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First report of a *Mikrocytos*-like parasite in European oysters *Ostrea edulis* from Canada after transport and quarantine in France

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ABSTRACT: As part of a disease resistance experiment, 112 apparently healthy European flat oysters *Ostrea edulis* L. were exported from Canada (Nova Scotia) into France to test their susceptibility to *Bonamia ostreae* infection. Twelve oysters died in transit and 17 others died within 2 wk of laboratory quarantine acclimation. All oysters were examined histologically, and the 17 that died during quarantine were assayed for microcells (*Bonamia* sp. and *Mikrocytos mackini*) using molecular techniques. A microcell parasite was detected in the connective tissue of 5 of the 112 oysters. Morphological appearance, tissue affinity and molecular characterization through PCR, *in situ* hybridization (ISH), fluorescence *in situ* hybridization (FISH) and sequencing revealed a protist related to *M. mackini*. This is the first report of a parasite of the genus *Mikrocytos* in a species belonging to the genus *Ostrea* from the Atlantic Ocean.

KEY WORDS: *Mikrocytos* · *Ostrea edulis* · *In situ* hybridization · PCR · Histology

INTRODUCTION

After the discovery of *Bonamia ostreae* in France (Pichot et al. 1980), in other European countries (Bannister 1982, Polanco 1984, Hudson & Hill 1991, McArdle et al. 1991, van Banning 1991, Cigarria 1997) and subsequent detection and retrospective recognition of this oyster pathogen in the US (Katkansky et al. 1969, Elston et al. 1986, Barber & Davis 1994), different tests of resistance to infection have been conducted with oysters collected from different geographical areas (Elston et al. 1986, Culloty et al. 2001, Montes 1991). As part of this research, flat oysters *Ostrea edulis* from Nova Scotia, Canada, were exported to France in June 2001. After careful histological examination of 60 specimens to check for the absence of lesions and/or pathogens, 112 adult oysters were exported to La Tremblade (France) and were maintained in strict quarantine at the IFREMER laboratory. To date, neither bonamiosis nor mikrocytosis have been reported in *O. edulis* from the east coast of Canada (OIE 2005a), whereas *Mikrocytos mackini* occurs on the southwest coast of Canada (Bower et al. 1997) and *Bonamia ostreae* was detected at one site on the west coast of Canada for the first time in 2004 (OIE 2005b). However, transplantation of oysters between the east and west coasts of Canada is not permitted.

Twelve oysters died in transit to La Tremblade, and within 2 wk after arrival an additional 17 oysters died in quarantine. Heart smears revealed the presence of a small protozoan parasite in 2 specimens that died in quarantine. The surviving oysters were then examined for this parasite. This paper is the first report of the presence of a microcell parasite in *Ostrea edulis* originating from Nova Scotia. Furthermore, it is the first report of a *Mikrocytos*-like infection in *Ostrea* oyster species in the Atlantic Ocean.

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MATERIALS AND METHODS

Oysters. The oysters from Nova Scotia were produced in a hatchery and transferred to open water after 4 mo. They were held in suspended culture for the duration of grow out and were ca. 3 yr of age when shipped to France (A. Mallet, pers. comm.). In April 2001, 60 adult *Ostrea edulis* were collected from this population and histological examination of tissue cross-sections and heart smears to check for the absence of lesions and/or pathogens was done at the Shellfish Health Laboratory of the Fisheries and Oceans Canada, Gulf Fisheries Center (DFO-GFC), Moncton, NB, Canada. Subsequently, 112 cohorts from the same site were shipped in June 2001 and were received after 6 d of transit and maintained in quarantine at the IFREMER laboratory, La Tremblade, France. The quarantine system used chlorine treatment for effluent water, but the incoming water was not treated. Temperature of the water was 14°C.

Following the detection of a presumptive *Mikrocytos* sp. in the oysters at IFREMER, 135 American oysters *Crassostrea virginica* and 275 *Ostrea edulis* were collected from several beds in Nova Scotia in late 2001 and in 2002. Of these, 3 samples totaling 189 *O. edulis* (46%) came from the initial site of sampling (Table 1). A total of 104 *O. edulis* from this site were examined by histology for pathogens and all of the oysters were examined by PCR for the presence of *Mikrocytos* sp. and *Bonamia ostreae* at DFO-GFC.

Histology. Oysters were opened and a standard section through the digestive gland, gills, labial palps and mantle was fixed in Davidson's fixative (Moore et al. 1953), paraffin embedded and sections (5 µm thick) stained with haematoxylin and eosin (H&E). A complete examination of the cross-section was performed at 100× magnification with further examination of any unusual pathology and specifically for the presence of microcells at 1000× magnification (OIE 2003).

Table 1. *Crassostrea virginica* and *Ostrea edulis.* Date of sacrifice of oysters examined for the presence of microcells. Oysters were collected from different sites in Nova Scotia

Date of sacrifice	No. of ind.	Species
November 2001	10	*C. virginica*
December 2001	10	*C. virginica*[a]
December 2001	150	*O. edulis*[b]
January 2002	40	*C. virginica*[a]
February 2002	30	*O. edulis*
April 2002	13	*O. edulis*
May 2002	3	*O. edulis*
May 2002	5	*O. edulis*[b]
June 2002	34	*O. edulis*[b]
June 2002	40	*O. edulis*
June 2002	75	*C. virginica*[a]

[a]Wild oysters were collected and held in quarantine for up to 6 yr before being sacrificed at the specified dates. All other oysters were sacrificed within 2 d of collection

[b]Oysters collected from the facility that provided *O. edulis* to France in June 2001

Heart smears. Ventricles of the oysters screened in Canada prior to shipment and those received in France were removed, blotted on tissue paper and then dabbed on a microscope slide. Cells were air-dried, fixed in methanol and stained with Hemacolor (Merck) rapid blood stain. Each slide was examined microscopically for 10 min at 1000× magnification.

DNA extraction. Genomic DNA was extracted from ca. 150 mg of combined muscle, digestive gland and heart tissue of 2 infected oysters in France. Tissues were suspended in 10 volumes of extraction buffer (NaCl 100 mM, EDTA 25 mM pH 8, sodium dodecyl sulphate [SDS] 0.5%) with Proteinase K (100 µg ml^{-1}). Following an overnight incubation at 50°C, DNA was extracted using a standard phenol/chloroform protocol followed by ethanol precipitation (Sambrook et al. 1989).

Genomic DNA was extracted from oysters subsequently collected in Canada by a similar method except that tissue samples were incubated in ca. 300 µg ml^{-1} of Proteinase K at 55°C overnight, followed by inactivation at 95°C for 10 min before standard phenol/chloroform/isopropanol and ethanol precipitation of the DNA. DNA was diluted if necessary to concentrations of 1 mg $µl^{-1}$. A selected set of samples were extracted a second time with a QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions.

PCR. PCR assays on the oysters examined after quarantine were done initially with primer pair Bo (Cochennec et al. 2000, Hine et al. 2001) and specific *Mikrocytos* genus primer pair MM (Carnegie et al. 2003). Subsequently, putative specific *Mikrocytos*-like primer pair MM-like and *M. mackini* primer pair MM-mack were used (Table 2). Reactions were performed in a 50 µl volume containing about 10 ng of DNA, 5 µl of 10× PCR buffer, 5 µl of 25 mM $MgCl_2$, 5 µl of 2 mM dNTP, 0.5 µl of each 100 µM forward and reverse primers and 1 unit of Taq DNA polymerase (Promega). Samples were denatured for 5 min at 94°C and cycled 30 times at 94°C for 1 min, 60.5°C (55°C for primer pair Bo) for 1 min, and 72°C for 1 min, followed by an extension of 10 min at 72°C and hold at 20°C. Amplified DNA (5 µl) was analyzed by electrophoresis in ethidium bromide stained 2% agarose gel. Positive control DNA from oysters infected respectively with *Bonamia ostreae* (provided by N. Cochennec, IFREMER) and *M. mackini* (provided by S. Bower, Department of Fisheries-Pacific Biological Station, DFO-PBS) was included in respective assays.

Table 2. Primer sequences (5′-3′), specificity and predicted amplicon sizes of PCR products

Primer pair	Primers sequence (forward and reverse)	Specificity	Product size (bp)	Source
Bo	Bo: CATTTAATTGGTCGGGCCGC Boas: CTGATCGTCTTCGATCCCCC	*Bonamia* sp.	300	Cochennec et al. (2000)
MM	Mikrocytos-F: AGATGGTTAATGAGCCTCC Mikrocytos-R: GCGAGGTGCCACAAGGC	*Mikrocytos* sp.	546–523[a]	Carnegie et al. (2003)
MM-like	Mmlike: CCTGTCCTATGTCGGGCAGG Mikrocytos-R: *idem*	*Mikrocytos*-like	222	Present study
MM-mack	Mmack: CATTGGAGGAGTCAGAGGGTG Mikrocytos-R: *idem*	*M. mackini*	268	Present study
Msp	Msp443F: ATACCTAAGCGTTACTGCGTTT Msp746R: TCGCCACTGGTAGTCCTGTTT	*Mikrocytos* sp.	305–281[a]	Present study
Msp-like	MmX-308F: TATGTCGGGCAGGGCAGCAA Msp842R: AGCAAAGTTTGTGGTTGGGAC	*Mikrocytos*- like	193	Present study

[a]Product size is shorter with putative *Mikrocytos*-like DNA

PCR assays on the oysters collected subsequently in Canada for pathogen screening were done with primer pair Msp, and occasionally additional primer pairs MM and Msp-like were used (Table 2). Reactions of 20 µl included up to 1 µg of DNA, 0.5 µM of each primer, 8 µl of 2.5× Eppendorf MasterMix® (Brinkmann), 2.75 mM of $MgCl_2$ and 5% DMSO. PCR conditions were 2 min at 94°C, 10 cycles of touchdown PCR (94°C for 40 s, 60°C – 1°C cycle^{-1} for 40 s, 72°C for 1 min) and 35 cycles at the lower annealing temperature (50°C), followed by a final extension at 72°C for 7 min and a hold at 20°C. Electrophoresis was performed either in 1.5% agarose gels, or 10% acrylamide gels, using 10 µl of PCR product.

DNA sequencing. Two different PCR products from 2 infected oysters were cloned using the TA Cloning kit (Invitrogen). A recombinant plasmid from each PCR product with putative small subunit ribosomal DNA (SSU rDNA) inserts was sequenced at Appligene (Laboratory Qbiogen). A basic local alignment search tool (BLAST) similarity search (Altschul et al. 1990) for significant homologous sequences was done against GenBank® (National Center for Biotechnology Information) using the sequence obtained. Multiple sequence alignments were performed using ClustalW (Thompson et al. 1997).

***In situ* hybridization (ISH).** Putative specific primer pair MM-like (Table 2) was used to produce a digoxigenin-labeled probe by PCR at the IFREMER laboratory. PCR-probe amplification was performed as above except that 2.5 µl of DIG dUTP (25 mM) was added to the mix. A positive control probe targeting eukaryotic SSU rDNA was also used (Le Roux et al. 1999). This putative *Mikrocytos*-like probe was tested by ISH on paraffin-embedded tissue sections following Cochennec et al. (2000). Briefly, 5 µm thick sections on aminoalkylsilane-coated slides were deparaffinized and rehydrated. Proteinase K (100 µg ml^{-1}) in Tris-EDTA buffer was applied for 30 min at 37°C, and sections were dehydrated by immersion in an ethanol series and air dried. Sections were incubated with 100 µl of hybridization buffer (4× SSC, 50% formamide, 1× Denhardt's solution, 250 µg ml^{-1} yeast transfer RNA (tRNA), 10% dextran sulfate) containing 20 ng of the digoxigenin-labeled probe. Sections were covered with *in situ* plastic coverslips and placed on a heating block at 95°C for 5 min, then cooled on ice and left overnight at 42°C in a humid chamber. Sections were washed twice for 5 min in 2× saline sodium citrate (SSC) at room temperature and once for 10 min in 0.4× SSC at 42°C. Detection was done with the digoxigenin (DIG) nucleic acid detection kit (Boehringer Mannheim) according to manufacturer's instructions. In addition to the *Mikrocytos*-like infected and uninfected *Ostrea edulis*, the specificity of the DIG-ISH probe was tested on tissue sections from *Bonamia ostreae*-infected *O. edulis* (provided by N. Cochennec, IFREMER), *M. mackini* experimentally infected *O. edulis* and *Crassostrea gigas* (provided by S. Bower, DFO-PBS).

A slightly different ISH method was developed at DFO–GFC for the detection of *Mikrocytos* sp. in tissue sections. The PCR probe was prepared in a similar manner, using a PCR DIG probe synthesis kit (Boehringer Mannheim), and primer pair Msp, according to manufacturer's instructions. Tissue sections were deparaffinized, rehydrated and equilibrated in phosphate buffered saline (PBS). Proteinase K was applied as described above for 10 min only and digestion was stopped with a PBS-glycine 0.2% bath. Sections were equilibrated in 2× SSC and prehybridization solution (idem as above except that 0.5 mg ml^{-1} sheared salmon sperm DNA was used instead of yeast tRNA) was applied for 1 h at 42°C in a humid chamber.

Prehybridization solution was drained and replaced with 25 µl of fresh solution containing 5 ng μl^{-1} of probe. Sections were coverslipped and placed on a heating block at 88°C for 7 min, cooled on ice and left overnight at 42°C in a humid chamber. Sections were washed twice in 2× SSC and 1× SSC for 5 min at room temperature, then twice in 0.5× SSC at 42°C. Detection was done similarly as above, using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) as substrate and Bismarck brown yellow 0.5% for counterstaining.

Another DIG ISH protocol was done at DFO-PBS using procedures described by Meyer et al. (2005). In this case, tissue sections from 3 of the flat oysters from the IFREMER quarantine facility were assayed.

Fluorescence in situ hybridization (FISH). FISH assays were done at DFO-PBS on 3 of the flat oysters from the IFREMER quarantine facility using procedures described by Carnegie et al. (2003).

RESULTS

Heart smear examinations and histology

Heart smear examinations of 17 oysters that died during the first 2 wk in quarantine revealed the presence of a few (<10) spherical basophilic organisms in 2 of the *Ostrea edulis*. An additional 3 infected oysters were found among the remaining 83 oysters sacrificed after 25 d in quarantine. Parasites were 3 to 5 µm in diameter, with an eccentric large nucleus and were located outside hemocytes (Fig. 1).

Histology slides sent to the Office International des Epizooties (OIE) reference laboratory for mikrocytosis (S. Bower, DFO-PBS) confirmed the presence of several small focal areas in vesicular connective tissue showing intracellular microcells, undistinguishable from *Mikrocytos mackini* (Fig. 2). However, it was noted that the host response was diffuse, unlike the intense focal haemocyte infiltration that is typically observed in natural mikrocytosis infections in *Crassostrea gigas,* and the microcells tended to locate near the periphery of the host cells unlike *M. mackini*, which tends to occur throughout the cytoplasm of vesicular connective tissue cells (S. Bower pers. comm.).

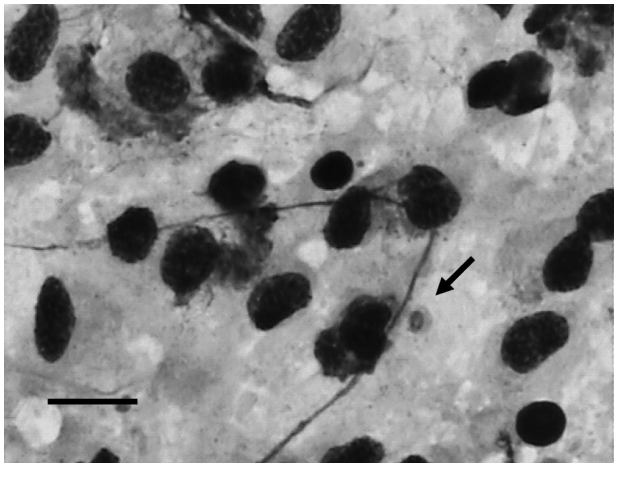

Fig. 1. *Ostrea edulis*. *Oysters* exported from Atlantic Canada and held in quarantine in France. Ventricular heart imprint, Hemacolor stain. Extracellular microcell (arrow). Scale bar = 10 µm

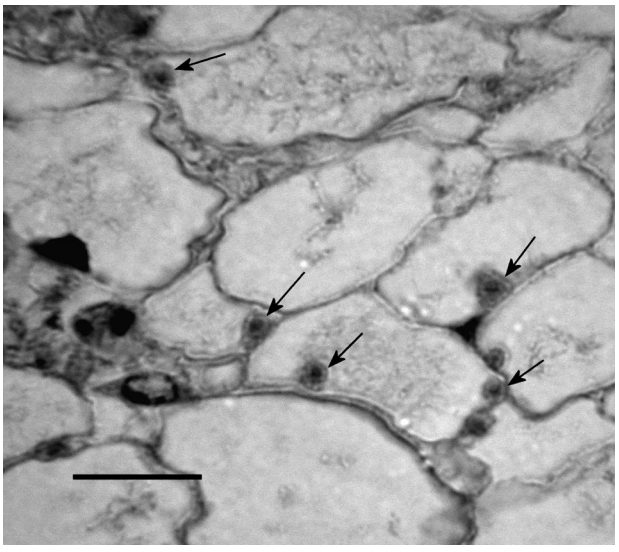

Fig. 2. *Mikrocytos*-like parasites infecting *Ostrea edulis.* Parasites (arrows) within vesicular connective tissue cells between gonad follicles of oysters exported from Atlantic Canada and held in quarantine in France. Haematoxylin and eosin stain. Scale bar = 10 µm

PCR amplifications and sequencing

Total DNA in tissue samples from 2 of the 5 infected oysters could not be amplified by PCR using primer pair Bo (Table 2) that was designed to amplify *Bonamia* spp. With *Mikrocytos* primer pairs MM and Msp (Table 2), an amplicon slightly shorter than the *M. mackini* amplified control was obtained (Fig. 3A,B, respectively). The PCR products obtained with primer pair MM were cloned and sequenced. These putative SSU rDNA gave a 523 bp sequence (deposited in Genbank, accession number DQ237912). A BLAST similarity search resulted in a close match (88% identity) with the SSU rDNA of *M. mackini* (accession no. AF477623). Alignment of these sequences revealed 2 major polymorphic regions (Fig. 4).

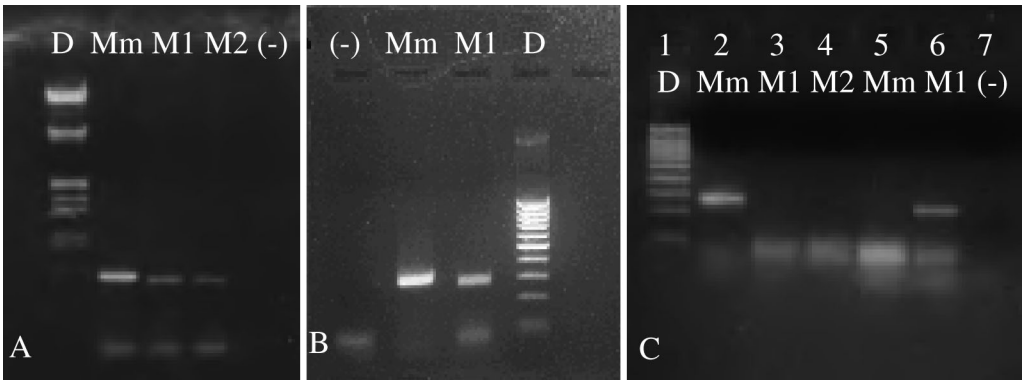

Fig. 3. Electrophoresis of PCR products using primer pair (A) MM, (B) Msp and (C) MM-mack in Lanes 2 to 4 and MM-like in Lanes 5 to 7. D: DNA marker; Mm: *Mikrocytos mackini*-infected oyster DNA; M1 and M2: *Mikrocytos* sp.-infected oysters; (–): no template (H_2O) negative control

Specific primers were generated to amplify the *Mikrocytos*-like sequence; primer pairs MM-like and Msp-like (Table 2). They produced the expected amplicon using the DNA extracts from oysters held in France (Fig. 3C; Msp-like data not shown). These primer pairs were not able to amplify *M. mackini* DNA. Similarly, MM-mack primer pair specific for *M. mackini* was generated to avoid amplification of *Mikrocytos*-like DNA, and no amplicon was seen in the infected oysters received in France, whereas a PCR product of the expected size was obtained using DNA from *M. mackini* infected oysters (Fig. 3C).

ISH and FISH assays

ISH was used to confirm the presence and identity of the microcells detected in heart smears and by histology in the flat oysters received in France. The *Mikrocytos*-like PCR probe produced with primer pair MM-like reacted with microcells in flat oyster tissue and showed the presence of parasites scattered throughout the connective tissue (Fig. 5). Conventional H&E staining of serial sections confirmed the identity of labeled cells (Fig. 2). It is important to note that the oysters examined were moribund, and tissue degradation may have reduced the number of parasites detected by both techniques. The probe reacted as well with positive control slides of *M. mackini* cells infecting *Ostrea edulis* and *Crassostrea gigas* (data not shown).

The *Mikrocytos*-like probe did not hybridize with *Bonamia ostreae* in *Ostrea edulis* tissue, whereas the *B. ostreae* probe did hybridize with *B. ostreae* in a subsequent tissue section from the same infected oyster (data not shown).

FISH assays performed on 3 presumptive *Mikrocytos*-like infected *Ostrea edulis* yielded a very weak positive signal of correct morphology in a few (<10) cells in 2 out of 3 oysters (data not shown). However, subsequent DIG ISH assays conducted at DFO-PBS resulted in a more convincing positive signal yet was still of variable intensity ranging from very weak to moderate in the same 2 out of 3 oysters (data not shown).

Additional oysters collected in Nova Scotia

Crassostrea virginica (n = 135) and *Ostrea edulis* (n = 275) oysters from Nova Scotia were assayed for *Mikrocytos* spp. (Table 1) in the months that followed initial reporting of a presumptive microcell parasite in the samples sent to France. They included oysters collected as part of investigations into reported field mortalities at sites in Nova Scotia. They were screened by PCR using primer pair Msp designed to detect *Mikrocytos* spp. (Fig. 3b). Positive signals were initially obtained from 13 specimens, but attempts to confirm their identity by repeating PCR assays with different primers were often unsuccessful. These occasional positive signals were generally weak. PCR analysis of DNA repeatedly extracted from the positive suspect oysters using QIAamp DNA mini kits was unsuccessful except for one individual. In addition, the few specimens where a PCR signal was obtained were further analyzed by ISH, but none gave a positive *in situ* signal, compared to *M. mackini*-infected controls, where a strong reaction was observed. The examination of 104 individual *Ostrea edulis* by histology did not reveal the presence of suspected pathogens. A subsample of 12 from the initial 60 oysters examined at DFO-GFC before transfer to France was also reexamined. In the absence of any unusual pathology, oysters were randomly selected and examined with ISH assays. Again, no positive results were obtained.

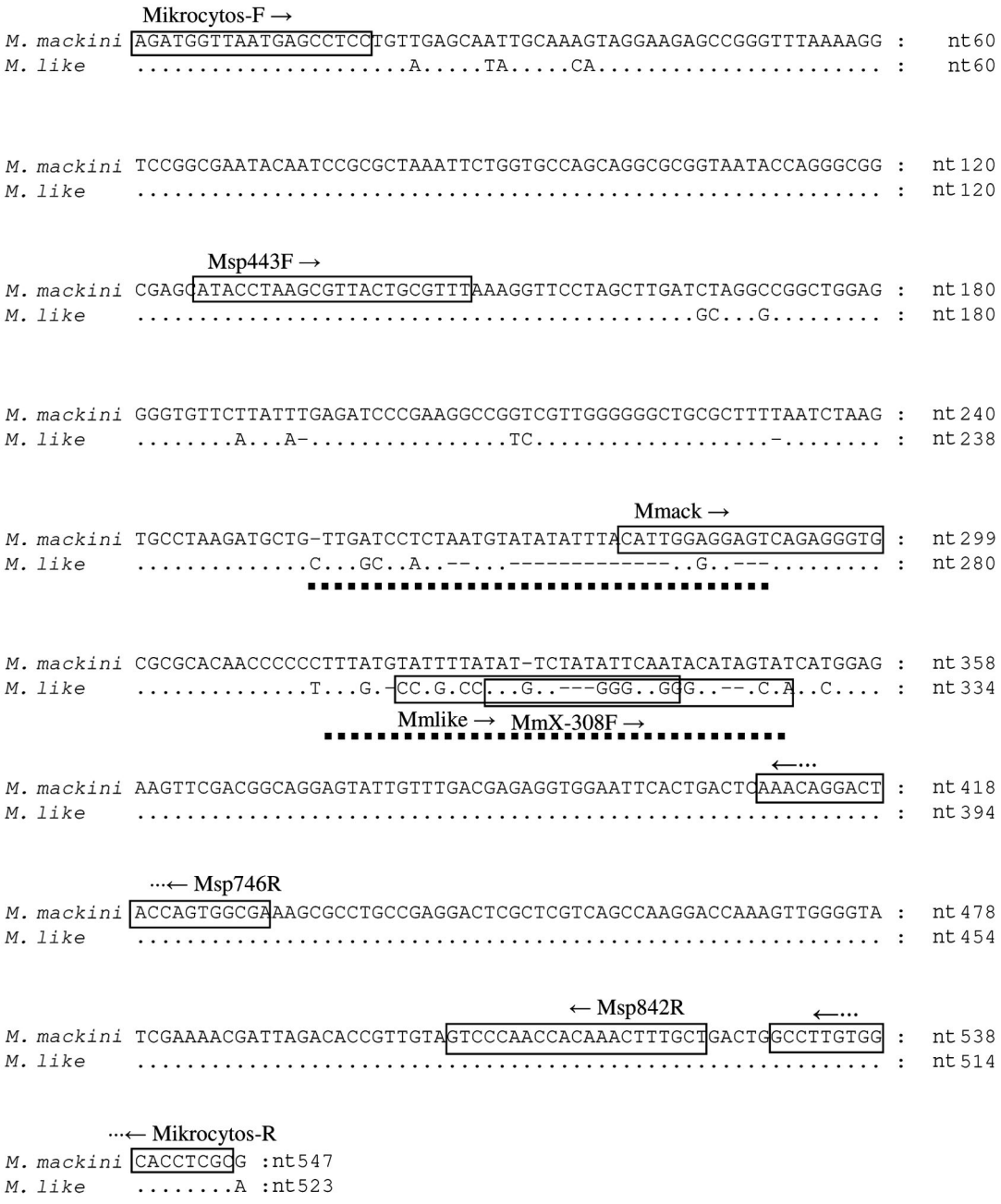

Fig. 4. Comparison of *Mikrocytos mackini* and *Mikrocytos*-like putative sequences. *Mikrocytos*-specific primers used in the present study enclosed in rectangles. Gaps shown by dashed lines. nt: nucleotide. Polymorphic regions are underlined with bold squares

DISCUSSION

The present study reports the presence of a microcell infection in European flat oysters *Ostrea edulis* from Nova Scotia maintained in quarantine in France. The quarantine facility uses chlorine treatment for outlet water, but no treatment was applied to inlet water. The occurrence of mortalities within 2 wk of importation suggests that this infection was not transmitted to the experimental oysters by inlet water. In addition, since 1986, regular screenings as part of the active French pathological surveillance program (REPAMO: Réseau de Pathologie des Mollusques) have not revealed *Mikrocytos*-like parasites in *O. edulis* and *Crassostrea gigas* in France. However, it has been shown experimentally that juvenile *C. gigas* (18.30 ± 3.46 mm in shell length) can become infected during 18 h of bath exposure to high levels of *M. mackini* (about 12 million

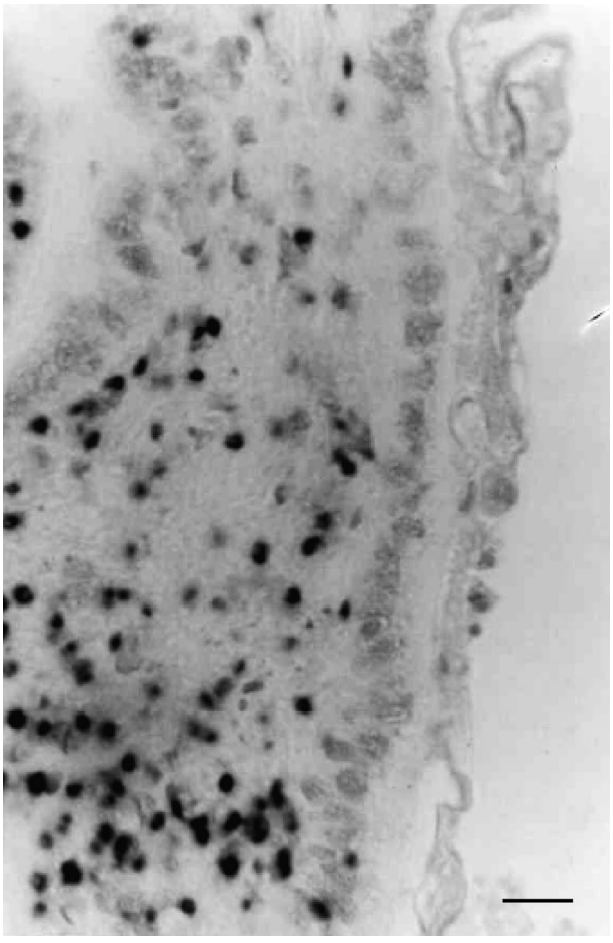

Fig. 5. *In situ* hybridization (ISH) on *Mikrocytos*-like infected *Ostrea edulis* sections. Microcells stained dark blue (dark spots here) and appear diffused in the connective tissue adjacent to the digestive gland. Scale bar = 20 µm

M. mackini in 1230 ml seawater), and the parasite was detected in the epithelium of the digestive tract at 2 h post-exposure (Bower et al. 2005). Although it is not the same oyster species, the same parasite, or conditions of exposure, we cannot definitely rule out the inlet water as the source of parasites or the possibility that naïve and stressed oysters became infected during quarantine.

It has been demonstrated that *Ostrea edulis* is a susceptible host of *Mikrocytos mackini* and may be more susceptible than *Crassostrea gigas*, which is a natural host of the pathogen (Bower et al. 1997). The intense focal haemocytic response of *C. gigas* may explain their relative resistance to *M. mackini*. The lack of response observed in *O. edulis* to the *Mikrocytos*-like parasite and absence of haemocyte infiltration may be an explanation for their higher susceptibility to the disease or that the infection was fairly recent and the disease response had not yet developed. However, death cannot be attributed to the *Mikrocytos*-like parasite without further investigations.

Experimental evidence suggests that mikrocytosis infections can remain subclinical for long periods, especially if the oysters are maintained at temperatures above 15°C (Bower et al. 1997). Laboratory experiments have shown that like *Crassostrea gigas*, *Ostrea edulis* can develop the disease when held at 10°C for 55 d following exposure. However, clinical disease does not develop and no pathogens are detectable by histology if *C. gigas* is held at 18°C for the same period of time (Bower et al. 1997). In Nova Scotia, oysters were collected in late spring, after a prolonged winter exposure to water temperatures of ca. 5°C. Water temperature in summer rises slowly to 12°C and reaches ca. 18°C by the end of summer; thus, persistence of this new microcell (assuming temperature preference characteristics similar to *Mikrocytos mackini*) in this area is plausible.

The small size of microcell parasites makes light infections difficult to detect by histology or cytology, and the infections seen in the present study presented no clinical signs in the oyster tissues. Furthermore, the microcells did not elicit a typical focal host response in the few individuals infected in this case compared to what can be seen in natural infections of *Mikrocytos mackini* in *Crassostrea gigas* (Farley et al. 1988) and *Ostrea edulis* (Bower et al. 1997), which renders detection even more difficult. The infected oysters presented a very low level of infection (<10 parasites) by conventional histology and cytology. Remaining oysters in quarantine were examined by heart smears and even fewer parasites were observed. However, this method is not adequate for the diagnosis of *Mikrocytos* sp. because the parasite usually resides in the vesicular connective tissue of the mantle and palps or in tissue of the adductor muscle. Using ISH, the microcells became easily detectable, and the intensity of infection appeared greater than seen by conventional histology (Fig. 5). Nevertheless, the infection was focal with very little host response and probably did not significantly contribute to the death of the infected individuals. This underlines the difficulty of detecting microcell parasites and the necessity of developing new diagnostic tools to complete and confirm their detection. The ISH assay developed during the present study was not specific and the *Mikrocytos*-like probe hybridized to both *M. mackini* and *Mikrocytos*-like, but it would be possible to modify the assay to a specific level by using an oligonucleotide probe designed from the variable region of *Mikrocytos*-like parasite as Stokes & Burreson (1995) used for the specific detection of *Haplosporidium nelsoni* (MSX).

PCR and sequencing results from the present study were used to demonstrate the presence of a new

microcell parasite. Although the segment amplified represents a short portion of the 18S gene, it is remarkably similar to *Mikrocytos mackini* with 88% homology. *M. mackini* is distantly related to other protists and stands alone at the base of the eukaryotic tree (Carnegie et al. 2003). The close sequence homology, and similarity at the microscopic level, suggest that the *Mikrocytos*-like parasite may retain several characteristics of its close relative. *M. mackini* is currently listed as a notifiable disease by the OIE (OIE 2005c).

A total of 137 *Crassostrea virginica* and 276 *Ostrea edulis* collected in Nova Scotia in the months that followed the initial report of a microcell parasite did not yield further infected individuals except for weak PCR results unconfirmed by visual identification. Possible explanations are the different sampling seasons, distance from initial site of sampling for some of the samples and lack of sustained stress in these additional samples compared to the oysters sent to France. Therefore, the presence of focal microcells infection reported here does not warrant a change of status, and *O. edulis* on the southeast coast of Canada remain free of OIE-listed diseases.

This is the first description of a new microcell infecting *Ostrea edulis* that is closely related to *Mikrocytos mackini*. Further investigations are needed to complete SSU rDNA sequence and ultrastructural studies. Given the economic impact possibly entailed by the presence of a parasite of this nature, it is necessary to continue to monitor *O. edulis* in both natural and aquaculture conditions.

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