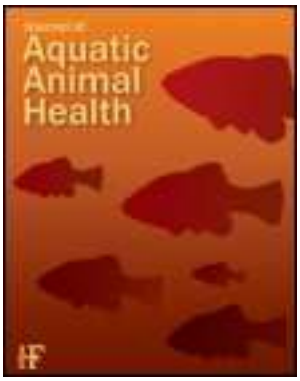

Journal of Aquatic Animal Health
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uahh20

Expanded Range and New Host Species of Mycobacterium shottsii and M. pseudoshottsii

Cynthia B. Stine [a] , John M. Jacobs [b] , Matt R. Rhodes [b] , Anthony Overton [c] , Mark Fast [d] & Ana M. Baya [a]

[a] University of Maryland, Virginia-Maryland College of Veterinary Medicine, College Park, Maryland, USA

[b] National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Cooperative Oxford Laboratory, Oxford, Maryland, USA

[c] Department of Biology, East Carolina University, Greenville, North Carolina, USA

[d] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York, USA

Version of record first published: 09 Jan 2011.

To cite this article: Cynthia B. Stine, John M. Jacobs, Matt R. Rhodes, Anthony Overton, Mark Fast & Ana M. Baya (2009): Expanded Range and New Host Species of Mycobacterium shottsii and M. pseudoshottsii , Journal of Aquatic Animal Health, 21:3, 179-183

To link to this article: http://dx.doi.org/10.1577/H09-005.1

Journal of Aquatic Animal Health 21:179–183, 2009

DOI: 10.1577/H09-005.1

[Communication]

Expanded Range and New Host Species of *Mycobacterium shottsii* and *M. pseudoshottsii*

CYNTHIA B. STINE*[1,2]
University of Maryland, Virginia–Maryland College of Veterinary Medicine, College Park, Maryland, USA

JOHN M. JACOBS[2] AND MATT R. RHODES
National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Cooperative Oxford Laboratory, Oxford, Maryland, USA

ANTHONY OVERTON
Department of Biology, East Carolina University, Greenville, North Carolina, USA

MARK FAST
School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York, USA

ANA M. BAYA
University of Maryland, Virginia–Maryland College of Veterinary Medicine, College Park, Maryland, USA

Abstract.—*Mycobacterium shottsii* and *M. pseudoshottsii* are recently described mycobacteria commonly isolated from Chesapeake Bay striped bass *Morone saxatilis*. However, their distribution in striped bass outside of the Chesapeake region and their ability to infect alternative hosts have not been described. Mycobacteria identified as *M. shottsii* (based on fatty acid methyl ester analysis and multigene sequencing) were isolated from striped bass collected in Albemarle Sound, North Carolina, and white perch *Morone americana* in the Rhode River, Maryland, and detected in striped bass from the New York Bight off Long Island, New York. *Mycobacterium pseudoshottsii* were isolated from white perch in the Rhode and Corsica rivers, Maryland, and detected in striped bass in the New York Bight. This work demonstrates that these mycobacteria can be found outside of the Chesapeake Bay as well as in hosts other than striped bass.

Mycobacteriosis has received considerable attention in the Chesapeake Bay region because of the elevated prevalence of disease in a key species, striped bass *Morone saxatilis* (Overton et al. 2003; Rhodes et al. 2004). Of great interest in this epizootic has been the isolation of two new species, *Mycobacterium shottsii* and *M. pseudoshottsii* (Rhodes et al. 2003, 2004, 2005). Whereas *Mycobacterium* spp. have been isolated previously from many species of fish in the Chesapeake Bay and globally, *M. shottsii* and *M. pseudoshottsii* have been reported only from wild Chesapeake Bay striped bass (Rhodes et al. 2004; Ottinger and Jacobs 2006; Kane et al. 2007; Jacobs et al. 2009b; Stine et al., in press).

Mycobacterium shottsii and *M. pseudoshottsii* are slow-growing species of the *M. tuberculosis* clade, most closely related to *M. marinum* and *M. ulcerans* (Kaattari et al. 2006). *Mycobacterium pseudoshottsii* has been shown to produce mycolactone, a macrolide toxin, similar to the necrotizing mycolactone of *M. ulcerans* (Ranger et al. 2006). Although the zoonotic potential of *M. shottsii* and *M. pseudoshottsii* remains unclear, their close relation to clinically significant members of the *M. tuberculosis* clade, and the capability of producing mycolactone by *M. pseudoshottsii*, warrant further surveillance. In this communication, we briefly describe the isolation or detection of these novel species in an alternative host and in geographically distinct regions of the Atlantic Coast. Prevalence data for these isolates will be presented in forthcoming reports from several large ongoing studies.

Methods

Adult striped bass were collected from Albemarle Sound, North Carolina (129 km south of the mouth of the Chesapeake Bay), in December 2006 and south of Rockaway Beach, Long Island, New York (403 km north of the mouth of the Chesapeake Bay), in November 2007. Adult white perch were collected from the Chesapeake Bay in the Rhode and Corsica rivers, Maryland, in July 2007.These collection efforts

* Corresponding author: cynthia.stine@fda.hhs.gov
[1] Present address: Food and Drug Administration, Laurel, Maryland, USA.
[2] Senior author

Received February 1, 2009; accepted July 2, 2009
Published online October 12, 2009

represent three distinct research projects planned to report on prevalence and associated factors in future publications.

Spleens and kidneys were removed aseptically from striped bass (Albemarle Sound), as were spleens from white perch (Chesapeake Bay). Samples were taken for full routine histology and preserved in 10% neutral buffered formalin until routine processing and aqueous acid-fast staining (McCollough 2008). After weighing, spleens were homogenized with 2 mL of Butterfield's phosphate-buffered solution and 200 μL was plated on Middlebrook 7H10 Agar supplemented with oleic acid, albumin, dextrose, catalase (OADC; Difco, Detroit, Michigan). Plates were checked for growth weekly for 12 weeks (incubated at 22 ± 2°C). Acid-fast colonies were isolated on Middlebrook 7H10 Agar and processed for gas-chromatographic fatty acid methyl ester (FAME) analysis (Sasser 1990; Stine 2008). Isolates were identified as *M. shottsii* when clustered within 10 units (Euclidean distance) of the type isolate of *M. shottsii* (American Type Culture Collection [ATCC] 700981) and identified as *M. pseudoshottsii* when clustered with the type isolate of *M. pseudoshottsii* (ATCC BAA-883) in FAME analysis (Stine 2008).

DNA was extracted from cultures by a simple boiling method previously described (Afghani and Stutman 1996). ATCC reference strains used for sequencing included *M. marinum* ATCC 927, *M. shottsii* ATCC 700981, and *M. pseudoshottsii* ATCC BAA-883. The exported repeated protein (*erp*) sequences for *M. marinum* (AF213153) and *M. ulcerans* (CP000325) were retrieved from GenBank. Internal transcribed spacer (ITS) sequences for *M. shottsii* and *M. pseudoshottsii* from this study have been submitted to GenBank (FJ899740 and FJ899741, respectively).

Culture was not attempted from New York striped bass. Spleen, liver, and anterior kidney were removed aseptically and processed for histology and polymerase chain reaction (PCR) analysis. Samples for PCR were flash-frozen on dry ice and stored at −80°C until further analysis. Tissues were thawed on ice and whole-tissue DNA was extracted by using the Wizard Genomic DNA Purification Kit according to the manufacturer's instructions (Promega Corp., Madison, Wisconsin). Tissue DNA preparations were screened for mycobacterial DNA by nested PCR before inclusion in multigene sequencing (Talaat et al. 1997).

All samples were subsequently screened for inclusion in the *M. tuberculosis* clade by using a real-time PCR assay targeting a genus specific region of the ribosomal 16–23S ITS region (van Coppenraet et al. 2004; Jacobs et al. 2009a). Sequence analysis of the *erp* gene was used to distinguish *M. shottsii* and *M. pseudoshottsii* from other members of the *M. tuberculosis* clade (Rhodes et al. 2005; Jacobs et al. 2009a). Isolates were identified as *M. shottsii* or *M. pseudoshottsii* when 100% similarity was seen in *erp* sequences with those of reference strains. For samples where only fish tissue was available, total DNA was set at 150 ng per reaction. When necessary, a nested approach was used for amplification of the *erp* gene as described in Jacobs et al. (2009a). Otherwise, boiled cells from isolates and spleenic tissue DNA were treated similarly for PCR and postprocessing. Positive samples were gel-extracted and purified with a Qiagen QIAquick Gel extraction kit (Qiagen, Inc., Valencia, California). Sequencing was performed at the University of Maryland, Center of Marine Biotechnology BioAnalytical Services Laboratory. All sequencing was performed on an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, California) and using the dye terminator method. Sequences were aligned to our own databases and those of GenBank by using the freeware BioEdit (Hall 1999).

Results

Mycobacterium shottsii (identified by morphology, FAME analyses and PCR) was recovered from spleen and kidney of Albemarle Sound striped bass and was detected in the spleen of Long Island striped bass (detected by PCR; Table 1). Colony morphology from the North Carolina isolates was similar to that in species descriptions (Rhodes et al. 2003) and in samples isolated previously in our laboratory from Chesapeake Bay striped bass. Recovered colony-forming units (CFUs) ranged from 33 to 1,200 CFU/g. *Mycobacterium shottsii* was also recovered from the spleen of a Rhode River white perch, the morphology being similar to that in species descriptions, at 25 CFU/g.

Mycobacterium pseudoshottsii was detected by PCR in Long Island striped bass and was isolated from Chesapeake Bay white perch (identified by morphology, FAME analyses, and PCR; Table 1). Again, colony morphology of the white perch isolates was similar to that in species descriptions (Rhodes et al. 2005) and in samples isolated previously in our laboratory from Chesapeake Bay striped bass. Recovered colonies ranged from 167 to 3000 CFU/g in Chesapeake Bay white perch.

The eight isolates identified as *M. shottsii* had 100% sequence identity to *M. shottsii* (GenBank AY496288), and the five isolates identified as *M. pseudoshottsii* had 100% sequence identity to *M. psuedoshottsii* (GenBank AY496289) for the *erp* gene (Table 1). Similarly, all sequences obtained from these isolates for the ITS were identical to previous GenBank submissions for *M. ulcerans* (CP000325) and *M. marinum* (CP000854).

TABLE 1.—Metrics for striped bass (SB) and white perch (WP) from which *Mycobacterium shottsii* and *M. pseudoshottsii* isolates were recovered or detected. Abbreviations are as follows: AFB = acid-fast bacilli; CFU = colony-forming units; and ND = not detected.

Isolate	Host	Capture location	Capture date	Estimated age (years)[a]	Sex[b]	AFB granulomas	Isolate detection method	CFU/g
				M. shottsii				
NCSB 5K	SB	Albemarle Sound	Dec 12, 2006	8	F	Skin	Culture	133
NCSB 8S	SB	Albemarle Sound	Dec 12, 2006	3	M	Negative	Culture	300
NCSB 14S	SB	Albemarle Sound	Dec 12, 2006	5	M	Skin, spleen	Culture	225
NCSB 24K	SB	Albemarle Sound	Dec 13, 2006	4	F	Skin	Culture	200
NCSB 38K	SB	Albemarle Sound	Dec 13, 2006	4	F	Spleen	Culture	33
NCSB 43S	SB	Albemarle Sound	Dec 13, 2006	4	F	Head kidney	Culture	1,200
NYSB 21S	SB	Rockaway Beach	Nov 14, 2007	5	M	Spleen, liver, head kidney	PCR	ND
CBWP 2–6S	WP	Rhode River	Jul 24, 2007	8	F	Negative	Culture	25
				M. pseudoshottsii				
NYSB 16S&K	SB	Rockaway Beach	Nov 14, 2007	6	M	Spleen, liver, head kidney	PCR	ND
NYSB 29S	SB	Rockaway Beach	Nov 14, 2007	4	F	Spleen, liver, head kidney	PCR	ND
CBWP 2–1S	WP	Rhode River	Oct 16, 2007	5	F	Spleen, liver, head kidney, heart	Culture	167
CBWP 1–9S	WP	Corsica River	Oct 23, 2007	5	F	Spleen, head kidney	Culture	3,000

[a] Estimates for striped bass from length-at-age data provided by the Maryland Department of Natural Resources, those for white perch from St. Pierre and Davis (1972).
[b] F = female, M = male.

Both sets of isolates grouped with respective ATCC reference isolates in FAME analysis.

Acid-fast bacilli were observed in granulomas in the skin, spleen, and head kidney of North Carolina striped bass, as well as in granulomas in the spleen, head kidney, liver, and heart of Chesapeake Bay white perch (Table 1).

Discussion

Mycobacterium shottsii, as identified by colony morphology, FAME analysis, and sequencing of both the ribosomal 16–23S ITS region and the *erp* gene, was isolated from six North Carolina striped bass and one Chesapeake Bay white perch and was detected by PCR in one New York Bight striped bass. In addition, *M. pseudoshottsii*, as identified by colony morphology, FAME analysis, and sequencing of both the ribosomal 16–23S ITS region and the *erp* gene, was isolated from two Chesapeake Bay white perch and detected in two striped bass from the New York Bight. This represents the first report of these recently described members of the *M. tuberculoisis* clade outside of the Chesapeake Bay region, and the first finding in a host other than striped bass. This communication reports only confirmed isolates from three separate studies; prevalence of these mycobacteria in these host populations will be presented in forthcoming full reports.

It is not surprising that *M. shottsii* and *M. pseudoshottsii* have been found in regions other than the Chesapeake Bay, but whether the detection results from transport of the bacteria to these new systems or from increased surveillance is unclear. An *M. shottsii*-like isolate (based on phenotypic characterizations) was described from a Delaware Bay striped bass; however, wildlife can move directly between the Delaware and Chesapeake bays by the Chesapeake and Delaware Canal (Ottinger et al. 2007). The current study places colonized fish at systems distant from the Chesapeake Bay, but the Atlantic coastal stock of striped bass undergoes a well-known seasonal migration along the Eastern seaboard of the United States that includes both New York and North Carolina waters (Kohlenstein 1981). Because the routes of infection and transmission of *M. shottsii* and *M. pseudoshottsii* are unknown, the reason for the presence of colonized fish in these systems is also unknown. Increased awareness and surveillance because of investigations of the epizootic in Chesapeake Bay may have allowed the detection of these bacteria in these new systems.

Likewise, in the Chesapeake Bay region, it is not entirely unexpected that *M. shottsii* and *M. pseudoshottsii* were isolated from white perch, a close relative of striped bass. The two host species share a similar trophic niche during early life history, occupy similar habitats for the first year of life, and spawn in similar areas with only slight temporal offset (Murdy et al. 1997). Although routes of transmission are not well understood, the close interaction of these genetically similar species in Chesapeake Bay may offer ample opportunity for a mode of infection common to both host species.

The ecological implications of host colonization with *M. shottsii* and *M. pseudoshottsii* are unknown; however, potential economic repercussions because of concerns over seafood safety could be significant. The expansion of the geographic range of *M. shottsii* and

M. pseudoshottsii and the number of host species colonized should focus attention on increasing the knowledge base on these organisms, including identifying potential risk factors for colonization and elucidating their roles in disease and in the environment. Studies on the distribution and abundance of mycobacteria in these host species in these systems are currently underway. Nonetheless, our findings should emphasize the importance of further comprehensive investigations that include other potential host species and geographic areas.

Acknowledgments

The authors thank Eric Diaddorio, Jim Saupe, and Ken Riley (East Carolina University) for collection assistance; Mark Sokolowski and Mike Taddeo (School of Marine and Atmospheric Sciences–Stony Brook) for assistance in field collections and histology processing; Jennifer O'Dwyer, Julia Socrates, Todd Abenante, and Michael Bernstein (New York State Department of Environmental Conservation [NYS DEC]) for collections and aging work; the University of Maryland Center of Marine Biotechnology Bio-Analytical Services Laboratory for sequencing isolates; Mamuka Kotetishvili for tree assistance; and the University of North Carolina Coastal Studies Institute Laboratory in Manteo, North Carolina, for providing work-up facilities. Protocols were approved by the Institutional Care and Use Committee of Stony Brook University, Stony Brook, New York. Portions of this study were supported by the National Oceanic Atmospheric Administration, National Ocean Service Cooperative Oxford Laboratory and by the NYS DEC under MOU AMOU177 (2007–2009). Support for C.S. was also provided by Program Development Funds from Maryland Sea Grant.

References

Afghani, B., and H. R. Stutman. 1996. Polymerase chain reaction for diagnosis of *M. tuberculosis*: comparison of simple boiling and a conventional method for DNA extraction. Biochemical and Molecular Medicine 57:14–18.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Jacobs, J. M., D. W. Howard, M. R. Rhodes, E. B. May, and R. M. Harrell. 2009a. Historical presence of mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*): 1975–1985. Diseases of Aquatic Organisms 85:181–186.

Jacobs, J. M., C. B. Stine, A. M. Baya, and M. L. Kent. 2009b. A review of mycobacteriosis in marine fishes. Journal of Fish Diseases 32:119–130.

Kaattari, I. M., M. W. Rhodes, S. L. Kaattari, and E. B. Shotts. 2006. The evolving story of *Mycobacterium tuberculosis* clade members detected in fish. Journal of Fish Diseases 29:509–520.

Kane, A. S., C. B. Stine, L. Hungerford, M. Matsche, C. Driscoll, and A. M. Baya. 2007. Mycobacteria as environmental portent in Chesapeake Bay fish species. Emerging Infectious Diseases 13:329–331.

Kohlenstein, L. C. 1981. On the proportion of the Chesapeake Bay stock of striped bass that migrates into the coastal fishery. Transactions of the American Fishery Society 110:168–179.

McCollough, C. B. 2008. Application of an aqueous acid-fast staining technique to detect pathogens of aquatic species. Biotechnic and Histochemistry 83(3–4):191–197.

Murdy, E. O., R. S. Birdsong, and J. A. Musick. 1997. Fishes of Chesapeake Bay. Smithsonian Institution Press, Washington, D.C.

Ottinger, C. A., J. J. Brown, C. L. Densmore, C. E. Starliper, V. S. Blazer, H. S. Weyers, K. A. Beauchamp, M. W. Rhodes, H. Kator, D. T. Gauthier, and W. K. Vogelbein. 2007. Mycobacterial infections in striped bass from Delaware Bay. Journal of Aquatic Animal Health 19:99–108.

Ottinger, C. A., and J. M. Jacobs, editors. 2006. USGS/NOAA Workshop on Mycobacteriosis in Striped Bass. U.S. Geological Survey, Scientific Investigations Report 2006-5214, and NOAA Technical Memorandum NOS NCCOS 41.

Overton, A. S., F. J. Margraf, C. A. Weedon, L. H. Pieper, and E. B. May. 2003. The prevalence of mycobacterial infections in striped bass in Chesapeake Bay. Fisheries Management and Ecology 10:301–308.

Ranger, B. S., E. A. Mahrous, L. Mosi, S. Adusumilli, R. E. Lee, A. Colorni, M. Rhodes, and P. L. C. Small. 2006. Globally distributed mycobacterial fish pathogens produce a novel plasmid-encoded toxic macrolide, mycolactone F. Infection and Immunity 74:6037–6045.

Rhodes, M. W., H. Kator, I. Kaattari, D. Gauthier, W. Vogelbein, and C. A. Ottinger. 2004. Isolation and characterization of mycobacteria from striped bass *Morone saxatilis* from the Chesapeake Bay. Diseases of Aquatic Organisms 61:41–51.

Rhodes, M. W., H. Kator, S. Kotob, P. van Berkum, I. Kaattari, W. Vogelbein, F. Quinn, M. M. Floyd, W. R. Butler, and C. A. Ottinger. 2003. *Mycobacterium shottsii* sp. nov., a slowly growing species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). International Journal of Systemic and Evolutionary Microbiology 53:421–424.

Rhodes, M. W., H. Kator, A. McNabb, C. Deshayes, J. M. Reyrat, B. A. Brown-Elliott, R. Wallace Jr., K. A. Trott, J. M. Parker, B. Lifland, G. Osterhout, I. Kaattari, K. Reece, W. Vogelbein, and C. A. Ottinger. 2005. *Mycobacterium pseudoshottsii* sp. nov., a slowly growing chromogenic species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). International Journal of Systemic and Evolutionary Microbiology 55:1139–1147.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Microbial ID, Inc., Newark, Delaware. Available: www.midi-inc.com/pdf/mis_Technote_101.pdf. (September 2009).

Stine, C. B. 2008. Mycobacteriosis in Chesapeake Bay striped

bass *Morone saxatilis*. Doctoral dissertation. University of Maryland, College Park.

Stine, C. B., A. S. Kane, and A. M. Baya. In press. Mycobacteria isolated from Chesapeake Bay fishes. Journal of Fish Diseases.

St. Pierre, R. A., and J. Davis. 1972. Age, growth, and mortality of the white perch, Morone americana, in the James and York rivers, Virginia. Chesapeake. Science 13:272–281.

Talaat, A. M., R. Reimschuessel, and M. Trucksis. 1997. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. Veterinary Microbiology 58:229–237.

van Coppenraet, E. S. B., J. A. Lindeboom, J. M. Prins, M. F. Peeters, E. C. J. Claas, and E. J. Kuijper. 2004. Real-time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of mycobacterial lymphadenitis in children. Journal of Clinical Microbiology 42:2644–2650.