



REVIEW

Inter-relationships of haplosporidians deduced from ultrastructural studies

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ABSTRACT: We reviewed papers reporting haplosporidian ultrastructure to compare inter-relationships based on ultrastructure with those based on molecular data, to identify features that may be important in haplosporidian taxonomy, and to consider parasite taxonomy in relation to host taxonomy. There were links between the following: (1) the plasmodia of an abalone parasite, *Haplosporidium nelsoni* and *Urosporidium crescens* in the release of haplosporosomes; (2) *H. costale* and *H. armoricanum* in haplosporosome shape and presence and shape of Golgi in spores; (3) basal asporous crustacean haplosporidians which form haplosporosomes from formative bodies (FBs) in vegetative stages — *H. nelsoni*, which forms haplosporosomes from FBs in plasmodial cytoplasm, and *H. louisiana*, *Minchinia* spp. and *Bonamia perspora*, which form haplosporosomes from FBs in spores; (4) crustacean haplosporidians, *Bonamia* spp. and *M. occulta* in the predominance of uni- and binucleate stages; and (5) lipid-like vesicles in sporoplasms of *H. costale*, *H. armoricanum*, *H. lusitanicum*, *H. pickfordi*, *H. montforti*, and *B. perspora*. In general, these relationships reflect phylogenies based on molecular studies. As well as spore form and ornamentation, haplosporogenesis in spores appears to be taxonomically important. Parasite and host taxonomy were linked in the infection of lower invertebrates by *Urosporidium* spp., the infection of oysters by *Bonamia* spp., and of molluscs by *Minchinia* spp. *Haplosporidium* spp. are patently an artificial, paraphyletic group probably comprising many taxa. Consequently, the taxonomy of haplosporidians needs a thorough revision.

KEY WORDS: Haplosporidia · Ultrastructure · Phylogeny · *Urosporidium* · *Haplosporidium* · *Minchinia* · *Bonamia*

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INTRODUCTION

The Phylum Haplosporidia is in the Kingdom Protista, super-group Rhizaria; this super-group also includes the Phyla Cercozoa, Foraminifera and Radiolaria, and *Gromia* spp. (Adl et al. 2005). It comprises 8 named species of *Urosporidium*, 23 named species of *Haplosporidium*, 4 named species of *Bonamia*, and 5 named species of *Minchinia*, as well as an un-named

Urosporidium sp. (Carballal et al. 2005), 3 un-named species of *Haplosporidium*, 1 un-named species of *Minchinia*, and 11 undesignated species (Burrenson & Ford 2004, Bearham et al. 2008a,b).

Bonamia has also been reported from *Ostrea chilensis* in Chile (Campalans et al. 2000), *O. puelchana* in Argentina (Kroeck & Montes 2005), and *Crassostrea ariakensis* in the eastern USA (Burrenson et al. 2004). Studies indicate that a spot prawn parasite (SPP) in

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Pandalus spp. (Reece et al. 2000, Bower & Meyer 2002), and another protistan from crabs (*Carcinus maenas*) (Stentiford et al. 2004), are also haplosporidians. A molecular study (Reece & Stokes 2003) places the multi-nucleate stage of a haplosporidian of New Zealand abalone (*Haliotis iris*) parasite (NZAP) (Hine et al. 2002), as phylogenetically basal to *Urosporidium* spp., *Haplosporidium* spp., *Bonamia* spp., and *Minchinia* spp.

This study is based on papers published on the ultrastructure of haplosporidians, and some unpublished observations by P. M. Hine. It does not deal with the ultrastructure of spore ornamentation, as this has recently been thoroughly reviewed by Burreson & Reece (2006), and in subsequent studies (Bearham et al. 2008a,b). It was undertaken to compare inter-relationships based on ultrastructure with those based on molecular data, to identify features that may be important in haplosporidian taxonomy, and to consider parasite taxonomy in relation to host taxonomy. Initially, it was intended to carry out a cladistic analysis of ultrastructure, but the unevenness in the data available for each species—particularly from the studies solely of the spore stage, or asexual *Bonamia* spp.—did not permit such an analysis. This study highlights the need for revision of the taxonomy of haplosporidians.

METHODS

Initially, 53 ultrastructural features of haplosporidians and their effects on host cells were identified (Table 1). The features in each species were determined (Table 2) by examination of published electron micrographs. Only 2 of the 53 features have been recorded from *Haplosporidium pickfordi*, but 24 features have been reported from *H. armoricanum*, and many features from other ostreid *Haplosporidium* spp. and *Bonamia* spp. When the number of similarities between species was calculated, most similarities were reported for the parasites of ostreid oysters

Table 1. Ultrastructural features of haplosporidians identified

Feature no.	Feature
Uni- and bi-nucleate stages	
1	Few uni- and bi-nucleate stages
2	Parallel arrays of sER in uni-nucleate stages
3	Smooth endoplasmic reticulum (sER), small vesicles, cisternae or microtubules between nuclei in diplokarya
4 ^a	Nuclear membrane-bound Golgi (NM-BG)
5 ^a	Anastomosing endoplasmic reticulum (aER)
6 ^a	Vesicles budding from the outer nuclear membrane
7 ^a	Indentations of the nuclear surface near NM-BG (INS)
8 ^a	Possession of a cytoplasmic reticulated structure (RS).
Plasmodia	
9 ^b	Nuclear division by fission
10	Amoeboid plasmodia
11	Lipid droplets present
12	Sparse sER and/or aER
13	Cytoplasmic aggregates of dense matter
14	Release of haplosporosomes from plasmodia
15	Large parallel arrays of sER
16 ^b	Mitochondria grouped closely around nuclei
17	sER/mitochondrial complexes
18	Polycomplex-like structures in the nuclei.
Sporogony	
19	Membranes delimiting sporoblasts develop in association with Golgi
20	Damage to surrounding tissues at sporulation
21	Formation of a syncytium
22	Floccular, filamentous or granular matter in vacuoles in the episporoplasm
23	Lucent vesicles in the episporoplasm
24	Lipid droplets in the episporoplasm
25	Microtubules in the episporoplasm.
Spores	
26	Microfilaments on the spore operculum
27	Golgi cisternae throughout the sporoplasm
28	Golgi occurring as flat cisternae parallel to the sporoplasm plasma membrane
29	Formative bodies (FBs) from the spherulosome (herein spherule) develop into haplosporosomes
30	FBs from the spherule do not develop into haplosporosomes
31	Haplosporosomes develop directly from the spherule
32	Sporoplasm vacuolated
33	Microtubules beneath sporoplasm plasma membrane
34	Microfibrillar network perpendicular to the sporoplasm plasma membrane
35	Dense or lipid-like inclusions released from the sporoplasm
36	Haplosporosomes released from the sporoplasm
37	Exsporulation in the host
38	Striated FBs in the sporoplasm
39	Cores develop in spherule-derived FBs which gain an outer membrane by budding, to become haplosporosomes
40	Dense aggregates in the sporoplasm
41	Lipid-like or membrane-bound dense vesicles (L-LDV)
42	Fibrous/filamentous spore coat
43	Vertical striations in the spore wall
44	Extrasporal microtubules
45	Spherule formed when spore wall begins to develop
46	Sporoplasm plasma membrane tight against the spore wall
47	Bundles of microfibrils
48	Acorn-like capped spores
49	Spores with filamentous wrappings
50	Spores with tails that are lost with the episporoplasm
51	Spores with amorphous ribbons terminating in a 4-pronged cap
52	Internal membrane in the haplosporosome in the shape of an axe-head
53	NM-BG in sporoplasm
^a Also seen at the plasmodia stage	
^b Also seen at the sporogony stage	

Table 2. Features present in each haplosporidian species. NZAP: New Zealand abalone parasite

Species	Features
NZAP	4, 5, 6, 7, 10, 11, 13, 14, 15, 16, 17, 20
<i>Urosporidium cannoni</i>	11, 21, 23, 25, 28, 31, 42, 48
<i>U. crescens</i>	3, 4, 6, 10, 11, 12, 14, 20, 21, 25, 27, 31, 36, 37, 48
<i>U. spisuli</i>	4, 6, 27, 31, 47, 48
<i>U. jiroveci</i>	21, 23, 31, 48
<i>Haplosporidium louisiana</i>	3, 4, 5, 6, 11, 12, 16, 18, 21, 22, 32, 37, 38, 39, 43, 49
<i>H. nelsoni</i>	3, 4, 5, 6, 9, 10, 11, 13, 14, 20, 30, 37, 43, 44, 46, 49
<i>H. costale</i>	1, 3, 4, 5, 6, 7, 11, 12, 13, 16, 20, 29, 35, 40, 42, 49, 52
<i>H. armoricanum</i>	1, 2, 4, 5, 7, 9, 11, 12, 13, 16, 17, 19, 22, 23, 24, 29, 33, 35, 41, 46, 47, 49, 52, 53
<i>H. comatulae</i>	11, 16, 18, 21, 22, 23, 24, 30, 32, 49
<i>H. ascidiarum</i>	11, 16, 19, 22, 29, 32, 42
<i>H. lusitanicum</i>	11, 19, 20, 24, 26, 30, 35, 36, 37, 41, 45, 46, 49
<i>H. pickfordi</i>	35, 41, 49
<i>H. montforti</i>	21, 41, 49
<i>Bonamia perspora</i>	1, 2, 4, 5, 8, 10, 11, 15, 16, 19, 24, 29, 36, 39, 41, 45, 47, 51, 53
<i>B. exitiosa</i>	2, 4, 5, 6, 7, 8, 11, 12, 13, 15, 16
<i>B. ostreae</i>	4, 5
<i>Minchinia dentali</i>	10, 20, 25, 26, 29, 36, 37, 50
<i>M. tapetis</i>	20, 26, 33, 34, 37, 39, 50
<i>M. teredinis</i>	1, 25, 34, 38, 39, 50
<i>M. chitonis</i>	25, 37, 38, 39, 50
<i>M. occulta</i>	7, 10, 11, 12, 24, 25, 29, 37, 40, 42, 44, 46, 47

(Table 3), because there is a correlation between the number of features recorded and the number of similarities to other species (Fig. 1). There is also disparity in the stages reported. Only the late sporulation stages and spores of *Urosporidium* spp., *H. edule*, *H. parisi*, *Minchinia teredinis* and *M. chitonis* are reported, while the NZAP, and asporous *Bonamia* spp. are only known from vegetative stages.

Therefore, recognising the limitations of quantitative comparison of features, a more qualitative approach was taken to identify patterns within genera and groups. Other possible sources of bias were

- Different authors may interpret what they see in different ways, or they may concentrate on some features more than others. This is here called the 'observer effect'. The studies of Perkins (1968, 1969, 1971, 1975, 1979) contain much information, which may result in more linkages between the species he described than between species described by other workers (Tables 2 & 3). For example, only Perkins (1968, 1969, 1971, 1975) has reported smooth endoplasmic reticulum (sER), small vesicles, cisternae or microtubules between nuclei in diplokarya.

- The presence of a feature can be relied upon, but the absence of a feature cannot, as it might just have not

Table 3. Features in common between species. The numbers heading each column of the table relate to the number given for each species in the second column. Numbers in **bold** show the most congruent species. *Bonamia ostreae* has too few features for worthwhile comparison here. NZAP: New Zealand abalone parasite

Species	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NZAP	1																					
<i>Urosporidium cannoni</i>	2	1																				
<i>U. crescens</i>	3	7	5																			
<i>U. spisuli</i>	4	1	2	4																		
<i>U. jiroveci</i>	5	0	3	3	2																	
<i>Haplosporidium louisiana</i>	6	5	2	9	1	1																
<i>H. nelsoni</i>	7	8	1	9	1	0	8															
<i>H. costale</i>	8	8	2	7	1	0	8	9														
<i>H. armoricanum</i>	9	7	2	4	2	1	7	7														
<i>H. comatulae</i>	10	2	3	2	0	2	7	3	4													
<i>H. ascidiarum</i>	11	2	2	1	0	0	4	1	3	5	4											
<i>H. lusitanicum</i>	12	2	1	2	0	0	3	6	5	8	4	3										
<i>H. pickfordi</i>	13	0	0	0	0	0	1	1	1	3	1	0	3									
<i>H. montforti</i>	14	0	1	1	0	1	2	1	1	2	2	0	2	1								
<i>Bonamia perspora</i>	15	5	1	5	2	0	5	4	5	11	3	4	4	0	0	8						
<i>B. exitiosa</i>	16	8	1	5	1	0	6	5	8	8	2	2	1	0	0	2	0	0	2	0	0	0
<i>Minchinia teredinis</i>	17	0	1	1	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0
<i>M. dentali</i>	18	2	1	4	0	0	1	2	0	1	0	1	4	0	0	1	0	0	4	0	3	4
<i>M. tapetis</i>	19	1	0	2	0	0	2	2	1	1	0	0	3	0	0	1	0	0	3	0	3	0
<i>M. chitonis</i>	20	0	1	2	0	0	3	1	0	0	0	0	1	0	0	1	0	0	4	0	3	0
<i>M. occulta</i>	21	4	2	6	1	0	9	6	5	9	2	2	4	0	0	5	4	1	3	2	2	0

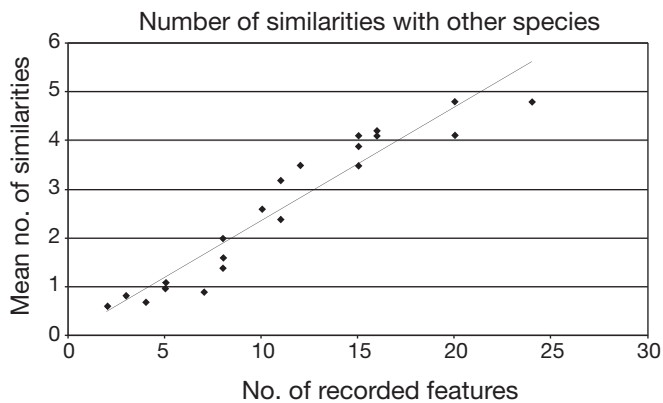


Fig. 1. Correlation of the mean number of recorded features and the mean number of similarities between species. The fewer the features reported, the less likelihood there will be of similarities, unless the few features reported are the same.

Except for spore ornamentation, this is seldom the case

been seen, or the material examined was unsuitable. For example, development of the spore walls at nodes has been reported for all *Haplosporidium* spp. and *Minchinia* spp., except *H. lusitanicum*, but spore wall development is almost certainly nodal in the latter species.

- Not all of the 53 features listed in Table 1 are likely to be of equal importance in identifying similarities and differences between species.

- The results vary depending on how much emphasis is put on a group of features, such as the many ultrastructural features in tails of *Urosporidium* spp.

- Assumption that a feature was permanently present, and not transient during a specific process (e.g. occurrence of microfilaments on the operculum during sporulation).

Not all described species are included in Tables 2 to 5 because descriptions of some species lack sufficient relevant information to be of use. *Haplosporidium cadomensis* Marchand & Sprague, 1979, is considered a synonym of *H. louisiana* Sprague, 1963, and the haplosporidian of *Crasostrea gigas* described by Comps & Pichot (1991), is regarded as *H. costale*-like. The multi-nucleate plasmodia of a haplosporidian of *Ostrea edulis* from St Philibert, Brittany, France (Bréhelin et al. 1982, Vivarès et al. 1982, Bonami et al. 1985) are regarded as those of *H. armoricanum*, which also occurs in *O. edulis* at that site (van Banning 1977, Hine et al. 2007). The *Haplosporidium* sp. reported from rock oysters *Saccostrea*

cucullata by Hine & Thorne (2002) was reclassified as *Minchinia* sp. on the basis of a molecular study (Bearham et al. 2007), and described as *M. occulta* Bearham et al. (2008b).

RESULTS AND DISCUSSION

Analysis of features and comparison with molecular phylogenies

Many features were only seen in 1 or 2 species, or were nearly ubiquitous. Little can be concluded from their occurrence and therefore they are not considered below.

Occurrence of aER, NM-BG, INS and cytoplasmic aggregates of dense matter

These 4 features may be constituents of the *trans*-Golgi network (Figs. 3 to 7 in Hine et al. 2002), and therefore the occurrence of one of these features may indicate the presence of the others. As well as in the NZAP, anastomosing endoplasmic reticulum (aER) occurs in 1 crustacean (*Haplosporidium louisiana*), and 6 haplosporidians infecting ostreid oysters (Table 4). Nuclear membrane-bound Golgi (NM-BG) occur in the NZAP, *Urosporidium spisuli*, *H. louisiana*, haplosporidians of ostreid oysters, and a crab haplosporidian

Table 4. Literature source electronmicrographs revealing the presence of 4 ultrastructural features— anastomosing endoplasmic reticulum (aER), nuclear membrane-bound Golgi (NM-BG), indentations of the nuclear surface (INS), and cytoplasmic aggregates of dense matter (cyto)—in haplosporidian species. NZAP: New Zealand abalone parasite

Haplosporidian species	Source figure(s) revealing feature				Source
	aER	NM-BG	INS	Cyto	
NZAP	3–7	3–7	2	12	Hine et al. (2002)
<i>Haplosporidium louisiana</i>	3				Perkins (1975)
<i>H. nelsoni</i>		17–19			Perkins (1979)
<i>H. costale</i>	1		9	6–9	Perkins (1969)
<i>H. armoricanum</i>			4	4	Cahour et al. (1980)
<i>H. armoricanum</i>		2			Bonami et al. (1985)
<i>H. armoricanum</i>		7,8	2,7	2	Hine et al. (2007)
<i>Bonamia ostreae</i>	2	2			Hervio et al. (1991)
<i>B. perspora</i>	30	29			Carnegie et al. (2006)
<i>B. exitiosa</i>	2,3	14		13	Hine & Wesney (1992)
<i>B. exitiosa</i>	6				Hine & Wesney (1994)
<i>B. exitiosa</i>	3				Hine et al. (2001)
<i>B. exitiosa</i>		3			Hine (1991)
<i>B. exitiosa</i>		3, 10, 12		4	Hine (1992)
<i>Urosporidium spisuli</i>		17			Perkins (1979)
<i>Minchinia occulta</i>			10	10	Hine & Thorne (2002)
<i>Carcinus maenas</i> parasite		18			Stentiford et al. (2004)

(Table 4). NM-BG occur in the spores of *Bonamia perspora* (Carnegie et al. 2006) and *H. armoricanum* (Hine et al. 2007). NM-BG were not reported from *Haplosporidium costale* by Perkins (1969), but they were not originally reported in *Haplosporidium nelsoni* either (Perkins 1968), only later (Figs. 17 to 19 in Perkins 1979), and may not have been observed in Perkins (1969). Similarly, NM-BG were also not reported from *Minchinia occulta* (Hine & Thorne 2002, Bearham et al. 2008b), but vegetative stages were rarely observed. Indentations in the nuclear surface (INS) have been reported in the NZAP and 3 ostreid haplosporidians (Table 4). INS have not been reported from *H. nelsoni* (Perkins 1968), *B. perspora* (Carnegie et al. 2006), or *B. ostreae* (Pichot et al. 1980). In the last case, this may be because INS are usually observed in plasmodia, and *B. ostreae* very rarely develops into plasmodia.

Cytoplasmic aggregates of dense matter, sometimes associated with the aER/NM-BG/INS complex, occur in the NZAP, *Haplosporidium costale*, *H. armoricanum*, *Minchinia occulta*, *M. chitonis*, and *Bonamia exitiosa* (Table 4). Perinuclear dense matter is rarely seen in *H. nelsoni* (P. M. Hine unpubl. obs.), but large cytoplasmic aggregates in which haplosporosomes develop—and from which they bud, acquiring the outer haplosporosome membrane—are a prominent feature of *H. nelsoni* plasmodia (Perkins 1968, 1979). They have not been reported from other *Haplosporidium* spp. Vesicles bud from the nuclear membrane into the aER/ NM-BG/INS complex in the NZAP (Fig. 7 in Hine et al. 2002), *Urosporidium spisuli* (Fig. 17 in Perkins 1979), *H. louisiana* (Perkins 1979), *H. nelsoni* (Fig. 18 in Perkins 1979), *H. costale* (Fig. 6 inset in Perkins 1969) and *B. exitiosa* (Fig. 16 in Hine & Wesney 1994).

Therefore, there appear to be many ultrastructural linkages between the NZAP, ostreid haplosporidians (*Haplosporidium nelsoni*, *H. costale*, *H. armoricanum*, *Bonamia* spp., *Minchinia occulta*), *H. louisiana*, and some *Urosporidium* spp., and *Minchinia* spp. This may reflect the number of studies carried out, and, as many of these studies have been by Perkins (1968, 1969, 1975, 1979) and Hine (Hine 1991, 1992, Hine & Thorne 2002, Hine & Wesney 1992, 1994, Hine et al. 2001, 2002, 2007, Stentiford et al. 2004, Carnegie et al. 2006), it may be due to the observer effect.

Haplosporogenesis

Patterns in haplosporogenesis may be taxonomically important. The direct development of haplosporosomes from the spherule links *Urosporidium* spp., and distinguishes the genus from other genera. Acquisition of an external membrane by cores budding from formative bodies (FBs) occurs in *Haplosporidium louisiana*, and *Bonamia perspora*, and is common in *Minchinia* spp. (Table 5). The FBs possess striated cores in *H. louisiana* (Perkins 1975, Marchand & Sprague 1979), *M. chitonis* (see Ball 1980) and *M. teredinis* (Hillman et al. 1990). Similarly, haplosporosomes develop from FBs in *M. occulta*, but they are not striated (Hine & Thorne 2002). A *Minchinia* sp. of *Mytilus galloprovincialis* also has electron dense vesicles, haplosporosomes, and large (300 to 500 nm) osmiophilic bodies in the spores, similar to those illustrated in *M. teredinis* (Fig. 3 in McGovern & Burreson 1990), but whether the haplosporosomes formed in, and budded from, the electron dense vesicles was not reported (Comps & Tigé 1997). Budding of cores from FBs to acquire an outer membrane and thus form haplosporosomes also occurs in *H. nelsoni*, but in the plasmodia,

Table 5. Haplosporogenesis. Literature sources (with their respective figures) revealing the occurrence of cores budding from formative bodies, and haplosporosome and lipid-like vesicle release in haplosporidian species. NZAP: New Zealand abalone parasite

Haplosporidian species	Fig. no.	Source
Cores budding from formative bodies		
<i>Haplosporidium louisiana</i>	28, 29	Perkins (1975)
<i>H. louisiana</i>	14, 15	Marchand & Sprague (1979)
<i>Bonamia perspora</i>	37	Carnegie et al. (2006)
<i>Minchinia chitonis</i>	1C, 1D	Ball (1980)
<i>M. tapetis</i>	13	Azevedo & Corral (1989)
<i>M. teredinis</i>	6, 7	Hillman et al. (1990)
Haplosporosomes released from plasmodia or sporonts		
NZAP	17	Hine et al. (2002)
<i>Urosporidium crescens</i>	5	Perkins (1971)
<i>H. nelsoni</i>	15	Perkins (1979)
<i>B. perspora</i>	39	Carnegie et al. (2006)
Release of lipid-like or membrane-bound dense vesicles		
<i>H. armoricanum</i>	9	Hine et al. (2007)
<i>H. costale</i>	9	Rosenfield et al. (1969)
<i>H. costale</i> -like	2b	Comps & Pichot (1991)
<i>H. lusitanicum</i>	3	Azevedo & Corral (1985)
<i>H. lusitanicum</i>	2	Azevedo et al. (1985)
<i>H. montforti</i>	11	Azevedo et al. (2006)
<i>H. pickfordi</i>	12	Burreson (2001)
<i>B. perspora</i>	39	Carnegie et al. (2006)
Axe-head shaped haplosporosomes		
<i>H. costale</i>	21	Perkins (1969)
<i>H. costale</i>	6	Rosenfield et al. (1969)
<i>H. costale</i> -like	2a	Comps & Pichot (1991)
<i>H. armoricanum</i>	6	Cahour et al. (1980)
<i>H. armoricanum</i>	11	Hine et al. (2007)

not the spores (Perkins 1968, 1979). The spore haplosporosomes of *H. costale*, a *H. costale*-like parasite from *Crassostrea gigas* (see Comps & Pichot 1991), and *H. armoricanum*, are axe-head shaped and ultrastructurally difficult to tell apart (Table 5).

Haplosporosomes are released from plasmodia in the NZAP, *Haplosporidium nelsoni*, and *Bonamia perspora*, and from sporonts and the sporoplasm in *Urosporidium crescens*. Lipid-like or membrane-bound dense vesicles (L-LDV), which may be released, occur at the periphery of the sporoplasm of gastropod haplosporidian species (*H. lusitanicum*, *H. pickfordi*, *H. montforti*); however, similar L-LDVs occur in, and may be released from, *H. costale*, an *H. costale*-like parasite, *H. armoricanum*, and *B. perspora* (Table 5). The genesis and function of L-LDVs, and their relationship to haplosporosomes, is unclear. Cytochemical studies on *H. lusitanicum* spores have shown that L-LDVs in the peripheral endosporoplasm, and dense bodies (DBs) in the episporoplasm, stain strongly for glycoproteins and for lipids (Azevedo & Corral 1985, 1989). Haplosporosomes in the same spores were positive for lipids in their internal membrane, and positive for glycoproteins in the external membrane and matrix. However, although L-LDVs in the endosporoplasm and DBs in the episporoplasm resembled each other cytochemically, haplosporosomes only occurred

in the endosporoplasm, making it unlikely that, either L-LDVs and DBs are the same, or that haplosporosomes develop from L-LDVs. The spherical bodies around the sporoplasm of *Minchinia dentali* (Fig. 44 in Desportes & Nashed 1983) appear to be different, having a composition similar to that of the cytoplasm.

Relationships based on ultrastructure

Taking these shared ultrastructural features and the numbers of similarities between species in Table 2 into account, a tree diagram was constructed to illustrate the most likely inter-relationships (Fig. 2). Species other than the 3 *Haplosporidium* spp. from gastropods, and *Minchinia* spp. have aER/NM-BG/INS complexes, except *M. occulta* which also has INS. *M. occulta* occurs predominantly as uni-nucleate and bi-nucleate *Bonamia*-like forms, leading Bearham et al. (2008b) to conclude that the single uni-nucleate stage may become more dominant in the life cycles of Haplosporidia in species more closely related to *Bonamia*. The occurrence of INS, predominance of uni-nucleate stages and occurrence in an ostreid host suggests that *M. occulta* is more closely related to *Bonamia* spp., than are other *Minchinia* spp. The NZAP (Fig. 12 in Hine et al. 2002), *B. perspora* (Fig. 30 in Carnegie et al. 2006) and *B. exitiosa* (Fig. 6 in Hine & Wesney 1994) possess a cytoplasmic reticulated structure, which may also be part of the aER/NM-BG/INS complex.

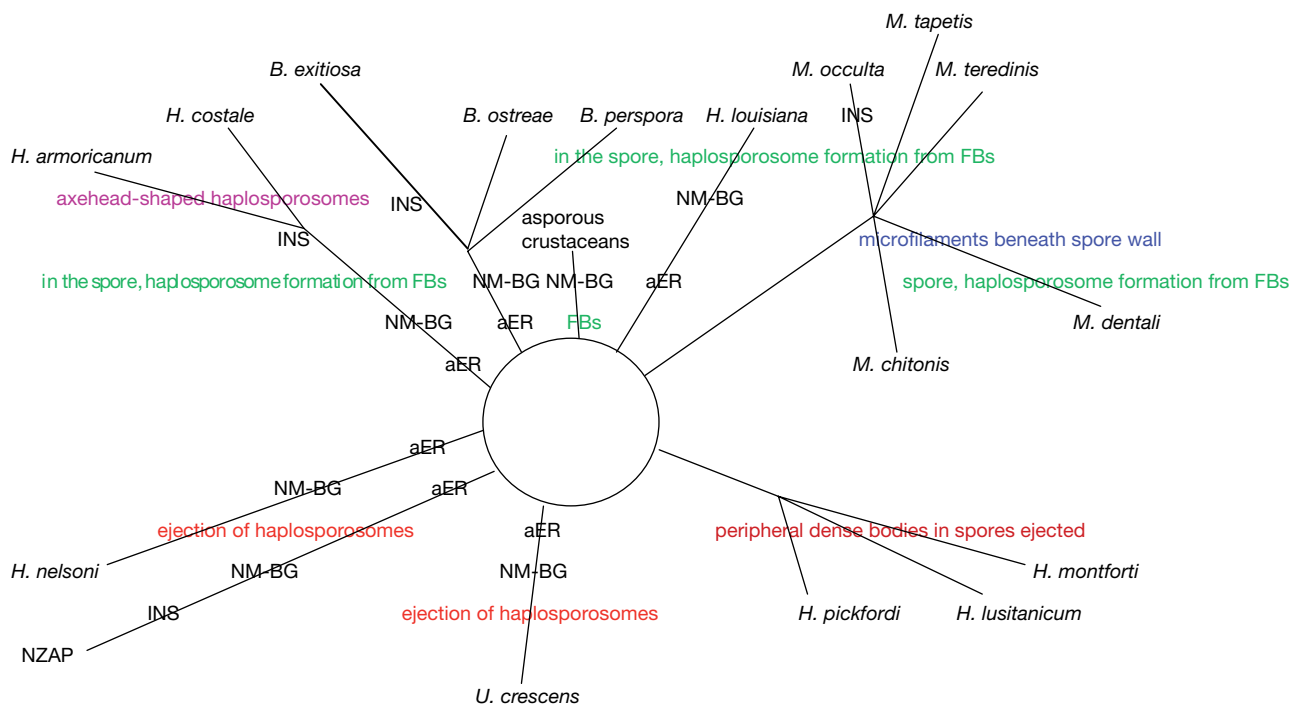


Fig. 2. The apparent haplosporidian inter-relationships, based on ultrastructural data. The features occurring in each species are given by the abbreviations crossing the lines leading to each species. Line length does not indicate relatedness. NM-BG: nuclear membrane-bound Golgi; INS: indentations in the nuclear surface; aER: anastomosing endoplasmic reticulum; FBS: formative bodies. See Table 2 for full species names

Bonamia perspora (Carnegie et al. 2006), *Haplosporidium louisiana* (Perkins 1975, Marchand & Sprague 1979), *Minchinia* spp., *H. armoricanum* and *H. costale*, undergo haplosporogenesis in the spore in 2 stages: production of vesicles by the spherule and their subsequent development into haplosporosomes. In particular, *B. perspora*, *H. louisiana*, *M. tapetis* and *M. teredinis*, form haplosporosomes by cores budding from the vesicles to acquire an outer haplosporosome membrane. *H. armoricanum* (Fig. 9 in Hine et al. 2007) and *B. perspora* (Carnegie et al. 2006) have NM-BG in the endosporoplasm. *H. armoricanum* (see Bachère & Grizel 1983), *B. perspora* (Carnegie et al. 2006), and *B. exitiosa* (Fig. 4 in Hine & Wesney 1994) have parallel arrays of sER in uni-nucleate stages.

Asporous haplosporidians from some crustaceans, including crabs (Newman et al. 1976, Stentiford et al. 2004), and prawns (Dyková et al. 1988), resemble *Bonamia ostreae* (see Brehélin et al. 1982), *B. exitiosa* (see Hine 1991), and possibly *B. roughleyi* (Cochennec-Laureau et al. 2003), in the development of uni-nucleate stages into multi-nucleate plasmodia, which may then divide to form more uni-nucleate stages. This may be due to reduction in the life cycles from spore-forming ancestors (Stentiford et al. 2004), such as *Haplosporidium louisiana* and *B. perspora*. A haplosporidian (Reece et al. 2000) from spot prawns *Pandalus platyceros*, with many stages that are not observed in other haplosporidians (Bower & Meyer 2002), has cytoplasmic bodies in the uni-nucleate stage that may be FBs preceding haplosporosomes.

The placing of species in Fig. 2 does not show several linkages because it is a 2-dimensional representation of a 3-dimensional structure. L-LDVs tight against the endosporoplasm membrane, or between the endosporoplasm and spore wall, not only occur in gastropod *Haplosporidium* spp. (*H. lusitanicum*, *H. pickfordi* and *H. montforti*), but also in *H. costale* (Rosenfield et al. 1969), *H. armoricanum* (Hine et al. 2007) and *Bonamia perspora* (Carnegie et al. 2006). *H. nelsoni* (Fig. 18 in Perkins 1968), *Minchinia occulta* (Fig. 17 in Hine & Thorne 2002), and *H. ascidiarum* (see Ormières & de Puytorac 1968) have extrasporal microtubules. *H. nelsoni* (Fig. 10 in Perkins 1968) and *H. armoricanum* (Fig. 3 in Hine et al. 2007) may undergo nuclear division by fission. *H. nelsoni* (Fig. 3 in Rosenfield et al. 1969) shares vertical striations in the spore wall with *H. louisiana* (Fig. 17 in Marchand & Sprague 1979; Fig. 8 in Perkins & van Banning 1981). *H. lusitanicum* (Fig. 5 in Azevedo 1984) is linked to *M. dentali* (Fig. 40 in Desportes & Nashed 1983) and *M. tapetis* (Fig. 5 in Azevedo & Corral 1989), by possession of opercular microfilaments. These may be transient and associated with exsporulation (Azevedo & Corral 1989), but they have not been observed on the operculae of *Haplo-*

sporidium spp. or *M. occulta* (Hine & Thorne 2002, Bearham et al. 2008b).

Comparison with molecular phylogenies

Fig. 2 resembles molecular phylogeny, in terms of the relationships of the NZAP to ostreid haplosporidians and to *Urosporidium* spp. (Reece et al. 2004, Azevedo et al. 2006, Carnegie et al. 2006). However, in Fig. 2, *Haplosporidium louisiana* is not basal to *U. crescens*, and ostreid haplosporidians, *Bonamia* spp. and *Minchinia* spp. (Carnegie & Cochennec-Laureau 2004, Reece et al. 2004, Azevedo et al. 2006, Carnegie et al. 2006), but it is placed between *Bonamia* spp. and *Minchinia* spp. The relationship with the latter and *H. costale* has recently been supported in phylogenies based on actin gene sequences (López-Flores et al. 2007), and on a prawn haplosporidian (Nunan et al. 2007). Future studies may confirm the placing of crustacean haplosporidians close to or between *Bonamia* spp. and *Minchinia* spp. The possible close relationship between the gastropod *Haplosporidium* spp. and *H. costale*, in possession of L-LDVs tight against the endosporoplasm plasma membrane, reflects the phylogenies of Reece et al. (2004), Azevedo et al. (2006), and Carnegie et al. (2006). Peripheral dense bodies and the axehead shape of haplosporosomes in spores suggests that *H. costale* and *H. armoricanum* are closely related, as in the phylogeny of Carnegie & Cochennec-Laureau (2004).

Haplosporidian molecular phylogenies and spore ornamentation (Burreson & Reece 2006) indicate that while *Urosporidium* spp., *Minchinia* spp., and *Bonamia* spp. are monophyletic, *Haplosporidium* spp. are paraphyletic (Burreson & Ford 2004, Burreson & Reece 2006). This is supported by ultrastructure. *Haplosporidium* species are each distinguished from others in the genus as follows: *H. nelsoni* by haplosporosome-formative areas in the plasmodial cytoplasm (Perkins 1968); *H. montforti* by filaments coming off the posterior of the spore, and from underneath the operculum; *H. hinei* by filaments arising from 2 posterior knobs that subsequently pass under the operculum (Bearham et al. 2008a); *H. edule* by spore ornamentation (Azevedo et al. 2003); *H. comatulae* by spore haplosporogenesis resembling *M. chitonis* (La Haye et al. 1984); *H. ascidiarum* by electron opaque inclusion bodies in the spores (Ciancio et al. 1999); and *H. parisi* by absence of haplosporosomes and possession of 'organites' in the spore (Ormières 1980). As the type *Haplosporidium* sp. (*H. scolopli* Caullery and Mesnil, 1899) was described from a polychaete, and *H. parisi* is the only species described ultrastructurally from a polychaete, *H. parisi* may resemble *H. scolopli*. If so, none

of the other ultrastructurally described *Haplosporidium* spp. belong in that genus, and—except for *H. costale*, *H. armoricanum* and the gastropod *Haplosporidium* spp.—none are congeneric. The spore ornamentation of *H. hinei* shows some similarities to *H. parisi* (see Bearham et al. 2008a). However, their sporoplasm ultrastructure is very different (Hine & Thorne 1998), suggesting that spore ornamentation alone is not a reliable taxonomic indicator. Similarly, among *Urosporidium* spp., the type species (*U. fuliginosum* Caullery and Mesnil, 1905) is the only species isolated from a polychaete, the other species deriving from helminths and a turbellarian (Burrenson & Ford 2004). Although *Minchinia* spp. are considered to be monophyletic, if spore ornamentation is a taxonomic indicator, the presence of microtubules over the surface (which might give rise to a ridge on the spore) and lack of episporic cytoplasmic extensions distinguishes *M. occulta* from other *Minchinia* spp., showing that there may be considerable variation within monophyletic groups.

Phylogenetic patterns in relation to the taxonomy of host species

Urosporidium spp. infect lower invertebrates, notably trematodes, but also a nematode, turbellarians and a polychaete (Burrenson & Ford 2004). *U. cannoni*, which infects a turbellarian, differs from other *Urosporidium* spp. in several respects: it has 11 to 13 tails, rather than the usual 1 to 3 of other species, an episporic fibrillar system of a network of complex microtubule-like fibrils, vermiform projections from the cyst wall, parallel Golgi cisternae, and spherical mitochondria (Anderson et al. 1993). Although *U. cannoni* almost certainly belongs in another genus, another *Urosporidium* sp. parasitizing a turbellarian resembles *U. spisuli* (see Carballal et al. 2005). *Haplosporidium costale*, *H. armoricanum* and *Bonamia* spp. are parasites of ostreid oysters. *H. lusitanicum*, *H. pickfordi* and *H. montforti* are all parasites of gastropods. In Fig. 2, the positions of haplosporidians from abalone, the NZAP and *H. montforti*, are distant from each other. However, the NZAP infects *Haliothis iris*, which is phylogenetically distant from all other *Haliothis* spp. (Coleman & Vacquier 2002), and probably diverged from them at the break-up of Gondwanaland. *Minchinia* spp. infect different classes of molluscs: *M. dentali* in a scaphopod, *M. chitonis* in a polyplacophoran, and *M. teredinis*, *M. occulta* (Bearham et al. 2008b) and *Minchinia* sp. (Comps & Tigé 1997) in different families of bivalves. *H. louisiana* is a parasite of decapods that, unlike other known haplosporidians of crustaceans (Newman et al. 1976, Dyková et al. 1988,

Bower & Meyer 2002, Stentiford et al. 2004, Nunan et al. 2007), forms spores. Observations on the other crustacean haplosporidians suggest that, for at least part of their life-cycles, they form large multi-nucleate plasmodia that internally divide to form uni-nucleate bodies, which separate and undergo karyokinesis to form multi-nucleate plasmodia, and the cycle is repeated.

Within the Phylum Haplosporidia, *Urosporidium* spp. are in the Family Urosporidiidae, and *Haplosporidium* spp. and *Minchinia* spp. are in the Family Haplosporidiidae (Perkins 2000). *Bonamia* spp. have not been clearly placed within this family, but the operculate spores of *B. perspora* (Carnegie et al. 2006) suggest that it is closer to *Haplosporidium* spp. and *Minchinia* spp. than to *Urosporidium* spp. Thus, the phylum comprises 2 families with 4 genera, yet both genotypic and phenotypic studies suggest considerable diversity that is not recognised in haplosporidian taxonomy, particularly within *Haplosporidium* spp. The conservative placing of diverse haplosporidians into just 2 families may reflect the initial lumping of the ultrastructurally very different paramyxians into the same Phylum Ascestospora by Sprague (1979) (Burrenson & Ford 2004). The Haplosporidia and Paramyxia were subsequently separated (Desportes & Perkins 1990), and the Phylum Haplosporidia is now recognised (Adl et al. 2005). There is an urgent need for a major taxonomic revision of the Haplosporidia based on molecular and ultrastructural studies, to clarify taxonomic relationships, in particular to provide a structure to accommodate the many diverse *Haplosporidium* spp.

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