Vol. 58: 261–264, 2004 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published March 10

NOTE

First record of the microsporidian parasite *Steinhausia mytilovum* in *Mytilus* sp. (Bivalvia: Mytilidae) from France

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ABSTRACT: *Steinhausia mytilovum* is a globally distributed microsporidian parasite which infects the oocytes of the blue mussels *Mytilus edulis* and *M. galloprovincialis*. Despite the intensive monitoring effort made on mussel populations, the parasite has not previously been reported in France. We report herein on the occurrence of *S. mytilovum* in *Mytilus* sp. from 1 cultured and 2 natural populations on the northern coast of France, thus extending the parasite's known distribution northwards. We also report on the observation in 1989 of *S. mytilovum* in *M. galloprovincialis* from the Golfe de Fos area in the Mediterranean Sea (South of France). *S. mytilovum* was observed in the European hybrid zone between *M. edulis* and *M. galloprovincialis*, which therefore renders the exact taxonomic status of the infected hosts unknown. The prevalence of the parasite was low, which suggests that its effect on mussel populations was probably limited.

KEY WORDS: *Mytilus edulis* · *Mytilus galloprovincialis* · *Steinhausia mytilovum* · Microsporidia · Parasite · Aquaculture

INTRODUCTION

Microsporidia are obligate intracellular parasites infecting many animal groups, including marine molluscs (Bower et al. 1994, Wittner & Weiss 1999). In bivalves, microsporidians have been recorded from several tissues of numerous species (Comtet et al. 2003). In particular, a few microsporidian species belonging to the genus *Steinhausia* have been described as infecting the oocytes of several bivalve species: *S. ovicola* in the European flat oyster *Ostrea edulis* (see Léger & Hollande 1917), *S. mytilovum* in the mytilid mussels *Mytilus edulis* and *M. galloprovincialis* (see Field 1921–1922, de Vincentiis & Renzoni 1963, Sprague 1965, Figueras et al. 1991), and *Steinhausia* spp. in the Sydney rock oyster *Saccostrea commercialis* (see Anderson et al. 1995), the tellinid clam *Macoma balthica* (see Farley 1977), the clam *Venerupis pullastra* (see Villalba et al. 1993) and the common cockle *Cerastoderma edule* (see Carballal et al. 2001, Comtet et al. 2003).

Steinhausia mytilovum (formerly *Haplosporidium mytilovum* and *Chytridiopsis mytilovum*) has a wide geographical range, occurring in both natural and cultured populations of *Mytilus edulis* and *M. galloprovincialis* from the Atlantic and Pacific coasts of the USA, Europe and Asia (Table 1). However, despite the intense survey of cultured mussel populations along the French coasts, *S. mytilovum* has never before been reported in France.

In this paper, we report for the first time the occurrence of *Steinhausia mytilovum* in French production areas and natural mussel populations.

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Table 1. *Steinhausia mytilovum*. Known distribution of the parasite in cultured (C) and natural (N) populations of *Mytilus* species

Host species	Location	Type	Sample size	Prevalence (%)	Source
M. edulis	Northeast coast of USA	–	–	–	Field (1921–1922)
	Maryland, northeast coast of USA	N	30 (females)	43[a,b]	Sprague (1965)
	East coast of USA, south of Cape Cod, MA	N	–	–	Farley (1988)
M. galloprovincialis	Marina del Rey, California, west coast of USA	N	50	5–10[c]	Hillman (1991)
	Gulf of Naples, west coast of Italy	–	–	10[c]	de Vincentiis & Renzoni (1963)
	Lake Fusaro, Bay of Bacoli, west coast of Italy	C	133	0–15[c]	Ceschia (pers. comm. 2003)
	Ria de Arosa, Ria de Ares-Betanzos, NW Spain	–	–	–	Gonzales et al. (1987)
	Ria de Arosa, NW Spain	C	330	7.5–12.5[a]	Figueras et al. (1991)
	Ria de Vigo, NW Spain	C	–	3.3–19.5[d]	Robledo et al. (1994)
	Rias of Galicia, NW Spain	C	2430	Up to 28.3[a]	Villalba et al. (1997)
	Mediterranean coast of Spain	N	174	17.6–50[a]	Sagristà et al. (1998)
	Thermaikos Gulf, North Aegean Sea, Greece	C	127 (females)	7.5–35.5[a]	Rayyan & Chintiroglou (2004)
	Northwest Black Sea	N/C	About 3000	Up to 76[a]	Rybakov & Kholodkovskaya (1987)
Mytilus sp.	Korea and Japan	–	–	–	Hine (1999)

[a]Calculated in relation to the number of females
[b]Observations made from fresh smears
[c]Calculated in relation to the total number of individuals
[d]Mode of calculation (total number of individuals vs females only) not known

MATERIALS AND METHODS

The present work was carried out within the framework of the French REPAMO network (IFREMER), which aims at surveying pathogens in harvested and cultured populations of molluscs from French production areas. As part of the sampling strategy of this survey program, samples of 30 living mussels were collected from 3 natural or cultured populations (see Fig. 1): *Mytilus edulis* specimens were sampled from a natural harvested subtidal population at Barfleur on 4 July 2000 (40 m depth), natural *M. galloprovincialis* were collected intertidally in the Baie de Saint-Brieuc (Le Rocher Martin, Plérin) on 13 June 2000, and cultured *M. galloprovincialis* were collected in Utah Beach (Normandy) on 2 May 2001. The latter originated from a 1996 spat collection in the Mediterranean Sea and were transferred to Utah Beach in 1997.

To evaluate their histopathological state, mussels were dissected and tissues fixed for 24 h in Davidson's fixative (Shaw & Battle 1957). They were dehydrated and embedded in paraffin. Sections of 2 to 3 µm thickness were cut, stained with hematoxylin-eosin and examined with light microscopy.

Due to its location in the oocytes, *Steinhausia mytilovum* infects only females or hermaphrodites. Thus, its prevalence was calculated only from female and hermaphrodite data (only 1 hermaphrodite was observed at Utah Beach) as previously done by Villalba et al. (1997) for *S. mytilovum* in *Mytilus galloprovincialis*. Neither males nor immature individuals were considered.

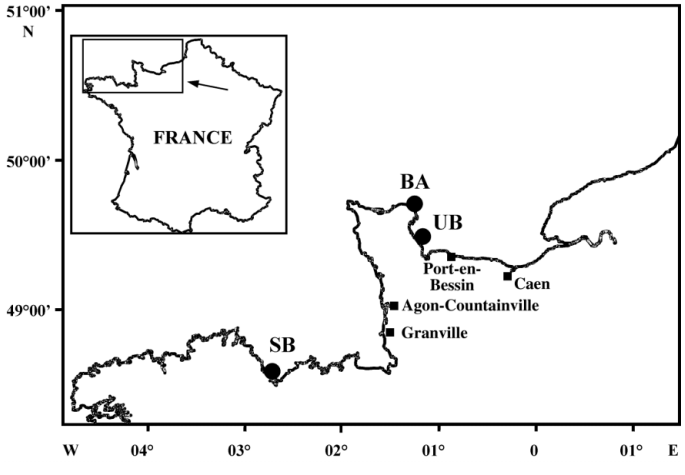

Fig. 1. Location of the sampling sites: Baie de Saint-Brieuc (SB), Barfleur (BA) and Utah Beach (UB)

RESULTS AND DISCUSSION

Four mussels were infected by *Steinhausia mytilovum* in the 3 populations (Table 2). The parasite appeared as cysts containing many spores and delimited by a thin membrane (Fig. 2). The cysts were mostly located in the oocyte cytoplasm, but a few were also observed in the nucleus. Most of the infected oocytes contained a single cyst, but multiple infections with 2 parasites in the cytoplasm were observed in a few cases. Prevalences were between 5.9 and 18.2% (Table 2) and were in the range of most of those previously reported (Table 1), although prevalences of up to 76% of the females examined were recorded in *M. galloprovincialis* from the northwest Black Sea (Rybakov & Kholodkovskaya 1987).

Table 2. *Steinhausia mytilovum*. Prevalence in female (and hermaphrodite) *Mytilus edulis* and *Mytilus galloprovincialis* from 3 French populations

Species	Location	Origin	Number of females[a]	Number of infected females	Prevalence (%)
M. edulis	Barfleur	Local recruitment	11	2	18.2
M. galloprovincialis	Saint-Brieuc	Local recruitment	17	1	5.9
M. galloprovincialis	Utah Beach	Mediterranean Sea	15	1	6.7

[a]Hermaphrodites included

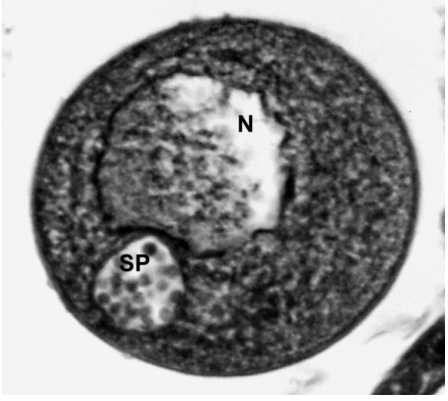

Fig. 2. *Steinhausia mytilovum* in *Mytilus edulis* from Barfleur. Histological section in gonadal tissue stained with hematoxylin-eosin (750×), showing a sporocyst (SP) located close to the host cell nucleus (N)

To our knowledge, this is the first published record of *Steinhausia mytilovum* in *Mytilus* spp. in France, and has increased the known geographic distribution of this parasite to the north. However, *S. mytilovum* was found in 1989 in *M. galloprovincialis* from Carteau (Golfe de Fos-sur-Mer, Mediterranean coast of France; Y. Pichot pers. comm.). Careful examination of additional *M. edulis* collected in the Barfleur population in 1999 and 2001 (6 and 16 females, respectively) did not reveal *S. mytilovum*. Moreover, despite the intensive monitoring effort made on mussel populations in France, within the national REPAMO network (between 1990 and 2001, 5041 mussels were analysed in France, including 2962 from the northern coast of France), *S. mytilovum* was never observed in samples other than those in the present study. This suggests that *S. mytilovum* has only recently been introduced to the northern coast of France. However, both its low prevalence (Table 2) and its overall low intensity of infection (a few oocytes per slide were infected, with the exception of the female from the Baie de Saint-Brieuc with more than 50 infected oocytes on a single slide) could also explain why it has only recently been detected. Several factors such as climate warming (Cook et al. 1998, Harvell et al. 2002) and transfer of infected hosts related to aquacultural practices (e.g. Naylor et al. 2001) are known to favour the geographic range extension of parasites, but our data did not allow us to propose such hypotheses in the case of *S. mytilovum*.

Steinhausia mytilovum was found in the European mosaic hybrid zone between *Mytilus edulis* and *M. galloprovincialis* (see Coustau et al. 1991, Daguin et al. 2001, Bierne et al. 2003), which therefore renders the exact taxonomic status of the infected individuals unknown. Thus, if mussels cultured at Utah Beach are *M. galloprovincialis* (originating from the Mediterranean Sea), natural mussel populations from Barfleur and Baie de Saint-Brieuc could in fact be the result of a mixing of *M. edulis*, *M. galloprovincialis* and hybrids. Indeed, in a natural population of the Baie de Saint-Brieuc (exact location unknown), *M. galloprovincialis* and *M. edulis* co-occur, and hybridisation has been shown to be partial (Coustau et al. 1991). Conversely, the Barfleur mussels are most likely *M. edulis*, as *M. edulis*-like genotypes are highly dominant in populations surrounding Barfleur, i.e. Granville, Agon-Coutainville, Port-en-Bessin and Caen (Fig. 1) (Coustau et al. 1991, Bierne et al. 2003).

The effect of *Steinhausia mytilovum* on its host is still unclear. A strong haemocytic host response has been reported in *Mytilus galloprovincialis* (see González et al. 1987, Bower & Figueras 1989, Figueras et al. 1991, Villalba et al. 1997, Sagristà et al. 1998), but no conclusive evidence has been reported regarding the viability of the infected oocytes. However, due to its location in the oocytes, numerous authors considered that *S. mytilovum* may affect the reproduction of their host (González et al. 1987, Bower & Figueras 1989, Figueras et al. 1991, Robledo et al. 1994, Villalba et al. 1997). In the present study, the prevalence of *S. mytilovum* was low and no haemocyte infiltration was observed, even in the case of high infection intensity (i.e. the female from Baie de Saint-Brieuc), which suggests a limited effect on mussel reproduction at the population level. Further monitoring of the mussel populations along the French coasts is needed in order to follow the evolution of the *S. mytilovum* extension and to better assess its potential impact on both the natural and cultured *Mytilus* sp. populations.

Acknowledgements. We are indebted to the staff of the Laboratoire côtier de Port-en-Bessin and Laboratoire côtier de Saint-Malo (IFREMER) for collecting samples. We acknowledge the coordinator of the REPAMO program, A. Thébault (IFREMER, Station de La Tremblade), for her assistance, Y. Pichot (IFREMER, Station de Sète) for reporting to us the occurrence of *Steinhausia mytilovum* in *Mytilus galloprovincialis* in the Golfe de Fos-sur-Mer and M.-A. Lekkas for improving the English of the text. We thank G. Ceschia (Istituto Zooprofilattico Sperimentale Delle Venezie), who provided us with unpublished data. Three anonymous reviewers provided helpful comments on the manuscript. This work was supported by IFREMER and CNRS.

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Editorial responsibility: Albert Sparks, Seattle, Washington, USA

Submitted: May 6, 2003; Accepted: November 25, 2003
Proofs received from author(s): February 19, 2004