Journal of Invertebrate Pathology 131 (2015) 32–42

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

New perspective on the haplosporidian parasites of molluscs

Isabelle Arzul[a], Ryan B. Carnegie[b,*]

[a] IFREMER, Laboratory of Genetics and Pathology, Av de Mus de Loup, 17390 La Tremblade, France
[b] Virginia Institute of Marine Science, College of William & Mary, P.O. Box 1346, Gloucester Point, VA 23062, USA

ARTICLE INFO

Article history:
Received 17 June 2015
Revised 24 July 2015
Accepted 27 July 2015
Available online 8 August 2015

Keywords:
Haplosporidium
Bonamia
Minchinia
MSX disease
Haplosporidiosis
Bonamiosis

ABSTRACT

The protist phylum Haplosporidia comprises over 40 described species with representatives infecting a range of mollusc hosts, including several ecologically and economically significant pathogens. Continuing exploration of haplosporidian diversity has added ten new species in recent years and brought the phylogenetics of the group into somewhat clearer focus, with monophyletic *Bonamia* and *Minchinia* lineages continuing to be supported. However, the addition of new sequences to phylogenetic analyses has left the paraphyletic genus *Haplosporidium*'s picture less resolved. It is not clear that even two genera will be enough to accommodate the species presently drawn to the *Haplosporidium* regions of the haplosporidian tree. In this review, we summarize recent findings in haplosporidian diversity and phylogenetics, and provide a synthesis of our understanding of the life cycles and environmental influences on haplosporidians, with particular emphasis on the important pathogens *Haplosporidium nelsoni* and *Bonamia ostreae*. Additionally, we consider the evolution of the "microcell haplosporidian" lifestyle of *Bonamia* parasites, and suggest that colonization of high-density oyster host populations in relatively stable euhaline marine environments may have been an important development favoring the evolution of the microcell haplosporidian life strategy.

1. Introduction

Since the emergence of *Haplosporidium nelsoni* (or MSX, for "multinucleate sphere unknown") in the Delaware Bay in 1957 and Chesapeake Bay in 1959 (Andrews, 1962; Haskin et al., 1966), haplosporidian parasites of molluscs have been considered major pathogens of concern for aquatic animal health managers and shellfish industries around the world. They were one of the first groups to be recognized as significant pathogens of bivalve molluscs, with only *Perkinsus marinus*, identified in the oyster *Crassostrea virginica* in the Gulf of Mexico in 1948 (at that time as *Dermocystidium marinum* (Mackin et al., 1950)), coming to our attention earlier. And the haplosporidians have been responsible for some of the most significant and consequential marine disease epizootics on record. The *H. nelsoni* outbreak along the Mid-Atlantic coast of the USA devastated oyster populations and caused significant economic disruption of coastal communities dependent on them. Oyster mortality associated with this outbreak exceeded 90% (Ford and Haskin, 1982; Haskin and Andrews, 1988), producing significant financial losses for the oyster industries in these estuaries (e.g., Haven et al., 1978), from which they only recently have begun to recover. The microcell haplosporidian *Bonamia ostreae* was no less impactful on oyster *Ostrea edulis* populations in Europe. First observed at Île Tudy in Brittany, France in 1979 (Pichot et al., 1980), *B. ostreae* caused substantial destruction of *O. edulis* populations in France before spreading through much of Atlantic coastal Europe where its activity was most intense (Van Banning, 1991; Hudson and Hill, 1991; Montes, 1990; McArdle et al., 1991). Damage from *B. ostreae* compounded that caused by *Marteilia refringens*, which emerged in *O. edulis* in France a decade earlier (Comps, 1970; Grizel et al., 1974), and by the gill iridovirus that drove the Portuguese oyster *Crassostrea angulata* essentially to commercial extinction (Marteil, 1968). The combination of these events led to the massive importation to France and then other European countries of the Pacific oyster *Crassostrea gigas* (Grizel and Héral, 1991; Ruesink et al., 2005), a landmark development in the diaspora of this now globally significant commercial species.

In the decade since the last review of the haplosporidians by Burreson and Ford (2004), ten new haplosporidian species have been described (Table 1) in addition to numerous observations of novel unnamed haplosporidians (Table 2), and our knowledge of key species like *H. nelsoni* and the *Bonamia* parasites has deepened. The objective of this paper will be to revisit the phylogeny of the haplosporidians based on the incorporation of these new records and to provide the first synthesis on *Bonamia* and the

---

* Corresponding author.
E-mail address: carnegie@vims.edu (R.B. Carnegie).

http://dx.doi.org/10.1016/j.jip.2015.07.014
0022-2011/© 2015 Elsevier Inc. All rights reserved.

Table 1
Haplosporidian species described since the publication of Burreson and Ford (2004).

Species	Host	Location	References
Bonamia perspora	Oyster *Ostrea stentina*	North Carolina, USA	Carnegie et al. (2006)
Minchinia occulta	Oyster *Saccostrea cuccullata*	Western Australia	Bearham et al. (2008a)
Minchinia mercenariae	Clam *Mercenaria mercenaria*	Virginia and New Jersey, USA	Ford et al. (2009b)
Haplosporidium montforti	Abalone *Haliotis tuberculata*	Galicia, Spain	Azevedo et al. (2006)
Haplosporidium hinei[a]	Pearl oyster *Pinctada maxima*	Western Australia	Bearham et al. (2008b)
Haplosporidium tuxtlensis	Gastropod *Siphonaria pectinata*	Veracruz, Mexico	Vea and Siddall (2011)
Haplosporidium raabei	Mussel *Dreissena polymorpha*	France, Germany, The Netherlands	Molloy et al. (2012)
Haplosporidium littoralis	Crab *Carcinus maenus*	England	Stentiford et al. (2013)
Haplosporidium patagon	Gastropod *Siphonaria lessonii*	Patagonia, Argentina	Ituarte et al. (2014)
Haplosporidium diporeiae	*Diporeia* spp. amphipods	Lakes Superior and Michigan, USA	Winters and Faisal (2014)

[a] Generic assignment made based on spore structure, with molecular data absent.

Table 2
Recent observations of undescribed haplosporidians.

Identification	Host	Location	References
Bonamia sp.	Oyster *Ostrea chilensis*	Chile	Campalans et al. (2000), Hill et al. (2014)
Bonamia sp.	Oyster *Ostrea edulis*	California, USA	Hill et al. (2014)
Bonamia sp.	Oyster *Dendostrea sandvicensis*	Hawaii, USA	Hill et al. (2014)
Minchinia sp.	Clam *Cyrenoida floridana*	Mississippi, USA	Reece et al. (2004)
Minchinia sp.	Mussel *Mytilus edulis*[a]	Wales, United Kingdom	Lynch et al. (2014b)
Urosporidium sp.	Trematode *Stictodora lari*	New South Wales, Australia	Reece et al. (2004)
Haplosporidium sp.	Oyster *Ostrea edulis*	The Netherlands, Ireland	Engelsma and Haenan (unpubl.), Lynch et al. (2013)
Not designated	Shrimp *Pandalus platyceros*	British Columbia, Canada	Bower and Meyer (2002), Reece et al. (2004)
Not designated	Clam *Ruditapes decussatus*	Galicia, Spain	Novoa et al. (2004)
Not designated	Polychaete *Syllis nipponica*[a]	Japan	Siddall and Aquado (2006)
Not designated	Shrimp *Litopenaeus vannamei*	Belize	Nunan et al. (2007)
Not designated	Shrimp *Litopenaeus vannamei*	Indonesia	Utari et al. (2012)

[a] Based on genetic detection without microscopic visualization.

haplosporidians, with particular attention to the haplosporidians that continues to be the most significant in terms of their impacts, *H. nelsoni* and *B. ostreae*.

2. Phylogeny

2.1. New perspective on haplosporidian interrelationships

Since the major phylogenetic analysis of the haplosporidians by Reece et al. (2004), the phylogeny of the group has continued to come into clearer focus with the continual characterization of new species. Reece et al. (2004) determined the relationships among fifteen haplosporidians for which SSU rDNA and actin gene sequences were available. They identified undescribed parasites of the spot prawn *Pandalus platyceros* (Bower and Meyer, 2002) and abalone *Haliotis iris* (Hine et al., 2002; Reece and Stokes, 2003) as basal to the established haplosporidian genera *Urosporidium*, *Haplosporidium*, *Minchinia*, and *Bonamia*, with *Urosporidium*, represented in their analysis by *Urosporidium crescens* and a *Urosporidium* sp. hyperparasitic in the trematode *Stictodora lari* from the whelk *Battilaria australis*, as basal to the other genera. The genera *Bonamia* and *Minchinia* were resolved as sister genera, the former including *B. ostreae* as well as *Bonamia exitiosa*, which we recognize *Bonamia* sp. and *Mikrocytos roughleyi* from the Reece et al. (2004) analysis to represent (Carnegie et al., 2014), and the latter represented by *Minchinia tapetis*, *Minchinia chitonis*, *Minchinia teredinis*, and an undescribed parasite from the clam *Cyrenoida floridana*. *Haplosporidium*, comprising the remainder of the sequences in the analysis, was paraphyletic, however, with *Haplosporidium costale* and the sister species *Haplosporidium pickfordi* and *Haplosporidium lusitanicum* forming a monophyletic clade sister to the *Bonamia*–*Minchinia* clade but with *H. nelsoni* and *Haplosporidium louisiana* basal to *Bonamia*–*Minchinia* and the other *Haplosporidium* species. Paraphyly of *Haplosporidium* has consistently been demonstrated in all subsequent analyses with additional sequences, and will require resolution through the creation of new genera to accommodate those presently in this genus; which lineage among these deserves to retain the *Haplosporidium* designation cannot be determined, however, without determination of the position of *Haplosporidium scolopli*, the type *Haplosporidium* species, a parasite of the polychaete *Scoloplos mülleri* (Caullery and Mesnil, 1899; Burreson and Reece, 2006).

The molecular characterization of additional haplosporidians has, in some ways, not dramatically altered the tree topology illustrated by Reece et al. (2004). More recent analyses have continued to identify the *P. platyceros* and *H. iris* parasites as basal haplosporidians, joined by an undescribed parasite of the clam *Ruditapes decussatus* in Spain (Novoa et al., 2004) but with the relationships among these three parasites not clearly resolved; *Urosporidium* as basal to the other established genera; and the derived genera *Bonamia* and *Minchinia* as reciprocally monophyletic (Azevedo et al., 2006; Carnegie et al., 2006; Siddall and Aquado, 2006; Bearham et al., 2007; Nunan et al., 2007; Ford et al., 2009a, 2009b; Vea and Siddall, 2011; Molloy et al., 2012; Stentiford et al., 2013; Engelsma et al., 2014; Ituarte et al., 2014). *Bonamia* is now represented additionally by *Bonamia perspora*, a parasite of the oyster *Ostrea equestris* (=*Ostrea stentina*, Shilts et al., 2007) in North Carolina, USA (Carnegie et al., 2006), for which there is weak evidence of a sister relationship to *B. ostreae* (Engelsma et al., 2014), and an undescribed *Bonamia* sp. from the oyster *Dendostrea sandvicensis* in Hawaii that appears to be the basal representative of this genus based on its SSU rDNA sequence (Engelsma et al., 2014; Hill et al., 2014; Fig. 1). *Minchinia* now includes *Minchinia occulta*, infecting oyster *Saccostrea cuccullata* in Western Australia (Bearham et al., 2008a) and *Minchinia mercenariae*, infecting clam *Mercenaria mercenaria* in Virginia, USA (Ford et al., 2009b). A parasite detected genetically in mussel *Mytilus edulis* from Wales appears to be a *Minchinia* species as well