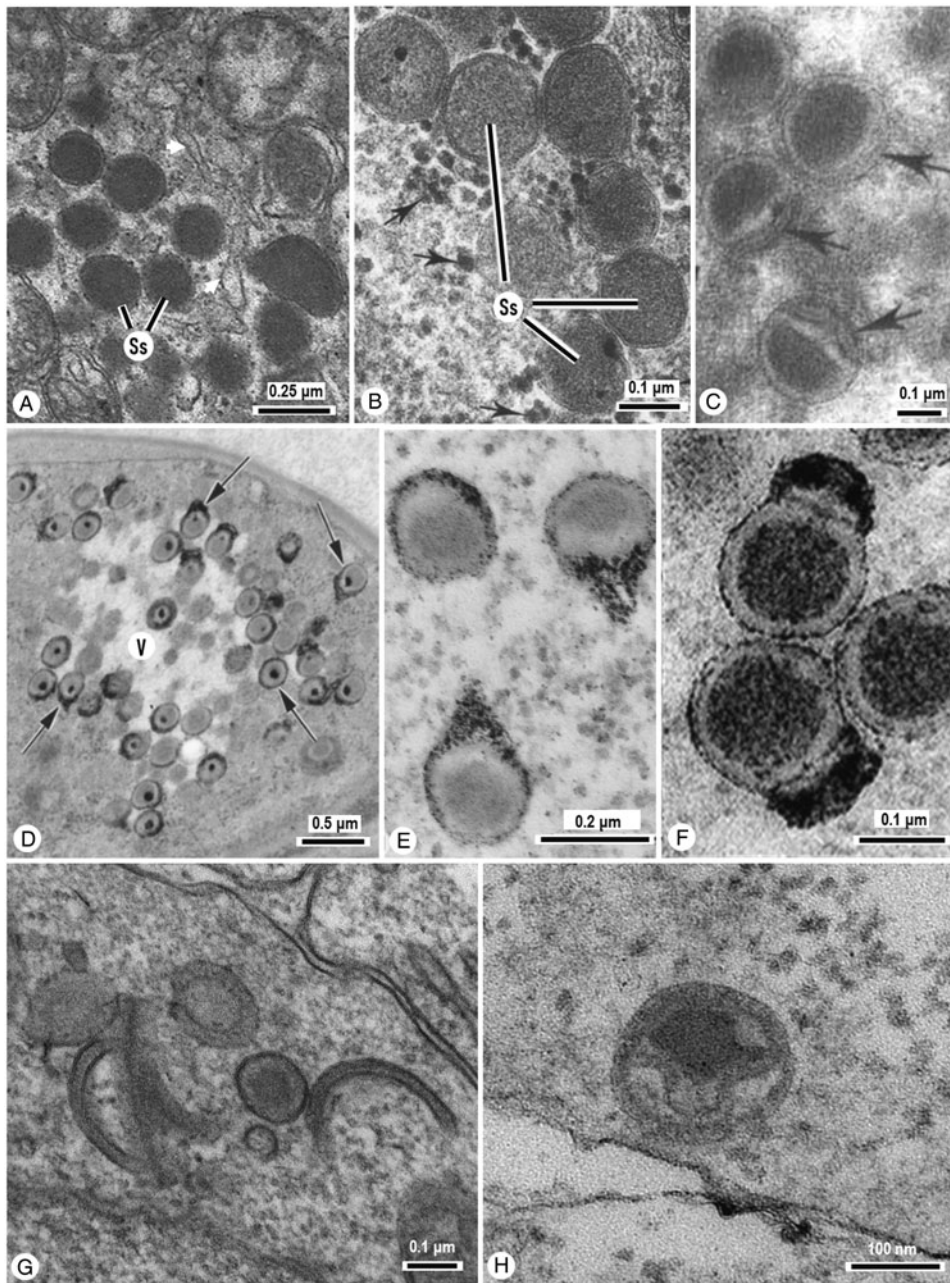
The haplosporosomes of the NZAP (Hine *et al.*, 2002) and *H. nelsoni* (Scro and Ford, 1990), and the sporoplasmosomes of *T. bryosalmonae* (Morris *et al.*, 2000) resemble each other in their positioning beneath the plasma membrane (Fig. 5A). However, they differ in details of their interaction with the plasma membrane. The outer membrane of the NZAP haplosporosomes appears to fuse with the plasma membrane, releasing the content between the 2 haplosporosome membranes and the intact cores. These intact cores are then endocytosed into lysosomes. The outer membrane of *H. nelsoni* haplosporosomes appears to fuse with the plasma membrane. Presumably, its contents are discharged, but there is no subsequent evidence of intact cores being endocytosed. The sporoplasmosomes of *T. bryosalmonae* may also fuse with the plasma membrane (Ferguson and Needham, 1978) and may (Smith *et al.*, 1984) or may not (Morris *et al.*, 2000) release their contents, or their lipid-rich content may interact with the plasma membrane (Morris *et al.*, 2000). Similarly, to the endocytosed cores of the NZAP, *T. bryosalmonae* may sometimes be engulfed by MVBs and enter the lysosomal pathway (Morris *et al.*, 2000). The discharge of haplosporosome contents from the NZAP and from plasmodia of *H. nelsoni* is

associated with the destruction of surrounding cells (Hine, 2020) which then nourish the parasites. The haplosporosomes *sensu* Perkins (1971) of haplosporidians and paramyxids are probably homologous.

### Concluding remarks

Haplosporosomes and sporoplasmosomes may have a common origin or similarities between them may be due to convergent evolution. Eukaryotic protists and cnidarians have deep evolutionary roots, both occurring in the Neoproterozoic (Van Iten *et al.*, 2014), with myxozoans occurring at the same time as basal bilaterians (Evans *et al.*, 2010), and the Malacosporea and the Myxosporea separating in the Cambrian (Kodádková *et al.*, 2015). If derived from a common origin the diversity of structure and evolution of a variety of functions may be expected, given the long evolutionary history of these organisms.

There is ultrastructural proof that haplosporosome exocytosis to destroying surrounding cells in the NZAP (Hine *et al.*, 2002) and of haplosporosomes containing nuclear material (Hine, 1992; Hine and Wesney, 1992; Hine *et al.*, 2002). However, there is no good evidence of the involvement of haplosporosomes or sporoplasmosomes in spore wall formation. Haplosporosomes containing nuclear material may indicate the presence of endogenous viral elements. Otherwise, the 3 groups form haplosporosomes/sporoplasmosomes for no apparent reason, despite their utilization of cellular resources. Our review highlights critical areas for further research. These include:



**Fig. 6.** Ultrastructural details of sporoplasmosomes in myxospores. (A) Sporoplasmosomes (Ss) and some cisternae of endoplasmic reticulum (white arrowheads) of a *Ceratomyxa tenuispora* myxospore. (B) Detail of some sporoplasmosomes (Ss) and glycogen particles (arrows) of a myxospore of *Henneguya amazonica*. (C) Sporoplasmosomes (arrows) of a myxospore of *Myxobolus braziliensis*. (D) Sporoplasmosomes (arrows) resembling the teardrop with an unusual electron density externally obtained from myxospore of *Henneguya friderici*. (E) Detailed aspect of some sporoplasmosomes shown in figure D. (F) Sporoplasmosomes, each with an eccentric, dense structure with a half crescent section located in a matrix formed by granular masses obtained from myxospore of *Myxobolus metyynnis*. (G) Sporoplasmosomes of *Sphaeromyxa balbiani* showing a variety of profiles from apparent tubes to circular structures indicating a cup-shaped structure of the mature sporoplasmosome. (H) Internal structure of *Myxobolus cotti* sporoplasmosome with an internal membrane containing electron-dense material.

- examining the relationship between *H. nelsoni* haplosporosomes and malacosporan sporoplasmosomes,
- determining the identity of the nuclear content in haplosporosomes of *B. exitiosa* and whether they may represent viral elements,
- the role of haplosporosomes in sporogony and spore wall formation,
- the structure and behaviour of sporoplasmosomes within proliferative, pre-sporogonic stages,
- determining any morphological similarities between sporoplasmosomes in myxospores, corresponding binucleate stages within annelid hosts and phylogenies,
- and the role of haplosporosomes and sporoplasmosomes in a wider range of species of each group.

Functional and genomic studies would clarify the role of these bodies in these groups, which include serious pathogens of fish and shellfish.

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