Vol. 85: 199–208, 2009
doi: 10.3354/dao02093

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 23

Ultrastructure of *Bonamia* sp. in *Ostrea chilensis* in Chile

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ABSTRACT: Oyster *Ostrea chilensis* samples were collected from Quihua Island, Chile, in December 2003 and February 2005, and examined in May 2004, and March, April and July 2005, for an ultrastructural comparison of the Chilean *Bonamia* sp. with other *Bonamia* spp. Only uni-nucleate stages were encountered, except in the July sample. The Chilean parasite differs from *B. perspora* in the apparent lack of a plasmodial stage and of sporulation. It resembles *B. ostreae* in size, the low number of mitochondrial profiles, and the prevalence and mean number of lipid droplets. It differs from *B. ostreae* in the greater prevalence of nuclear membrane-bound Golgi (NM-BG), associated haplosporogenesis, and smaller size of haplosporosomes. The Chilean *Bonamia* sp. resembles *B. exitiosa* in the number of haplosporosomes, prevalence of lipid droplets, anastomosing endoplasmic reticulum and NM-BG, presence of circles of smooth endoplasmic reticulum (sER), confronting cisternae (CC), and cylindrical CC (CCC). It also appears to have a similar developmental cycle to *B. exitiosa* with larger forms occurring in winter (August). The circles of sER, CC, and CCC have only been reported from *B. exitiosa*, and it appears that Chilean *Bonamia* sp. and *B. exitiosa* are more closely related than they are to *B. perspora* or *B. ostreae*. Similarities in ultrastructure and developmental stages between New Zealand and Chilean parasites suggest that the 2 species are related, and that the Chilean *Bonamia* sp. is either *B. exitiosa*, a sub-species of *B. exitiosa*, or a separate species closely related to *B. exitiosa*.

KEY WORDS: Chile · *Bonamia exitiosa* · Ultrastructure · Taxonomy

INTRODUCTION

The haplosporidian genus *Bonamia* comprises 4 described species, all of which infect oysters (Ostreidae). *B. ostreae* Pichot et al., 1980 infects *Ostrea edulis* on the west and east coasts of the U.S. and around Europe, and may also infect *O. angasi* (see Bougrier et al. 1986), *O. puelchana* (see Pascual et al. 1991), *O. chilensis* (see Grizel et al. 1983), and *Crassostrea ariakensis* (= *C. rivularis*; see Cochennec et al. 1998). It causes mortalities among oysters in its putative endemic area in the eastern US (Carnegie & Barber 2001), and massive epizootics occurred around Europe following its importation from North America (Grizel et al. 1988, Carnegie & Cochennec-Laureau 2004). *B. exitiosa* (Hine et al. 2001, Berthe & Hine 2004) infects *O. chilensis* in New Zealand and *O. angasi* in Australia (Corbeil et al. 2006), and it has had a devastating impact on wild oyster fisheries in New Zealand (Cranfield et al. 2005). A third species, originally described as *Mikrocytos roughleyi* (Farley et al. 1988), associated with mortalities (Roughley 1926) among Sydney rock oysters *Saccostrea glomerata* in southeast Australia, is now recognised as *B. roughleyi* (Cochennec-Laureau et al. 2003). The spore-forming species *B. perspora* infects native oysters (*O. stentina* = *Ostreola equestris*) in the eastern US (Carnegie et al. 2006). *Bonamia* spp. also occur in *Ostrea chilensis* in Chile (Campalans et al. 2000), and *O. puelchana* in Argentina (Kroeck & Montes 2005, Kroeck et al. 2008). *Bonamia* may be translocated by shipping (Howard 1994), which would explain the presence of Australasian *B. exitiosa* in *C.*

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ariakensis near a port in the eastern US (Burreson et al. 2004, Bishop et al. 2006), and the recent identification of *B. exitiosa* in Spain (Abollo et al. 2008).

The ultrastructure of *Bonamia ostreae* is known from several studies that have reported uni-nucleate dense and light forms (Comps et al. 1980, Pichot et al. 1980, Balouet et al. 1983, Grizel 1985, Bucke 1988, Friedman et al. 1989, Chagot et al. 1992, Mourton et al. 1992, Montes et al. 1994). The ultrastructure of *B. roughleyi* is poorly known (Farley et al. 1988, Cochennec-Laureau et al. 2003). There have been several ultrastructural studies on *B. exitiosa* in *Ostrea chilensis* from New Zealand (Hine 1991b, 1992, Hine & Wesney 1992, 1994a,b, Hine et al. 2001). *B. exitiosa* has an annual pattern of infection (Hine 1991a,b), in which very light infections with uni-nucleate forms occur in early spring (September to October), dense uni-nucleate stages in January to April, larger intermediate forms in May to June and amoeboid plasmodial forms in July to August. In July and August, large senescent forms contain cylindrical confronting cisternae (CCC; Hine & Wesney 1992).

Bonamia spp. are termed 'microcells' (Farley et al. 1988, Carnegie & Cochennec-Laureau 2004), because of their small size (2.0 to 6.0 µm), which makes it impossible to identify them by normal light microscopy, unless using sensitive and specific *in situ* hybridisation (Carnegie et al. 2003), which is not generally available. Different species of *Bonamia* need to be identified because they are very pathogenic and may be moved by hull fouling on ships. Molecular studies imply (Abollo et al. 2008), or state (Corbeil et al. 2006, López-Flores et al. 2007) that the Chilean *Bonamia* sp. is *B. exitiosa,* and the phylogenetic tree of Carnegie et al. (2006) places Chilean *Bonamia* sp. with Southern Hemisphere *B. exitiosa,* separated from Northern Hemisphere *B. ostreae* and *B. perspora*. The latter species are placed together despite the fact that *B. perspora* is spore-forming, but there is no indication of sporulation by *B. ostreae*. Here we report the ultrastructure of the Chilean *Bonamia* sp. and discuss its taxonomic affinities.

MATERIALS AND METHODS

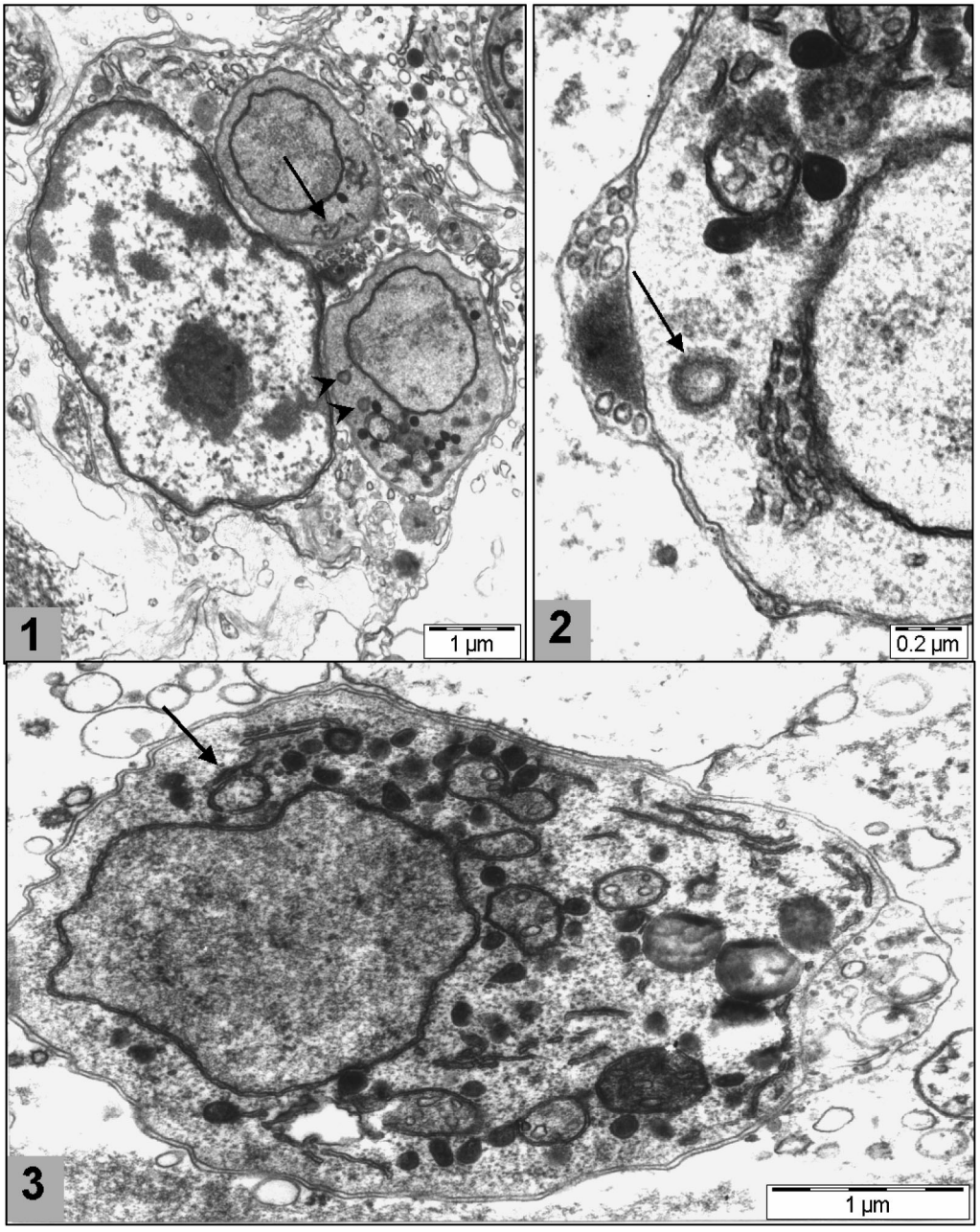
Chilean oysters *Ostrea chilensis* were collected in December 2003 in Quihua, Calbuco, Chile (41° 45′ S), and transported to the facilities of the Universidad Católica del Norte in Coquimbo (29° 57′ S). They were sampled for transmission electron microscopy (TEM) in May (n = 20) and August (n = 17) 2004, at a water temperature of 13°C. A second group of oysters was shipped to Coquimbo in February 2005, of which half were kept at 19°C and half at ambient temperature (13 to 17°C). These were sampled for TEM on 3 March (n = 11) at 17°C, 26 April (n = 24) at 19°C, 13 July (n = 22) at 13°C and 25 July (n = 7) at 19°C. The August sample was too poorly fixed for measurements other than cell size. Heart imprints were prepared and stained with Hemacolor™ (Merck), and the oysters with the heaviest infections were selected for TEM.

Small (1 mm^3) pieces of digestive gland and gills were fixed for TEM in 3% glutaraldehyde in 0.2 M cacodylate buffer with 1.75% NaCl for 90 min at 4°C, and post-fixed for 2 h in 1% reduced OsO_4 (1:1 mixture of 3% aqueous $KFe[CN]_6 \cdot 3H_2O$ and 2% OsO_4). After washing 3 times with buffer, they were rinsed twice in distilled H_2O, stained for 1 h en bloc with 2% aqueous uranyl acetate, dehydrated in an ethanol series, washed in acetone and embedded in Medcast (Pelco). Semi-thin sections (1 µm thick) were cut on an ultratome and stained with toluidine blue (1 g toluidine blue, 0.5 g methylene blue, 100 ml distilled water [dH_2O]). Thin sections (90 nm thickness) were cut with a diamond knife, collected on copper grids, and stained with 2% aqueous uranyl acetate and lead citrate (Reynolds preparation: mix 1.33 g lead nitrate, 1.76 g sodium citrate, 30 ml dH_2O for 30 min, to which were added 8 ml of 1N NaOH and dH_2O to make 50 ml, pH 12). The sections were viewed and photographed on a Zeiss EM 900 electron microscope at 50 kV.

The measurements of cell size, nuclear size and the size of haplosporosomes given in the tables were calculated as the means (±SD) of the longest dimension and the dimension at right angles to it, divided by 2. The nucleus:cytoplasm (N:C) ratio was the mean of nucleus/nuclei diameter(s) expressed as a percentage of the mean cell diameter.

RESULTS

Only uni-nucleate stages were observed (Fig. 1), except for a few bi-nucleate stages in July 2005. Diplokaryotic and plasmodial stages were not observed. Dimensions are given in Table 1. Uni-nucleate stages were usually round to ovoid, rarely irregular, with a central spherical or ovoid slightly irregular nucleus and intranuclear microtubules, and 19% had an eccentric nucleolus sometimes causing a slight protrusion of the nuclear surface. Prominent nuclear membrane-bound Golgi (NM-BG), often with a beaded appearance, occurred in 45% of parasites sectioned (Fig. 2), and haplosporogenesis involving intermediate haplosporosome-like bodies (H-LBs) occurred in 23% of *Bonamia* sp. sectioned. Haplosporosomes were round to ovoid in shape, and varied greatly in number per section (Table 1). Mitochondria had a swollen, or rarely dense, appearance, often lying



Figs. 1 to 3. *Bonamia* sp. infecting *Ostrea chilensis*. Fig. 1. Agranular haemocyte containing 2 phagocytosed *Bonamia* sp., the top one of which has cytoplasmic membranes resembling anastomosing endoplasmic reticulum (aER; arrow). The lower *Bonamia* sp. shows the affinity of haplosporosomes for mitochondrial membranes, and 2 circular profiles of smooth endoplasmic reticulum (sER) are present (arrowheads). May sample. Fig. 2. Nuclear surface showing the well-developed nuclear membrane-bound Golgi (NM-BG), a nearby haplosporosome-like body (arrow), and lipid-like vesicles between the parasite and the phagosome membrane. May sample. Fig. 3. Developing uni-nucleate stage showing the reduced nucleus:cytoplasm (N:C) ratio (27%), NM-BG or aER (arrow) near a slight depression in the nucleus, and a lipid droplet. July sample

against mitochondrial membranes, but less often against the nuclear membrane. In 14% of cells, anastomosing endoplasmic reticulum (aER) lay close to the nuclear membrane (Fig. 3). Lipid droplets were not common in the uni-nucleate stage (Fig. 3, Table 1).

Monthly dimensions and parameters of *Bonamia* sp. are given in Table 2. A sample was also collected in August 2004, but only size of the cell (2.4 ± 0.6 µm; range 1.8–3.9; n = 18), size of the nuclei (1.3 ± 0.2 µm; range 1.1–1.5) and the number of haplosporosomes (20 ± 12;

Table 1. *Bonamia* sp. infecting *Ostrea chilensis*. Dimensions and parameters of the uni-nucleate stage of *Bonamia* sp. in *O. chilensis* in Chile. The uni-nucleate stage is based on all samples; the bi-nucleate stage in based on the July 2005 sample. NM-BG: nuclear membrane-bound Golgi; H-LB: haplosporosome-like body; sER: smooth endoplasmic reticulum; aER: anastomosing endoplasmic reticulum

Parameter	All uni-nucleate (n = 89)	All bi-nucleate (n = 9)	Total (n = 98)
Size (µm)			
Mean length × width	2.5 ± 0.4 × 1.8 ± 0.3	3.4 ± 0.7 × 2.2 ± 0.4	2.5 ± 0.5 × 1.8 ± 0.3
Mean diameter	2.1 ± 0.4	2.8 ± 0.9	2.2 ± 0.5
Range, length × width	1.9–3.8 × 1.3–2.5	2.6–4.8 × 1.4–2.9	1.9–4.8 × 1.3–2.9
Nucleus size (µm)			
Mean length × width	1.4 ± 0.2 × 1.1 ± 0.1	1.4 ± 0.3 × 1.0 ± 0.2	1.4 ± 0.2 × 1.1 ± 0.2
Mean diameter	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3
Range, length × width	1.0–2.0 × 0.8–1.4	1.1–2.4 × 0.8–1.5	1.0–2.4 × 0.8–1.5
Mean nucleus:cytoplasm ratio	36 ± 7	37 ± 5	36 ± 7
Prevalence (%) of intranuclear microtubules	30	40	31
Mean no. and prevalence (%) of NM-BG	0.5 ± 0.5, 47	0.3 ± 0.5, 17	0.4 ± 0.5, 45
Mean no. and prevalence (%) of haplosporogenesis at NM-BG	0.3 ± 0.4, 22	Too few	0.2 ± 0.4, 23
Haplosporosomes			
Mean no. (range)	12 ± 7 (0–32)	19 ± 5 (13–27)	14 ± 8 (0–32)
Mean diameter (nm)	136 ± 20	128 ± 20	136 ± 20
Mean diameters, length × width (nm)	148 ± 17 × 124 ± 14	139 ± 11 × 116 ± 21	148 ±17 × 123 ± 15
Range of diameters, length × width (nm)	115–195 × 86–155	127–154 × 92–134	115–195 × 86–159
H-LBs			
Mean no. (range)	0.2 ± 0.6 (0–3)	0.1 ± 0.4 (0–1)	0.2 ± 0.5 (0–3)
Prevalence (%)	18	17	17
Mean size (nm)	154 ± 23	Too few	154 ± 23
Mean (range) of mitochondrial sections	2.0 ± 1.4 (0–8)	3.2 ± 0.8 (2–4)	2.0 ± 1.5 (0–8)
Proportion (%) of short sER	82	67	81
aER (%)	23	2	14
Proportion (%) of long sER	18	33	19
Prevalence (%) of circular sections of sER	8	0	7
Prevalence (%) of lipid droplets	26	63	24
Mean no. (range) of lipid droplets	0.3 ± 0.6 (0–4)	0.7 ± 0.5 (0–1)	0.3 ± 0.6 (0–4)

range 10–40), could be determined, because of poor fixation. Despite this there was a slight increase in cell size, increase in the prevalence of intranuclear microtubules, NM-BG and increase in the prevalence and mean numbers of H-LBs, between March and July samples, with peaks in May. These peaks may reflect seasonal changes, or that the May sample was from 2004, and the others from 2005. There was a slight decline in haplosporosome size over that period. Large uni-nucleate stages occurred in July and August (Table 3, Fig. 3). Karyokinesis by elongation of the nucleus to form a dumbbell shape (Fig. 4) dividing into bi-nucleate cells (Fig. 5) was observed in July samples. Bundles of parallel microfilaments were rarely observed in the cytoplasm (Fig. 6). Occasionally, circular profiles of smooth endoplasmic reticulum (sER), 202 to 267 nm in diameter, occurred in the cytoplasm, but some cells had several abnormal HLB-like structures (Fig. 7). *Bonamia* sp. sampled in August sometimes contained confronting cisternae (CC) or CCC of sER (Fig. 8).

With increase in cell size there was a reduction in N:C ratio and circular sections of sER, and slight reduction in haplosporogenesis and H-LBs, and an increase in the mean number of haplosporosomes, mitochondrial sections and lipid droplets (Table 4).

DISCUSSION

Chilean *Bonamia* sp. resembles *B. ostreae* in size, the number of mitochondrial profiles and the prevalence and mean number of lipid droplets (Table 5). The slight protrusion of the eccentric nucleolus also occurs in *B. ostreae* (Fig. 5 in Chagot et al. 1992), and NM-BG and an aER-like structure occur in *B. ostreae* (Fig. 2 in Hervio et al. 1991). Although aER and NM-BG have only been illustrated by Hervio et al. (1991), they occurred in 6 of 23 (27%) uni-nucleate *B. ostreae* examined from the Netherlands (M. Engelsma & M. Hine unpubl.). Chilean *Bonamia* and *B. ostreae* occur predominantly as uni-nucleate stages, less commonly as bi-nucleate forms (Brehélin et al. 1982). *B. ostreae* has larger haplosporosomes than other *Bonamia* spp. (Table 5), which are spherical rather than the ovoid

Table 2. *Bonamia* sp. infecting *Ostrea chilensis*. Seasonal pattern in the occurrence of *Bonamia* sp. in Chile. NM-BG: nuclear membrane-bound Golgi; H-LB: haplosporosome-like body; sER: smooth endoplasmic reticulum; aER: anastomosing endoplasmic reticulum

Parameter	March 2005, n = 11	April 2005, n = 24	May 2004, n = 20	July 2005, n = 30
Size (µm)				
Mean length × width	2.3 ± 0.2 × 1.7 ± 0.2	2.6 ± 0.3 × 1.8 ± 0.2	2.4 ± 0.3 × 1.9 ± 0.3	2.7 ± 0.6 × 1.8 ± 0.3
Mean diameter	2.0 ± 0.3	2.2 ± 0.5	2.2 ± 0.4	2.2 ± 0.7
Range, length × width	1.9–2.7 × 1.5–1.9	2.0–3.1 × 1.3–2.4	2.1–2.9 × 1.4–2.5	2.0–4.8 × 1.4–2.9
Nucleus size (µm)				
Mean length × width	1.4 ± 0.3 × 0.9 ± 0.1	1.5 ± 0.3 × 1.0 ± 0.2	1.4 ± 0.1 × 1.2 ± 0.1	1.5 ± 0.3 × 1.0 ± 0.2
Mean diameter	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	1.2 ± 0.3
Range, length × width	1.1–1.9 × 0.8–1.1	1.0–1.9 × 0.8–1.4	1.0–1.7 × 1.0–1.4	1.1–2.4 × 0.8–1.5
Mean nucleus:cytoplasm ratio	35 ± 10	32 ± 6	39 ± 7	36 ± 6
Prevalence (%) of intranuclear microtubules	17	13	47	33
Mean no. and prevalence (%) of NM-BG	0.4 ± 0.5, 38	0.3 ± 0.5, 39	0.7 ± 0.5, 65	0.5 ± 0.5, 40
Mean and prevalence (%) of haplosporogenesis at NM-BG	0.4 ± 0.6, 45	0.1 ± 0.3, 10	0.4 ± 0.5, 43	0.2 ± 0.4, 22
Haplosporosomes				
Mean (range) of numbers	12 ± 7 (4–17)	13 ± 5 (5–24)	11 ± 6 (0–21)	16 ± 7 (2–32)
Mean diameter (nm)	137 ± 19	152 ± 16	134 ± 18	129 ± 21
Mean diameters, length × width (nm)	148 ± 19 × 125 ± 11	153 ± 15 × 123 ± 10	145 ± 16 × 123 ± 13	140 ± 15 × 117 ± 20
Range of diameter, length × width (nm)	128–182 × 110–142	130–175 × 103–139	116–186 × 93–155	115–168 × 86–159
H-LBs				
Mean no. (range) of *Bonamia* with H-LBs	0.1 ± 0.3 (0–1)	0.0 ± 0.2 (0–1)	0.5 ± 0.8 (0–3)	0.3 ± 0.6 (0–2)
Prevalence (%)	9	4	31	19
Mean size (nm)	–	Too few	159 ± 19	154 ± 28
Mean no. of mitochondrial sections	2.0 ± 1.1	1.3 ± 0.8	1.9 ± 1.2	2.9 ± 1.8
Prevalence (%) of aER	43	8	33	23
Proportion (%) of sections of short sER	18	100	78	79
Proportion (%) of sections of long sER	82	0	22	21
Prevalence (%) of circular sections of sER	0	8	16	7
Prevalence (%) of lipid droplets	22	28	15	30
Mean no. (range) of lipid droplets	0.3 ± 0.5, 0–1	0.3 ± 0.6, 0–2	0.2 ± 0.4, 0–1	0.4 ± 0.9, 0–4

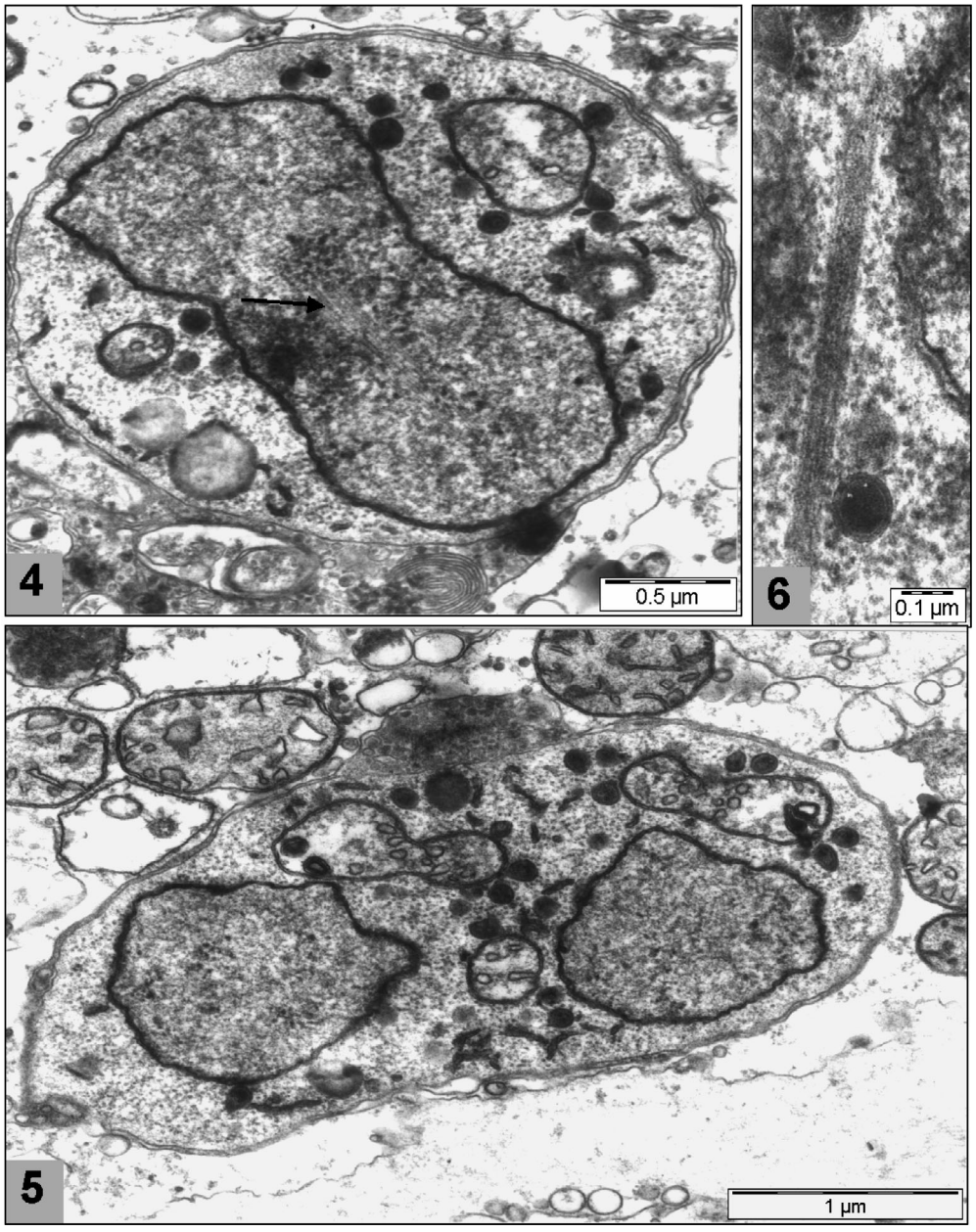
haplosporosomes of *B. exitiosa*, making it ultrastructurally easy to distinguish. The large (<40 µm) plasmodia with haplosporosomes 130 to 320 nm in diameter in *Ostrea edulis* from the estuary of the Saint-Philibert River, Morbihan, Brittany (Bonami et al. 1985) may be those of *Haplosporidium armoricanum*, as the latter is endemic in the estuary (van Banning 1977, Vivarès et al. 1982), and it has haplosporosomes 131 to 278 nm in diameter (Hine et al. 2007). The Chilean *Bonamia* sp. is unlike *B. perspora* in the scarcity of vegetative stages and presence of multinucleate plasmodia and spores in the latter (Carnegie et al. 2006).

Table 3. *Bonamia* sp. infecting *Ostrea chilensis*. Distribution (%) of Chilean *Bonamia* sp. in relation to parasite size groups in monthly samples

Month	Size (µm)			
	1.6 – 2.0	2.1 – 2.5	2.6 – 3.0	> 3.1
March (n = 11)	64	36		
April (n = 24)	34	57	9	
May (n = 20)	35	60	5	
July (n = 28)	25	50	21	4
August (n = 17)	35	41	12	12

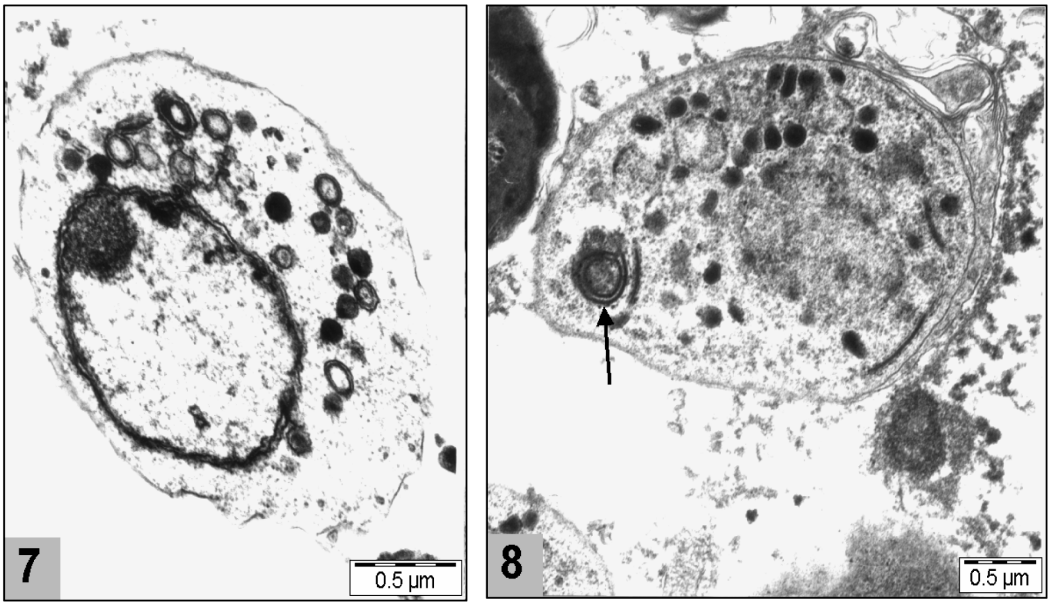
Chilean *Bonamia* sp. and *B. exitiosa* resemble each other in the presence of circles of sER, CC and CCC, which have only been reported from *B. exitiosa* in New Zealand (Hine & Wesney 1992), and circular sER occurs in Australian *B. exitiosa* (M. Hine unpubl. obs.). In New Zealand *B. exitiosa*, they occur in large uninucleate forms in autumn and early winter (Hine & Wesney 1992), and also occur in large forms in Chilean *Bonamia* sp. (Table 4). Their greater abundance in *B. exitiosa* than in Chilean *Bonamia* sp. may be related to size, as *B. exitiosa* develops into larger sizes than Chilean *Bonamia* sp. (Table 5).

The developmental pattern in the sequence of months given in Table 2 is artificial, as the different



Figs. 4 to 6. *Bonamia* sp. infecting *Ostrea chilensis*. Fig. 4. Cell in the early stages of division. Note the intranuclear microtubules (arrow). July sample. Fig. 5. Bi-nucleate cell containing short sections of sER and a lipid droplet. July sample. Fig. 6. Bundle of parallel microfilaments in the cytoplasm of one cell. July sample

months were sampled in different years, and the oysters were held at varying temperatures. There is no indication of an annual pattern of *Bonamia ostreae* infection, and sampling of the Gravelingen in the Netherlands showed the presence of only uni-nucleate and bi-nucleate forms throughout the year (M. Engelsma & M. Hine unpubl. obs.). In a *Bonamia* sp. resembling Australian *B. exitiosa* (Bishop et al. 2006), salinity (Audemard et al. 2008a) and temperature (Audemard et al. 2008b) affect the kinetics of infection, and temperature may cause parasite seasonality (Carnegie et al. 2008) and affect transmission (Audemard et al. 2008b). However, it is unknown whether this seasonality is accompanied by an annual developmental cycle. The annual developmental pattern of *B. exitiosa* in *Ostrea chilensis* (see Hine 1991a,b) occurs in 20 to 50 m deep oyster beds in Foveaux Strait, New Zealand, where the annual temperature only varies by 5 to 8°C (Garner 1961, Cranfield 1968), and therefore it may be endogenous. Enlargement of the Chilean



Figs. 7 & 8. *Bonamia* sp. infecting *Ostrea chilensis*. Fig. 7. *Bonamia* sp. showing abnormal ring-like structures, some resembling haplosporosome-like bodies (H-LBs), suggesting abnormal haplosporogenesis. April sample. Fig. 8. Uni-nucleate stage with confronting cisternae (CC) and cylindrical confronting cisternae (CCC; arrow). August sample

Bonamia sp. uni-nucleate stage occurred in autumn (April, May) and winter (July, August), as in New Zealand *B. exitiosa* (Hine 1991a,b), despite sampling in different years and the oysters being held at different temperatures. Irrespective of this, circular profiles of sER have only been observed in Australian, New Zealand and Chilean *Bonamia* sp.

Chilean *Bonamia* sp. and *B. exitiosa* are dissimilar in cell size, haplosporosome size, number of mitochondrial sections and mean number of lipid droplets (Table 5), and in the prevalence of NM-BG in the uni-nucleate stage (47% in the Chilean parasite, 15% in *B. exitiosa*). Also, large *B. exitiosa* in winter appear amoeboid and have indentations in the nuclear surface, large parallel arrays of sER and phagosomes that resemble multivesicular bodies (Hine 1991b, Hine & Wesney 1992, 1994a). While large Chilean *Bonamia* sp. may be amoeboid and have suggestions of nuclear indentations (Fig. 3), none of the other features were observed. Rarely, *B. exitiosa* in Australia (M. Hine unpubl.) and

Table 4. *Bonamia* sp. infecting *Ostrea chilensis*. Dimensions and parameters of *Bonamia* sp. in relation to parasite mean diameter. NM-BG: nuclear membrane-bound Golgi; ; aER: anastomosing endoplasmic reticulum; H-LB: haplosporosome-like body; CC: confronting cisternae; CCC: cylindrical confronting cisternae; sER: smooth endoplasmic reticulum

Parameter	Size (µm)		
	1.6–2.0	2.1–2.5	2.6–3.0
Nucleus:cytoplasm ratio (range)	39 ± 8 (27–54)	33 ± 6 (22–44)[a]	29 ± 10 (21–42)[a]
Mean no. and prevalence (%) of NM-BG	0.5 ± 0.5, 54	0.3 ± 0.5, 28	0.6 ± 0.5, 56
Mean no. and prevalence (%) of haplosporogenesis	0.4 ± 0.5, 44	0.3 ± 0.5, 25	0.3 ± 0.5, 25
Mean no. and prevalence (%) of aER	0.3 ± 0.5, 29	0.2 ± 0.4, 16	0.4 ± 0.5, 38
Mean no. and prevalence (%) of H-LBs	0.3 ± 0.6, 19	0.2 ± 0.6, 14	0.2 ± 0.4, 22
Intranuclear microtubules (%)	0.4 ± 0.5, 38	0.2 ± 0.4, 16	0.6 ± 0.5, 57
No. (range) of haplosporosomes	10 ± 7 (0–24)	13 ± 6 (4–30)	18 ± 6 (12–32)
Mitochondrial profiles (range)	1.6 ± 1.4 (0–6)	2.1 ± 1.2 (0–6)	3.4 ± 2.1 (1–8)
Parallel microfilaments (%)	4	0	0
CC and/or CCC (%)	4	0	11
sER short (%)	83	72	100
sER long (%)	17	28	0
sER circles (%)	0.1 ± 0.3, 10	0.1 ± 0.3, 5	0
Mean no. and prevalence (%) of lipid droplets	0.1 ± 0.3, 11	0.2 ± 0.5, 20	1.1 ± 1.3, 67

[a]Without the bi-nucleate or dividing stages

Table 5. Comparison of some parameters of *Bonamia* spp. to the Chilean *Bonamia* sp.

Parameter	*B. perspora*	*B. exitiosa*[a]	*B. exitiosa*[a]	*Bonamia* sp.	*B. ostreae*[b]
Location	Eastern USA	New Zealand	Australia	Chile	Europe, USA
Host species	*Ostreola equestris*	*Ostrea chilensis*	*Ostrea angasi*	*Ostrea chilensis*	*Ostrea edulis*
Size (µm)	3.2 ± 1.0	3.2 ± 0.5	2.8 ± 0.4	2.2 ± 0.5	2.1 ± 0.4
No. haplosporosomes	5 ± 3	29 ± 17	10 ± 4	14 ± 8	9 ± 4
Size haplosporosomes (nm)	137 ± 19	148 ± 11	156 ± 15	136 ± 20	188 ± 21
No. mitochondria	6 ± 2	4 ± 1	4 ± 1	2 ± 2	1 ± 1
Mean no. lipid droplets	1.0 ± 1.4	0.5 ± 0.9	0.5 ± 0.8	0.3 ± 0.6	0.4 ± 0.6
% with lipid droplets	75	32	30	24	30

[a]M. Hine unpubl. data; [b]M. Engelsma & M. Hine unpubl. data

New Zealand (Hine et al. 2001) have a symmetrical vacuolated stage, which was not observed here, possibly because of its rareness and the relatively small sample size of Chilean *Bonamia* sp. A reticulated structure in small uni-nucleate stages of New Zealand *B. exitiosa* (see Hine & Wesney 1992), and in plasmodia of *B. perspora* (Carnegie et al. 2006) was not observed in Chilean *Bonamia* sp. The parallel bundles of microfilaments seen here have been reported from spores of *B. perspora* (Carnegie et al. 2006) and *Minchinia occulta* (Bearham et al. 2008) (= *Haplosporidium* sp.; Hine & Thorne 2002), but not from New Zealand *B. exitiosa,* despite intensive investigation.

Therefore, the Chilean *Bonamia* sp. and *B. ostreae* are similar in some features, but it can readily be ultrastructurally distinguished from *B. ostreae*, which has larger round haplosporosomes, and from *B. perspora* by the predominance of vegetative stages and lack of spores. In the high degree of haplosporogenesis, reflected in the prevalence of NM-BG, the numbers of haplosporosomes, the development into larger uninucleate forms and the presence of sER circles, CC and CCC, suggest that it more closely resembles *B. exitiosa*, as also indicated by molecular phylogeny (Carnegie et al. 2006). However, the Chilean parasite differs from New Zealand *B. exitiosa* and the less studied Australian species in its smaller size, number and size of haplosporosomes, fewer mitochondrial profiles and fewer lipid droplets (Table 5).

These data can be interpreted in 3 ways. Firstly, the Chilean parasite is *Bonamia exitiosa,* and *B. exitiosa* may be considered a pan-Southern Hemisphere species showing ultrastructural and genetic diversity in different regions and different hosts, in which the host may influence the development of the parasite. This interpretation is implied by some molecular phylogenies (Corbeil et al. 2006, López-Flores et al. 2007, Abollo et al. 2008). Secondly, it may be regarded as a sub-species of *B. exitiosa,* due to ultrastructural and genetic divergence from the Australasian *B. exitiosa.* Possible support for this comes from the existence of Pliocene (5 million years ago) *Ostrea chilensis* in New Zealand, but more recent Holocene (10 000 years ago) fossil *O. chilensis* in Chile. Thirdly, the Chilean *Bonamia* sp. may be a smaller, separate species, showing greater affinities to Southern Hemisphere *B. exitiosa* than to *B. perspora* or *B. ostreae.* Essentially these 3 interpretations are similar, but differ in degree of separation. The phylogenetic placing of the Chilean *Bonamia* sp. with *B. exitiosa* isolates, and separate from Northern Hemisphere species (*B. perspora, B. ostreae*) by Carnegie et al. (2006), supports all of these interpretations. Further ultrastructural studies on developmental patterns and multi-gene phylogenetic analyses will clarify the inter-relationships of Southern Hemisphere *Bonamia* spp.

Acknowledgements. We thank F. Vargas from the TEM facilities of Universidad de Valparaíso for his skilful technical assistance. This study was partially financed by grant FIP-2003-27 (Chile).

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Editorial responsibility: Sven Klimpel, Düsseldorf, Germany

Submitted: December 8, 2008; Accepted: May 23, 2009
Proofs received from author(s): July 21, 2009