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1 **A review of the characteristics of the dinoflagellate parasite *Ichthyodinium***
2 ***chabelardi* and its potential effect on fin fish populations**

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19 Key words: Osteichthyes; Syndiniophyceae; *Amyloodinium ocellatum*; *Saprolegnia*
20 *parasitica*; *Sphaerothecum destruens*; X-cell species

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24 Abstract

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26 This paper focuses on the biology and ecological impacts of *Ichthyodinium chabelardi*
27 (Phylum Dinophyta, Class Syndiniophyceae, Order Syndiniales), a virulent endobiotic
28 parasite of yolk sacs and young larvae of many species of marine fin fish. Its infections
29 have been observed in warm and temperate open oceanic environments and crowded
30 marine fish tanks. The prevalence of *I. chabelardi* and the range of its host fishes is not
31 well-studied, and our understanding of its life cycle is incomplete. Here we describe what
32 is known about *I. chabelardi* infections in fish and we compare this with several other
33 protistan parasites of fish, including *Amyloodium ocellatum*, *Saprolegnia parasitica*,
34 *Sphaerothecum destruens*, and the “X-cell” clades *Gadixcellia* and *Xcellia*, all of which
35 are considered emerging generalist parasites infecting a wide variety of fin fish species.
36 Recent findings suggest that rising seawater temperatures might lead to higher infection
37 rates in fishes, and we expect that these changing conditions could also expand the ranges
38 of some of these parasitic species. Thus, it is essential that the fishing industry effectively
39 monitor fish tanks and water in the surrounding environments for the presence of
40 zoosporic parasites including *I. chabelardi* in order to take steps to prevent large losses in
41 these fisheries.

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45 **Introduction**

46 **Objectives of this review**

47 This review focuses on the marine dinoflagellate parasite *Ichthyodinium chabelardi*
48 Hollande & J. Cachon. The genus *Ichthyodinium* (Class Syndiniophyceae) consists of one
49 species, *I. chabelardi*. This species is a virulent, generalist, endobiotic parasite of embryos
50 and young larvae of many species of marine fin fish (Class Osteichthyes) living and
51 reproducing in the ichthyoplankton, primarily in tropical and warm temperate oceanic
52 ecosystems (Stratoudakis *et al.* 2000; Shadrin *et al.* 2010b). *I. chabelardi* has been
53 intensively studied in only a few host fin fish species. Most of the fin fish which carry this
54 parasite have been poorly investigated, even though some of the host fish species are
55 economically important food resources. Because there is some evidence that the
56 prevalence of other types of parasitic infections may increase with rising seawater
57 temperatures (van West 2006; Gozlan *et al.* 2014; Ercan *et al.* 2015), it is extremely
58 important to understand their mechanisms of infectivity, potential future distributions, and
59 begin to explore approaches to mitigation before they become an even greater threat to
60 fisheries.

61 Until now, no review of the literature on this parasite has been available. In contrast,
62 several compilations have been written about a closely related host generalist parasitic
63 protist belonging to the *Hematodinium* genus (also in the Class Syndiniophyceae)
64 (Stentiford and Shields 2005; Small and Pagenkopp 2011). The infectious agent of the
65 'bitter crab disease' (BCD) or the 'bitter crab syndrome' infects many commercially
66 important host species around the world including crabs, lobsters and amphipods. This
67 parasite has also been implicated in several outbreaks in fishery and aquaculture facilities
68 leading to substantial annual economic losses (Shields 1994; Stentiford and Shields 2005;

Small *et al.* 2012). Given that *I. chabelardi* is also a generalist parasite infecting several different species of fish hosts with high prevalence levels, we predict that it, too, could have a significant impact on commercial fin fish populations.

In this review we firstly discuss the phylogeny, biology and ecology of *I. chabelardi* focusing on its morphology and life cycle and the pathology of its infections in host fishes. We then describe the putative geographical expansion and host shift of this emerging disease and its potential ecological roles in marine food webs. We briefly compare the biology of *I. chabelardi* with four other emerging zoosporic parasites which commonly infect fin fish: *Amyloodium ocellatum*, *Saprolegnia parasitica*, *Sphaerothecum destruens* and “X-cell” parasites. Finally, we appraise the future possibility of large losses in production of marine fin fish to epidemics in fish farms unless measures are implemented to prevent the spread of parasitic infections.

Phylogenetic position of *Ichthyodinium chabelardi*

Baldauf (2003, 2008) divided all eukaryotic organisms into eight supergroups based upon consensus phylogeny. The supergroup Alveolata consists of a very diverse range of marine and freshwater protists belonging to three lineages: dinoflagellates, apicomplexans and ciliates. Presently the dinoflagellates (Phylum Dinozoa) include the following large clades based on both molecular and morphological characteristics: Phylum Perkinsozoa (Norén *et al.* 1999), Class Noctiluciphyceae (Gómez *et al.* 2010), Class Dinophyceae (core dinoflagellates) (Pascher 1914), and Class Syndiniophyceae (to which *Ichthyodinium chabelardi* belongs) (Loeblich III 1976). There are also several small clades, one of which includes the genus *Oxyrrhis* (Lowe *et al.* 2010).

92 Within the alveolates there are phototrophic, heterotrophic, and mixotrophic species. Most
93 of the core dinoflagellates are thought to be photosynthetic (including mixotrophs), but
94 there are also some heterotrophic species. Approximately half of all known dinoflagellate
95 species in all classes taken as a group are heterotrophic (Lessard and Swift 1986). Some
96 species of heterotrophic dinoflagellates contain plastids that lack a functional
97 photosynthetic apparatus (Robledo *et al.* 2011). The Phylum Perkinsozoa, Class
98 Syndiniophyceae, Class Blastodiniophyceae, Class Noctiluophyceae and the *Oxyrrhis*
99 clade are all groups of heterotrophic protists which lack functional chloroplasts and have
100 diverged into clades that are currently considered separate from the Class Dinophyceae
101 (core dinoflagellates) (Gómez *et al.* 2010; Bachvaroff *et al.* 2014).

102 *I. chabelardi* belongs to Class Syndiniophyceae which is composed of diverse lineages of
103 mainly endobiotic parasites, with several clades consisting entirely of uncultured species
104 (Moon-Van der Staay *et al.* 2001; Guillou *et al.* 2008). The Syndiniales are exclusive to
105 marine environments. Because of their endoparasitic lifestyle, they have unusual
106 adaptations, making them almost unrecognizable as dinoflagellates when inside their
107 hosts. The Syndiniales include at least five major marine alveolate (MALV) groups,
108 typically referred to as MALV I, II, III, IV, and V (but sometimes interchangeably as
109 Syndiniales I – V) (Guillou *et al.* 2008; Chambouvet *et al.* 2011). MALV II is currently
110 recognized as the most diverse clade and contains some of the more thoroughly studied
111 species, including numerous members of the genus *Amoebophrya*. Most members of
112 MALV II have been identified as parasites of dinoflagellates, although some also infect
113 radiolarians. Members of MALV IV infect other metazoans including *Hematodinium*, and
114 *Syndinium*, two well-characterized parasites of crustaceans. MALV III and MALV V
115 currently consist entirely of environmental sequences, but form well-supported clades

(Guillou *et al.* 2008). Finally, MALV I includes *Ichthyodinium* spp. as well as members that infect ciliates (Euduboscquellidae) (Coats 1988; Coats and Heisler 1989; Harada *et al.* 2007; Jung *et al.* 2016). A schematic representation of the position of MALV I relative to other members of Syndiniales and Dinophyceae is provided in Figure 1A.

Ichthyodinium chabelardi was originally described as a syndinid dinoflagellate by Hollande and Cachon (1952) with additional observations on its morphology and biology (Hollande and Cachon, 1953). Morphologically, *I. chabelardi* shares key features with other dinoflagellates. These include trichocysts, condensed chromatin in the nucleus of trophic stages, a dinospore (or zoospore) with two lateral flagella, and an alveolate pellicle synthesized inside alveolar membranes (Hollande and Cachon 1952; Gestal *et al.* 2006; Simdyanov *et al.* 2016).

The phylogenetic placement of *I. chabelardi* within the Alveolata superphylum remains controversial. Gestal *et al.* in 2006 using molecular methods placed this parasite at the base of the Perkinsozoa lineage, renaming the genus *Perkinsoide* (Gestal *et al.* 2006). However, analysis of the SSU and LSU regions show poor support for this change, and zoospore ultrastructure is more similar to other groups of dinoflagellates than the Perkinsozoa. As with recent authors (Mori *et al.* 2007; Skovgaard *et al.* 2009; Simdyanov *et al.* 2016), we retain the placement of *I. chabelardi* in the Syndiniophyceae until additional work is done on its taxonomic affinities.

Although only one species has been formally described thus far, genetic analysis of the SSU rDNA of the parasitic protists infecting a mix fish larvae of eight different species reveal slight differences in the sequences, which suggests that there could be at least two different ribotypes (98% of identity - 22 nt/ 868bp) in the sample (Sørensen *et al.* 2014).

These form separate clades within MALV I – 3 when aligned with other environmental SSU rDNA sequences (Figure 1B). These two ribotypes have been detected to date in distinct oceanic regions (Figure 2). These results provide the evidence that this group might be more complex than previously described and composed of genetically distinct parasites, as has been characterized for other syndinean parasites such as *Amoebophrya* spp. (Guillou *et al.* 2008).

Life cycle of *I. chabelardi*

Ichthyodinium chabelardi was originally described as an infectious agent of sardines from the Mediterranean Sea (Alger Bay) by Hollande and Cachon (1952), who made additional observations on its morphology and biology (Hollande and Cachon 1953). These descriptions reveal a complex life cycle that might differ depending on the parasite and/or host species, with infections in some hosts appearing to have two generations of schizonts while others have been observed with three (Figure 3). Subsequently, free-swimming zoospores are released which can then infect new hosts.

The first evidence of infection appears in the yolk sac of embryos after gastrulation (stages VI to XI, Dulčić 1998; Meneses *et al.* 2003). Infected eggs have no sign of penetration on their surface, suggesting that the parasite may pass through the hole for sperm penetration before the hole is completely closed (Yuasa *et al.* 2007).

Three successive stages of schizonts have been described by Hollande and Cachon (1953):

Primordial schizonts: The smallest stages of the parasite appear to be unicellular spheres (maximum 1 to 3 per egg) with a diameter of approximately 8-15 µm, less than 20 µm

(Hollande and Cachon 1952, 1953; Meneses *et al.* 2003; Yuasa *et al.* 2007; Sorensen *et al.* 2014). These trophocytes absorb the vitellus material of the host in a central vacuole and remain uni-nucleated, although mitotic divisions following by transversal divisions may occur at this stage. Then, the nucleus undergoes multiple mitotic divisions without subsequent cytoplasmic division and the primordial schizont rapidly becomes a large multinucleated structure of 100-140 μm . The cytoplasm forms cylindrical projections around each nucleus and each of these units become a secondary schizont (~20-30 μm) that eventually separates from the rest in a budlike manner.

Secondary schizonts: These are cylindrical or in a racket-like shape, about 20 μm in length (Hollande and Cachon 1953). Lecithin starts to concentrate in these forms. This early secondary schizont first grows, then begins to divide by longitudinal bipartitions. As the posterior poles of the two daughter cells remain attached, the parasites assume a triangular form, resembling to a 'rosace'. Secondary schizonts can then form a long cord (up to 1–2 mm) in layers of successive groups of eight cells connected by their poles (Hollande and Cachon 1953).

Last generation of schizonts: Oblong (cylindrical) schizonts are released from the cord, which become spherical after a series of divisions. At this phase, the yolk sac is opaque and entirely occupied by uni-nucleated parasitic cells. This last generation of schizonts produce zoosporangia, which are released outside the host, in the water, generally after hatching. The yolk sac breaks causing the death of the newly hatched larvae. In some larvae, the parasite burst occurs immediately after hatching, while it takes more than 10 h after hatching in others (Mori *et al.* 2007). The number of sporangia released from a yolk-sac larva of the yellowfin tuna *Thunnus albacares* has been estimated to be about 4×10^4 cells (Yuasa *et al.* 2007).

In the water, the sporangia undergo one to two divisions and become flagellated (Hollande and Cachon 1953). These spores have trichocysts (Gestal *et al.* 2006), which are known to be involved in the host attachment in other Syndiniales (Miller *et al.* 2012). Nuclei of released spores exhibit numerous nuclear pores and have chromatin masses, permanently condensed in circular or oval profiles of DNA. In some cases, structures like rhoptries and microtubule-organizing centres, similar to specific apicomplexan characteristics, have been observed at this developmental phase (Gestal *et al.* 2006). These structures may have similar function during the host invasion as the dense bodies and striated strips observed in *Amoebophrya* (Miller *et al.* 2012). Spores remain actively swimming for a few days, but cannot survive in a free-living state beyond that (Hollande and Cachon 1953). Spores of different sizes (Skovgaard *et al.* 2009) seem to correspond to different generations of division (Shadrin *et al.* 2015). The fate of the parasite outside of the host is not known at present.

Variations observed from this complex life cycle: The three generations of schizonts, first described on sardines from the Mediterranean Sea, were also observed in the mackerel off Portugal (Meneses *et al.* 2003). However, secondary schizonts seem to be absent on sardines from Portugal (Borges *et al.* 1996; Gestal *et al.* 2006) and several other host species, such as *Plectropomus leopardus* from Japan (Mori *et al.* 2007) and *Thunnus albacore* (Yuasa *et al.* 2007). In this case, primary schizonts lead directly to the last generation schizonts, without producing the rosace and cord. No zoosporangium was formed in *Plectropomus leopardus* from Japan (Mori *et al.* 2007); instead, schizonts themselves transformed into zoospores within 10 minutes after release.

Host range and geographical distribution

Parasitic species appear to be highly ubiquitous in the world's oceans. Known parasites comprised more than half of both the richness and abundance of sequences within the pico-nanoplankton size fraction collected in the TARA Oceans expedition, which sampled at 68 stations across the world's oceans (de Vargas *et al.* 2015). Within the TARA study, the Syndiniales featured prominently and over 2000 species-level OTUs were assigned to the MALV I clade (which includes *Ichthyodinium* spp.). Additionally, parasitic interactions were the most common type of interaction identified from network analysis of this global sequence dataset (Lima-Mendez *et al.* 2015).

There is evidence that parasitism by certain Syndiniales species could play a role in regulating the populations of their hosts. This has even been suggested as a means of controlling harmful algal blooms since many toxic dinoflagellates have been identified as Syndiniales hosts (Taylor 1968). In the Penzé estuary in France, Chambouvet *et al.* (2008) consistently found successive blooms and declines of four different dinoflagellate species, with prevalence of different Syndiniales species corresponding to or shortly lagging each of the four dinoflagellate blooms. It is clear that in certain cases Syndiniales species can heavily impact the dynamics of their host populations, and given the incredible diversity of hosts, their regulatory effects on a wide range of marine organisms, including fishes, are understudied.

Microscopic and molecular analysis of pelagic samples of ichthyoplankton have revealed the presence of *I. chabelardi* and relatives in the yolk sacs of fertilized eggs, embryos and larvae in various species of fish at widely distributed sites in the South China Sea (Shadrin *et al.* 2010 a and b), South Pacific (Yuasa *et al.* 2007) and in the North Eastern Atlantic and the Mediterranean (Dulčić 1998; Skovgaard *et al.* 2009). Up to now, *I. chabelardi* is reported to infect at least 13 different marine fish species including several commercially

important species such as *Maurolicus muelleri* (Guelin) and *Sparus aurata* L., *Trachurus trachurus* L., *Micromeesistius poutassou* (Risso) and *Engraulis encrasicolus* L. and *Scophthalmus morhua* L. (see Table 1 for more details on observations in certain host species, Figure 2 for geographical distribution, and the Aquasymbio website: <http://www.aquasymbio.fr/fr>).

The presence of this pathogen has not been reported elsewhere in the ocean in wild host fish species but has become a pest in some fish farms (Mori *et al.* 2007; Yuasa *et al.* 2007). No data has been collected for the prevalence of *I. chabelardi* at most other sites at which general surveys for dinoflagellates have been conducted. Most sampling sites where *I. chabelardi* has been detected have been in warm water. The only surveys in cold water were near Denmark (Sørensen *et al.* 2014). It is therefore clear that *I. chabelardi* and relatives are generalist parasites infecting many bony fish species and are widely distributed throughout the oceans. However, most of the studies thus far focus on few ecosystems and broader surveys are required today to establish the global distribution of these parasites.

Infectivity and ecological significance

The prevalence of *Ichthyodinium* parasites in ichthyoplankton samples has been investigated in various euphotic zones (North Atlantic, Mediterranean, South China and South Pacific waters). These shorter-term studies highlight a surprisingly high prevalence (Pedersen *et al.* 1994; Stratoudakis *et al.* 2000; Skovgaard *et al.* 2009). For example, field studies in the North Atlantic revealed infection prevalences of 37% in sardine eggs in 1999 and 46% in mackerel eggs in 2000 (Stratoudakis *et al.* 2000; Meneses *et al.* 2003).

In the South Pacific the prevalence was nearly 100% for yellow fin tuna in 2007 (Yuasa *et al.* 2007).

More recently, Shadrin *et al.* (2010b) confirm this high prevalence at Nha Trang Bay, Vietnam between 2001 and 2010 detecting *Ichthyodinium*-like parasite in representatives of eight families of fish. These long-term observations reveal also an increase of parasitic prevalence year after year leading to a substantial proportion of fish mortality at early developmental stages. Hence, infection prevalence was no more than 1% in 1993, but reached up to 98% in 2006. Similarly, a recent study on the leopard coral grouper fish in southern Japan reveal that parasitic prevalence attributed to *Ichthyodinium* sp. ranged in the same batch from 1.9% in 1990 to 31.8% in 1995 (Mori *et al.* 2007).

Shadrin *et al.* (2010a) have suggested that infection by *I. chabelardi* has been responsible for the significant disappearance of populations of fish species in warm water off the coast of Vietnam. The quantitative effect of infection by this parasite elsewhere awaits further long-term investigation. However, present data suggest that *I. chabelardi* is an emerging pathogen and is spreading into new marine ecosystems and possibly into freshwater ecosystems (Sørensen *et al.* 2014).

Taken all together, these findings data suggest that the impact of this parasite on fish host populations is still today underestimated and long-term investigation is urgently needed.

Other emerging eukaryotic parasites of fish

Ichthyodinium chabelardi is not alone in its role as an emerging threat to fisheries. Several other protists of differing phylogenetic origins, and different life cycles and modes of

infection, also present a challenge for fishery management. Here we briefly describe the mechanisms and effects of five other eukaryotic parasites.

Amyloodium ocellatum (Class Dinophyceae, Order Peridiniales) is known to infect over 100 species of fin fish worldwide causing marine velvet disease or amyloodiniosis (Landsberg *et al.* 1994, see also the Aquasymbio website: <http://www.aquasymbio.fr/fr>). *A. ocellatum* is a virulent euryhaline ectobiotic parasite that tolerates varying osmotic conditions and temperatures and is commonly observed in warm and temperate oceanic environments including coral reefs and especially in crowded fish pens and aquaria (Noga 1989; Levy *et al.* 2007). This species can grow at temperatures of up to 40 °C (Kuperman and Matey 1999). The details of its life cycle have been worked out, and there do appear to be seasonal, temperature-related dynamics of infection prevalence (Kuperman and Matey 1999). Its free-swimming dinospore stage attaches to the gills and skin of hatched fish (Kuperman and Matey 1999) rather than the eggs as does *I. chabelardi*.

Saprolegnia parasitica (Phylum Oomycota) and *Sphaerothecum destruens* (Phylum Mesomycetozoa) are known to be significant emerging parasites and are very serious threats for production in populations of both wild and farmed fin fish in freshwater ecosystems (van West 2006; Rowley *et al.* 2013; Gozlan *et al.* 2014; Sarowar *et al.* 2014). Both species have been spread widely by resistant carriers into new ecosystems where these pathogens have formerly never been observed.

Several pathotypes of *Saprolegnia* can act as opportunistic and aggressive parasites of egg, larval and adult stages of many economically important species of fish such as salmon, trout and catfish, and are responsible for significant economic losses in salmon hatcheries. Thoen *et al.* (2015) found many different ITS-based genotypes among 89

isolates of *Saprolegnia* recovered from water samples and egg, larval and adult salmon tissues from 26 salmon hatcheries along the coast of Norway. A limited number of species of *Saprolegnia* (four species) were found in tissues of salmon: *diclina*, *ferax*, *hypogyra* and *parasitica*. *S. diclina* (sub-clade IIIB) clearly dominated and accounted for 79% of the recovered species. These data indicate considerable genetic variation within the populations of *Saprolegnia* pathogens, but the relationship to pathogenicity is not known.

Sphaerothecum destruens was spread from China to Europe by a healthy freshwater carrier species, the top-mouth gudgeon (*Pseudorasbora parva*) (Gozlan, 2014). Recently the rate of spread within parts of Europe has been estimated to be very rapid (Ercan *et al.* 2015). This pathogen is a generalist, can infect a wide range of hosts and can cause high mortalities, especially in commercially important fish species such as Chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*).

The “X-cell” clades *Gadixcellia* and *Xcellia* (Superphylum Alveolata) are parasites associated with tumor-like formations in teleost fishes, including several groups of commercial interest (Freeman *et al.* 2017). X-cells were originally believed to be abnormal, diseased host cells, but cells extracted from lesions in the gills and skin of various fishes reveal them to be a related group of parasitic protists. The phylogeny of these parasites is newly characterized and shows an unexpected level of divergence between the two robust x-cell clades within the Phylum Perkinsozoa (Alveolata). Both clades are basal to the Perkinsids. Fishes with x-cell infections have been observed in the Atlantic, Pacific, and Southern oceans (Evans and Tupmongkol 2014; Freeman *et al.* 2017), but the parasites have not yet been observed, nor their sequences detected, in seawater independently of their hosts. In the common dab, diseased fish had difficulty with respiration and much lower survival than healthy fish (Diamant and Vicar 1987).

It is clear that *Ichthyodinium chabelardi* is not the only infectious disease threatening the future survival of important fisheries. Many diseases have been described. Moreover, eukaryotic parasites have been under-studied and neglected so far despite their major role in fish disease. Some methods have been proposed to control the spread of these parasites in controlled environments including reduced salt concentration and various chemicals, though some of these (like malachite green) have been banned due to their toxicity to humans (van West 2006). Further knowledge of each of these parasites, especially their early detection and predicted responses to changing ocean conditions, will be of crucial importance to the future of fish production.

Future perspectives and conclusions

There are plans in the future to build new hatcheries for production of marine fin fish species such as cod along the coasts where appropriate. Countries such as Norway, Scotland, Canada and southern Australia currently have only salmon hatcheries filled with cold freshwater. These facilities could be easily modified for marine fish using cold seawater. However, a dramatic increase of disease caused by *Saprolegnia* in freshwater fish tanks has already been observed (van West 2006). This appears to be a result of two factors: the mean temperature of the ocean is predicted to increase significantly with global warming, and furthermore the use of malachite green in the tanks to prevent the growth of parasites has been banned internationally because of its toxicity to humans (van West 2006).

It is often concluded that geographical range and prevalence of emerging infectious diseases has increased tremendously over the last 20 years (Harvell 1999, 2002; Sutherst

2001). Climate change, habitat destruction, and introduction of exotic species (hosts and parasites) have been blamed for this increase. Actually, it has recently been observed that the combination of introduction of diseased fish with the rise of seawater temperature has significantly increased the prevalence of diseases of fin fish in general (van West 2006; Gozlan 2014; Ercan *et al.* 2015). We therefore predict that the ocean temperature increase will provide more favourable environmental conditions for parasite growth and will inexorably lead to geographical expansion of infectious agent populations like *I. chabelardi* and other protozoan parasites. This movement could impact population dynamics and may drive a decrease of or even cause extinction of commercially important fish population stock. It is not known whether fish could have a designated area to provide protection from infection by this parasite, but cold water in the oceans near the poles could be a reservoir if there is sufficient oxygen present.

It is essential that the fishing industry effectively monitor fish tanks and water in the surrounding environments for the presence of parasites and takes steps to prevent large losses in fish production. At present efforts to measure the prevalence of these parasites appear to be inadequate. Molecular methods may provide tools for early detection (for example, PCR of environmental samples using *Ichthyodinium*-specific primers), but further research is required to develop new procedures for monitoring and controlling infections. Mori *et al.* (2007) were able to prevent horizontal transfer of infection by using UV-sterilized or ozone-treated seawater. Methods such as these might warrant further exploration for fish tank settings.

Pathogenic species such as *I. chabelardi* and the others discussed in this paper infect a wide variety of host species that may favor their spread. Generalist parasites have been responsible for huge losses in populations of plants and animals in agriculture,

aquaculture and the environment (Phillips *et al.* 2008). All of the species discussed in this review are examples of emerging pathogens and equally important to the economy of the aquaculture industry (Stentiford *et al.* 2012) in a world in which food production must be increased to keep up with human population growth. They are all spread easily and rapidly by highly infective free-living stage the zoospores from infected to uninfected susceptible host species of fish and could cause epidemics especially in highly dense host populations such as in fish farms.

Figure Legends

Figure 1. (A) Generalized schematic of Dinophyceae phylogeny with designation of the five major clades within Syndiniales. MALV I, to which *Ichthyodinium* spp. belong, is highlighted. Adapted from Guillou *et al.* 2008. (B) Partial SSU rDNA phylogeny (Maximum Likelihood) of *Ichthyodinium* spp. together with closely related environmental sequences (MALV I clade 3, following the nomenclature by Guillou *et al.* 2008), showing the two clusters described within the genus. Environmental sequences are labeled by their GenBank accession number following by the name of the clone. Sequences were retrieved from GenBank using BLAST and aligned with referenced *Ichthyodinium* spp. sequences using the online version of MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>), and considering the secondary structure of RNA. RAxML (Randomized Axelerated Maximum Likelihood) was performed using the GTRCAT substitution model, using a random number seeds, and 100 replicates for the bootstrap analyses.

Figure 2. Known global distributions and hosts of the two established ribotypes of *Ichthyodinium* sp.

Figure 3. Life cycle of *I. chabelardi* as observed in mackerel eggs (from Meneses et al. 2003).

Table 1. Some examples of pathotypes of *Ichthyodinium chabelardi* reported as parasites of fish species. Recently identities have been confirmed by both light microscopic studies and molecular methods.

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Conflict of Interest

The authors declare no conflict of interest.

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