

# Further Bacteriological Analysis of Annual Pheasantshell (Actinonaias Pectorosa) Mussel Mortality Events in the Clinch River (Virginia/Tennessee), USA, Reveals a Consistent Association with Yokenella Regensburgei

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### **REGULAR ARTICLE**

# FURTHER BACTERIOLOGICAL ANALYSIS OF ANNUAL PHEASANTSHELL (ACTINONAIAS PECTOROSA) MUSSEL MORTALITY EVENTS IN THE CLINCH RIVER (VIRGINIA/ TENNESSEE), USA, REVEALS A CONSISTENT ASSOCIATION WITH YOKENELLA REGENSBURGEI

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### **ABSTRACT**

Pheasantshell (Actinonaias pectorosa) mussels in the Clinch River (Tennessee/Virginia, USA) have declined dramatically in recent years. The bacterium Yokenella regensburgei was first isolated with high prevalence from Pheasantshells during the peak of a 2017 mortality event, but it was not identified after mortality subsided a few months later. Since 2017, Pheasantshell mortality in the Clinch River has occurred each autumn. We extended the investigation of culturable bacterial communities in the Clinch River during mussel mortality events in 2018, 2019, and 2020 and examined the spatial and temporal distribution of bacterial genera among Pheasantshells, as well as among other unionid mussels. We identified Y. regensburgei each year, almost exclusively during active mortality events. The significance of Y. regensburgei remains unclear, but the continued association of this bacterium with mussel mortality events warrants further study.

KEY WORDS: Actinonaias pectorosa, Pheasantshell mussel, Unionidae, Yokenella regensburgei, Clinch River, Virginia, mortality event

### **INTRODUCTION**

Large-scale mortality events and declines in mussel populations have occurred with increasing frequency in recent decades (Strayer et al. 2004). Clear explanations, such as toxic spills, have been identified in some cases; in others, disease has been suspected, but not confirmed (Neves 1987; Haag 2019). Beginning in summer 2016, biologists observed a mass mortality event affecting numerous mussel species in the

Virginia and Tennessee portions of the Clinch River. Subsequent investigations revealed that mortality recurred seasonally from late summer to early autumn. Although many species were observed dead or moribund, the Pheasantshell (*Actinonaias pectorosa*) mussel was affected disproportionately. Pheasantshell initially was among the most abundant species in the Clinch River, but population sizes declined 50–80% across multiple sites after mortality events (Richard 2018). In response to the mortality event in the Clinch River and a contemporaneous multispecies mortality event in Big Darby Creek, Ohio, USA, a research group was formed to

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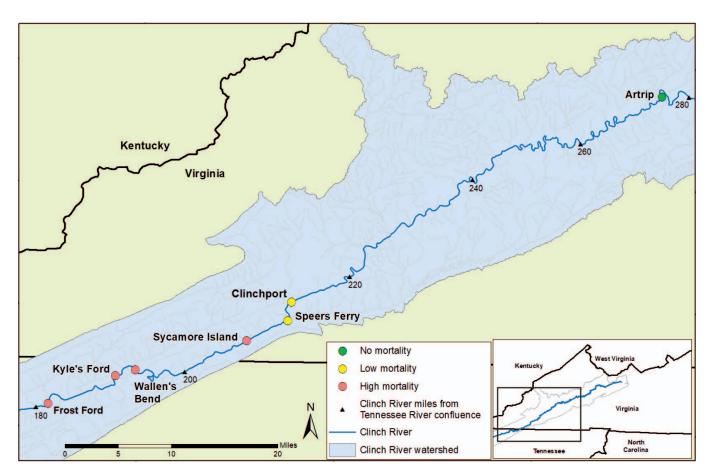


Figure 1. Locations where hemolymph samples were collected from mussels in the Clinch River, USA. Inset map shows location of the study area in southwestern Virginia and northeastern Tennessee.

study the events and gather baseline data to identify potential pathogens (Leis et al. 2018). The group reported a picorna-like virus from a Wabash Pigtoe (*Fusconaia flava*) in the upper Mississippi River (Goldberg et al. 2019); 17 novel viruses, including a densovirus associated with moribund mussels in the Clinch River (Richard et al. 2020); and a novel gonadotropic microsporidian (Knowles et al. 2022). They also conducted molecular and culture-based evaluations of the bacterial composition of mussel hemolymph from several river systems in the eastern United States (Leis et al. 2019; Richard et al. 2021).

In a previous study, we examined culturable bacteria associated with a 2017 mussel mortality event in the Clinch River (Leis et al. 2019). We identified many bacterial genera, but only *Yokenella regensburgei* was detected with high prevalence in Pheasantshells while mortalities were occurring, and it was not present a few months later after mortality subsided. This bacterium was previously identified from a mussel mortality event in the Tennessee River (Starliper et al. 2011), but whether it plays a direct role in such events remains unknown. Since 2017, episodic mortality of Pheasantshells has continued each autumn in the Clinch River. We investigated bacterial communities in the Clinch River during mussel mortality events in 2018, 2019, and 2020 and examined the

spatial and temporal prevalence of bacterial genera among Pheasantshell and other unionid species.

### **METHODS**

We collected samples from live and moribund mussels at seven sites in the Clinch River in 2018, 2019, and 2020 (Fig. 1 and Table 1). After observing mussel mortality in autumn 2016 and 2017, we established a series of sampling sites within and upstream of the zone of observed mortality and began sampling in summer 2018. We sampled six sites monthly from August to October 2018. High rainfall forced us to abandon planned sampling events in November and December 2018. In 2018, we sampled Pheasantshell and Mucket (Actinonaias ligamentina); the annual Pheasantshell mortality event began in September and no moribund Muckets were observed (Table 1). In 2019, we observed a mortality event that began in September and sampling occurred at Sycamore Island while the event was ongoing in October. We sampled moribund Pheasantshells and apparently healthy individuals of Mucket, Pocketbook (Lampsilis ovata), Threeridge (Amblema plicata), Kidneyshell (Ptychobranchus fasciolaris), Wavyrayed Lampmussel (Lampsilis fasciola), and Purple Wartyback (Cyclonaias tuberculata). We observed

Table 1. Isolation and prevalence of *Yokenella regensburgei* in Clinch River, USA, mussels from 2018 to 2020. *A. ligamentina = Actinonaias ligamentina*; *A. pectorosa = Actinonaias pectorosa*; *A. plicata = Amblema plicata*; *P. fasciolaris = Ptychobranchus fasciolaris*; *L. fasciola = Lampsilis fasciola*; *C. tuberculata = Cyclonaias tuberculata*; *E. brevidens = Epioblasma brevidens*; *L. oyata = Lampsilis oyata*. *N* = number of individuals sampled.

Sampling Month and Year	Location	Mussel Species (N)	Active Mortality Observed	Prevalence of Yokenella (%)
August 2018	Sycamore Island	A. pectorosa (2)	No	0
Tugust 2010	Sycamore Island	A. ligamentina (2)	No	0
	Wallen's Bend	A. pectorosa (2)	No	50
	Wallen & Bella	A. ligamentina (2)	No	0
	Kyle's Ford	A. pectorosa (2)	No	0
	11,10 8 1 816	A. ligamentina (2)	No	0
	Frost Ford	A. pectorosa (2)	No	0
	11050 1 010	A. ligamentina (2)	No	0
September 2018	Artrip	A. pectorosa (3)	No	0
	Speers Ferry	A. pectorosa (3)	No	0
	Sycamore Island	A. pectorosa (5)	Yes	60
	Wallen's Bend	A. pectorosa (5)	Yes	40
	Kyle's Ford	A. pectorosa (6)	Yes	33
October 2018	Artrip	A. pectorosa (3)	No	0
	Speers Ferry	A. pectorosa (4)	Yes	25
		A. ligamentina (1)	No	0
	Sycamore Island	A. pectorosa (6)	Yes	0
	•	A. ligamentina (1)	No	0
	Kyle's Ford	A. pectorosa (6)	Yes	17
October 2019	Sycamore Island	A. pectorosa (2)	Yes	50
		A. ligamentina (4)	No	0
		L. ovata (2)	No	0
		A. plicata (2)	No	0
		P. fasciolaris (1)	No	0
		L. fasciola (2)	No	0
		C. tuberculata (1)	No	0
October 2020	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa (7)	Yes	86
	Sycamore Island	A. pectorosa (9)	Yes	89
		A. ligamentina (1)	Yes	100
		E. brevidens (1)	Yes	100

mortality in October 2020 and collected targeted samples consisting of moribund Pheasantshells combined from three adjacent sites: Speers Ferry, Sycamore Island, and Clinchport. Later in the month, we also sampled moribund Pheasantshells, Muckets, and Cumberlandian Combshells (*Epioblasma brevidens*) from Sycamore Island.

In 2018 and 2019, we collected hemolymph from the anterior adductor muscle of each mussel by slightly opening the shell with a child nasal speculum, placing a stopper between the shells, and drawing out a hemolymph sample with a 1-mL syringe and 25-gauge needle. After collecting each sample, we immediately plated and streaked approximately 100  $\mu$ L of hemolymph onto sterile tryptic soy agar culture plates (Becton Dickinson, Le Pont de Claix, France). Plates were shipped overnight to the U.S. Fish and Wildlife Service, La Crosse Fish Health Center, La Crosse, Wisconsin. We incubated the plates at 21°C for 7–14 d. After incubation, we

used a sterile, disposable loop to remove morphologically unique colonies from each plate; placed them in a microcentrifuge tube; and extracted DNA by using the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA). We subjected the extracted DNA to 16S rRNA gene PCR by using the same primers used by Leis et al. (2019), followed by Sanger sequencing (Eton Biosciences, Union, NJ, USA). We then edited and assembled the sequences de novo by using the default parameters in Geneious v11.1.5 (https://www.geneious.com/download/ previous-versions/#geneious-r11-dot-1 [accessed August 19, 2022]), and we identified resulting contig sequences through megaBLAST searches in the National Center for Biotechnology Information database (https://blast.ncbi.nlm.nih.gov/Blast. cgi [accessed August 19, 2022]). In 2020, moribund mussels were wrapped in wet towels and sent on ice to the La Crosse Fish Health Center for processing as described above. Because

Table 2. Prevalence of the six most common bacterial genera and samples yielding no bacterial isolates in moribund and healthy Pheasantshell (*Actinonaias pectorosa*) mussels collected in Clinch River, USA, from 2018 to 2020. An asterisk (\*) indicates statistically significant differences in prevalence between healthy and moribund mussels (Fisher's exact test:  $P \le 0.002$ ). N = number of individuals sampled.

	Yokenella* (%)	Aeromonas* (%)	Bacillus (%)	Pseudomonas (%)	Microbacterium (%)	Acinetobacter (%)	No Isolates (%)
Healthy $(N = 33)$	9.1	0.0	24.2	21.2	21.2	9.1	27.3
Moribund $(N = 34)$	64.7	26.5	11.8	11.8	8.8	8.8	11.8

Pheasantshell was the primary species observed in moribund condition, we used Fisher's exact tests to examine whether there were nonrandom associations between frequently observed bacterial genera and healthy or moribund Pheasantshell samples. For each bacterial genus present in six or more Pheasantshells, as well as for the condition of "no bacterial growth observed," we set up a  $2 \times 2$  contingency table with categories of bacteria presence/absence and healthy/moribund mussels. Pheasantshell samples within the moribund and healthy groups were pooled across all sites and dates from the study. The results of each Fisher's exact test indicate whether there was a statistically significant association between the presence of a particular bacterial genus and Pheasantshell health status.

### **RESULTS**

We examined a total of 91 mussels (67 Pheasantshells, 15 Muckets, 1 Cumberlandian Combshell, 1 Purple Wartyback, 2 Wavyrayed Lampmussels, 1 Kidneyshell, 2 Threeridges, and 2 Pocketbooks), including 49 healthy and 42 moribund individuals, from the Clinch River during 2018, 2019, and 2020. Bacteria were isolated from 80% (73 of 91) of the mussels sampled; 18 mussel samples yielded no bacterial isolates. All the cultured colonies were identifiable, except for two isolates from Muckets sampled on August 16, 2018, and October 25, 2018.

Across all sampling seasons, we identified 190 isolates belonging to 46 bacterial genera from 91 individual mussel hemolymph samples (49 apparently healthy, 42 moribund; Appendix A1). Most bacterial genera were observed only rarely, with 39 of the 46 genera present in three or fewer individual mussels and one present in four individuals (Appendix A1). The six most common genera identified were (in order of decreasing abundance) Yokenella, Bacillus, Microbacterium, Pseudomonas, Aeromonas, and Acinetobacter. The most common isolates for healthy mussels were Bacillus (27%; 13 of 49), Microbacterium (20%; 10 of 49), and Pseudomonas (16%; 8 of 49), with all other genera present in four or fewer individuals. The most common isolates for moribund mussels were Yokenella (57%; 24 of 42), Aeromonas (26%; 11 of 42), and Bacillus (14%; 6 of 42), with all other genera present in four or fewer individuals. Yokenella was observed in only three healthy individuals, whereas Aeromonas was never observed in healthy individuals. The prevalence of Yokenella and Aeromonas was significantly

higher in moribund than healthy Pheasantshells (Fisher's exact test: P < 0.0001 and P = 0.0021, respectively; Table 2). The prevalence of the other four most common genera and the prevalence of samples yielding no bacterial isolates were not significantly different between moribund and healthy Pheasantshells (Table 2).

We observed *Y. regensburgei* each year during active mortality events in the Clinch River. Sequences identified as *Y. regensburgei* shared >99.3% similarity and were between 636 and 1,375 bp (Appendix A1). In 2018, *Y. regensburgei* was present in Pheasantshells at Speers Ferry, Sycamore Island, Wallen's Bend, and Kyle's Ford, all of which are sites where moribund mussels were observed (Table 1). The bacterium was not isolated from apparently healthy Muckets sampled at these sites or from any samples collected at Artrip, an upstream site where Pheasantshell mass morality has not been observed. All detections of *Y. regensburgei* in Pheasantshells occurred during periods of active mortality, except for one isolation from Wallen's Bend on August 16, 2018, which preceded our first observations of mortality by several weeks.

In 2019, *Y. regensburgei* was isolated from Pheasantshell, but not from six other mussel species; active mortality of Pheasantshells was also observed (Table 1). In 2020, during sampling that targeted moribund mussels, *Y. regenburgei* was isolated from 86% of Pheasantshells at three sites on October 7 and from 89% of Pheasantshells at Sycamore Island on October 20. *Yokenella regensburgei* also was isolated from moribund Muckets and Cumberlandian Combshell on October 20 (Table 1).

Aeromonas was detected only in 2020, when it was present in 11 of 18 moribund mussels collected. In one of these samples, two Aeromonas isolates were the only bacteria cultured, whereas in the remaining 10 samples containing Aeromonas, it co-occurred with Yokenella.

The prevalence of *Bacillus* spp. did not differ between apparently healthy mussels (44%; 16 of 36) and moribund mussels (17%; 6 of 36; Fisher's exact text: P = 0.1986).

### **DISCUSSION**

The consistent association of *Y. regensburgei* with mussel mortality events and moribund mussels was one of the strongest and most conspicuous patterns of bacterial occurrence in our samples. We isolated *Y. regensburgei*, generally with high prevalence, during mortality events in every year of our study, and it was previously isolated during a mortality

event in 2017 (Leis et al. 2019). Furthermore, it was rarely identified when mortality events were not occurring or at sites where mortality has not been observed (Artrip). The only occurrence of *Y. regensburgei* outside of a mortality event was its detection in an apparently healthy Pheasantshell on August 16, 2018, at Wallen's Bend; this may have represented an incipient occurrence at the onset of mussel mortality, which was observed a few weeks later at this site.

Yokenella regensburgei, along with predominantly Hafnia alvei, was identified from Ebonyshell (Reginaia ebenus) during mortality events in the Tennessee River, Alabama (2006 and 2008), and H. alvei was previously identified from the Clinch River (Starliper et al. 2008, 2011). Hafnia alvei and Y. regensburgei both are enteric bacteria that share similar biochemical characteristics, making separation of the two species uncertain by using traditional laboratory diagnostic methods (Lo et al. 2011). It is unclear whether Starliper et al. (2011) used molecular or biochemical techniques to identify Y. regensburgei and H. alvei. Furthermore, the Analytical Profile Index database (Biomérieux, Marcy-l'Étoile, France; https:// www.biomerieux-diagnostics.com/sites/clinic/files/9308960-002-gb-b-apiweb-booklet.pdf [accessed December 5, 2022]) used by Starliper et al. (2008) would have been unable to identify Y. regensburgei because that species is not included in the database, but H. alvei is included. Because of this limitation, it is possible that Y. regensburgei was present at higher prevalence during the Tennessee River mortality event. Our molecular methods should have allowed accurate separation of the two species, but neither we nor Richard et al. (2021) detected *H. alvei* in samples of mussel hemolymph from the Clinch River.

Despite the consistent association of Y. regensburgei with mussel mortality events, its role in these events is unclear. At least two scenarios could explain this association. The first scenario is that this bacterium is pathogenic. Preliminary histopathology work does not support pathogenicity (S. Knowles, unpublished data), but additional research is needed to confirm this result. The second scenario is that Y. regensburgei opportunistically colonizes mussels that are stressed and of compromised health due to a separate insult, such as exposure to environmental toxins or degraded water quality (see Leis et al. 2019). Richard et al. (2021) found a shift in bacterial communities of mussel hemolymph when mussels exhibit signs of apparent disease. An important question is whether there is a relationship between Y. regensburgei and Clinch densovirus 1 or other viruses identified from the Clinch River (Richard et al. 2020). For example, are these organisms pathogenic, or does a separate environmental factor (e.g., toxins, thermal stress, changes in water chemistry or algal communities) result in an immunocompromised state that allows unchecked bacterial growth and viral replication? Another important question is whether Y. regensburgei is consistently associated with mussel mortality events in other watersheds. Future work evaluating the importance of this bacterium would involve the development of a diagnostic assay to rapidly identify Y. regensburgei in mussels, which could also be used to search for potential environmental sources or reservoirs and to better understand the seasonality of its occurrence. In addition, in vivo infection trials are needed to evaluate the pathogenicity of *Y. regensburgei* to Pheasantshell and other mussel species, alone and in combination with other factors.

Although the prevalence of *Bacillus* spp. did not differ significantly between healthy and moribund mussels, there was a suggestive trend of higher prevalence in healthy mussels, a trend also noted by Leis et al. (2019). Members of *Bacillus* have several characteristics that, hypothetically, could be considered beneficial to freshwater mussels (see Leis et al. 2019). The lack of a significant difference in the prevalence of *Bacillus* between healthy and moribund mussels could be due to the persistence of these bacteria in moribund mussels after the onset of disease. Additional studies are needed to evaluate potential associations of *Bacillus* spp. with mussel health.

The strong pattern of co-occurrence between Aeromonas and Yokenella in 2020 is intriguing because it also was observed by Richard et al. (2021) (their study included samples from Clinch River mussels in 2017–2018) and Leis et al. (2019) (their study included samples from Clinch River mussels in 2017). Both studies found high Aeromonas spp. and Yokenella prevalence associated with moribund mussels from mortality sites, and the two genera often co-occurred in samples. However, Richard et al. (2021) found high Aeromonas spp. prevalence in 2018 samples from Clinch River mussels, whereas we observed Aeromonas spp. only in samples collected in 2020. It is possible these discrepancies are due to differences between metagenomics and culturebased techniques, differences in sampling strategy, or other factors. Gill et al. (2022) observed an increase in potentially pathogenic Aeromonas in gut samples from Plain Pocketbook (Lampsilis cardium) after experimental exposure to mixed agricultural contaminants. It is possible that the Aeromonas represents late-stage opportunistic infections of individuals previously stressed by pathogens, contaminants, or other stressors. Future field studies and experimental infection challenges would aid our understanding of the role of these bacteria in mussel mortality events.

### **DATA AVAILABILITY STATEMENT**

Data for this study are available in Leis et al. (2022) (https://doi.org/10.5066/P9SARYP3 [accessed December 5, 2022]).

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We thank John Fisher from the National Conservation Training Center Library (Shepherdstown, WV, USA) for help locating references. All collections were conducted with required federal, state, and local permits. Samples of Cumberlandian Combshell were collected under the authority of 50 CR 17.21(c)(3)(iii). The use of trade, firm, or product

names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The findings and conclusions in this article are those of the authors and the U.S. Geological Survey and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Appendix A1. Bacteria identified from mussels in the Clinch River, USA. Isolate refers to the number assigned to the mussel sample (number) and the isolate identification (letter). Location refers to the sampling locations as defined in Figure 1. Mussel species abbreviations are as follows: A. ligamentina = Actinonaias ligamentina; A. pectorosa = Actinonaias pectorosa; A. plicata = Amblema plicata; P. fasciolaris = Ptychobranchus fasciolaris; L. fasciola = Lampsilis fasciola; C. tuberculata = Cyclonaias tuberculata; E. brevidens = Epioblasma brevidens; L. ovata = Lampsilis ovata. Genus and species refer to the identifications of bacteria made through BLAST searches in the National Center for Biotechnology Information (NCBI) database (see text). Percent similarity represents the similarity of the isolate sequence to sequences in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi [accessed August 19, 2022]). Length refers to the number of contiguous base pairs in the sequence for each isolate. Accession number refers to the top match or matches identified through the BLAST search of the NCBI database.

		Mussel			%		
Isolate	Location	Species	Genus	Species	Similarity	Length	Accession No(s).
August	16, 2018						
1B	Sycamore Island	A. ligamentina	Microbacterium	testaceum	100	901	MK618600.1
26A	Wallen's Bend	A. ligamentina	Exiguobacterium	antarcticum/acetylicum	100	817	MK478815.1/MH719376.1
26B	Wallen's Bend	A. ligamentina	Unidentifiable				
26C	Wallen's Bend	A. ligamentina	Bacillus	mycoides	100	882	CP037992.1
26D	Wallen's Bend	A. ligamentina	Bacillus	pumilus/zhangzhouensis	100	890	MK696261.1/MK131335.1
27A	Wallen's Bend	A. ligamentina	Bacillus	megaterium/aryabhattai	100	893	MK027252.1/MK618612.1
45A	Kyle's Ford	A. ligamentina	Bacillus	cereus/wiedmannii/thuringiensis	100	778	MK696545.1/MK696254.1/MK696253.1
45C	Kyle's Ford	A. ligamentina	Pseudox anthomonas	japonensis/mexicana	99.6	842	KX588601.1/KF135463.1
45D	Kyle's Ford	A. ligamentina	Novosphingobium	barchaimii	99.8	1212	KM019861.1
45E	Kyle's Ford	A. ligamentina	Fictibacillus	phosphorivorans/nanhaiensis	100	895	MG547923.1/MG049786.1
45F	Kyle's Ford	A. ligamentina	Bacillus	cereus/mycoides/pseudomycoides/ anthracis	99.7	766	MK285635.1/MG694513.1/MG198676.1 MH261153.1
45G	Kyle's Ford	A. ligamentina	Bacillus	mycoides	99.9	816	MK719967.1
45I	Kyle's Ford	A. ligamentina	Pseudomonas	rhodesiae	100	907	MG571697.1
47A	Kyle's Ford	A. ligamentina	Acinetobacter	guillouiae/lwoffii	100	899	MK070050.1/MH930396.1
47B	Kyle's Ford	A. ligamentina	Acinetobacter	guillouiae/johnsonii	99.9	904	MH144279.1/MG788346.1
47C	Kyle's Ford	A. ligamentina	Curtobacterium	sp.	99.2	587	MK704290.1
47D	Kyle's Ford	A. ligamentina	Microbacterium	oxydans	99.7	906	KX083528.1
1D	Sycamore Island	A. ligamentina	Streptomyces	sp.	99.8	908	MH053444.1
2A	Sycamore Island	A. ligamentina	Bacillus	megaterium	100	781	MH071287.1
2B	Sycamore Island	A. ligamentina	Microbacterium	marinum	99.8	901	MF373495.1
2D	Sycamore Island	A. ligamentina	Agrococcus	terreus	99.8	790	MH934923.1
10A	Sycamore Island	A. pectorosa	Arthrobacter	sp.	99.9	707	MG860243.1
10B	Sycamore Island	A. pectorosa	Pseudarthrobacter	defluvii/siccitolerans/scleromae	100	708	MH910272.1/MF681877.1/KY496253.1
13A	Sycamore Island	A. pectorosa	Microbacterium	sp.	90.4	463	HM352378.1
13B	Sycamore Island	A. pectorosa	Bacillus	pumilus/zhangzhouensis	100	1413	MH045860.1/MG651573.1
17A	Wallen's Bend	A. pectorosa	Pantoea	sp.	99.9	900	MH769349.1
17B	Wallen's Bend	A. pectorosa	Yokenella	regensburgei	99.7	705	KR537290.1
17D	Wallen's Bend	A. pectorosa	Microbacterium	oleivorans	99.9	919	HF952706.1
17E	Wallen's Bend	A. pectorosa	Yokenella	regensburgei	100	788	LC383918.1
18A	Wallen's Bend	A. pectorosa	Bacillus	pumilus/zhangzhouensis	100	810	MK696262.1/MK131335.1
18B	Wallen's Bend	A. pectorosa	Curtobacterium	luteum/citreum	100	845	MF959445.1/LT986192.1
18C	Wallen's Bend	A. pectorosa	Bacillus	pumilus/zhangzhouensis	100	890	MK696261.1/MK131335.1
33A	Kyle's Ford	A. pectorosa	Acinetobacter	soli	100	823	MK241870.1
34A	Kyle's Ford	A. pectorosa	Bacillus	cereus/thuringiensis	100	864	KT241012.1/FJ463780.1
34C	Kyle's Ford	A. pectorosa	Pantoea	dispersa	100	872	MF681826.1
34C	Kyle's Ford	A. pectorosa	Erwinia	sp.	100	872	MG681219.1
34D	Kyle's Ford	A. pectorosa	Bacillus	cereus/bingmayongensis/ pseudomycoides	100	875	MK285635.1/MK120869.1/MH578628.1
34E	Kyle's Ford	A. pectorosa	Arthrobacter	pascens/globiformis	99.9	902	HQ530516.1/HQ455821.1
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49A	Frost Ford	A. ligamentina	Bacillus	megaterium	99.3	667	LC269278.1
49B	Frost Ford	A. ligamentina	Bacillus	toyonensis/cereus/thuringiensis	100	816	MK611646.1/MK592620.1/MK583935.1
49C	Frost Ford	A. ligamentina	Bacillus	cereus/mycoides/pseudomycoides	99.9	1368	MK285635.1/MG694513.1/MG198676.1
49D	Frost Ford	A. ligamentina	Bacillus	idriensis	99.4	964	MK240437.1
49E	Frost Ford	A. ligamentina	Lysinibacillus	sphaericus/xylanilyticus/ boronitolerans	99.9	893	MG928532.1/MG905851.1/MF111565.1
49F	Frost Ford	A. ligamentina	Bacillus	thuringiensis	98.5	1103	EU161995.1
49G	Frost Ford	A. ligamentina	Bacillus	pumilus	99.8	509	MK521054.1
49H	Frost Ford	A. ligamentina	Bacillus	mycoides	100	743	MK217080.1
49I	Frost Ford	A. ligamentina	Bacillus	altitudinis/pumilus/ stratosphericus	99.1	980	MK521060.1/MH118525.1/MH910298.1
63A	Frost Ford	A. pectorosa	Bacillus	thuringiensis	99.3	1049	JF421247.1
63B	Frost Ford	A. pectorosa	Bacillus	thuringiensis	98.5	1104	MK491010.1

# Appendix A1, continued.

Isolate	Location	Mussel Species	Genus	Species	% Similarity	Length	Accession No(s).
63C	Frost Ford	A. pectorosa	Bacillus	megaterium/aryabhattai	99.6	826	MH244337.1/MH177254.1
63D	Frost Ford	A. pectorosa	Lysinibacillus	parviboronicapiens/sphaericus	99.8	889	KY038722.1/MF111586.1
63E	Frost Ford	A. pectorosa	Lysinibacillus	sphaericus/fusiformis	98.3	514	JQ835283.1/JQ834699.1
63F	Frost Ford	A. pectorosa	Exiguobacterium	acetylicum/indicum	99.7	732	MK606065.1/MK294298.1
63G	Frost Ford	A. pectorosa	Nocardiopsis	alba	100	802	MH333283.1
	er 26, 2018	The President of the Pr	7				
15A	Speers Ferry	A. pectorosa	Acinetobacter	radioresistens	100	908	MK127547.1
15B	Speers Ferry	A. pectorosa	Citricoccus/Micrococcus	sp.	100	773	MF063312.1/JQ072036.1
15C	Speers Ferry	A. pectorosa	Pseudomonas	monteilii	99.7	310	MH484604.1
15D	Speers Ferry	A. pectorosa	Pseudomonas	putida	100	669	MK064155.1
17A	Speers Ferry	A. pectorosa	Bacillus	cereus/bingmayongensis/	99.9	878	MK285635.1/MK120869.1/MH578628.1
	1 ,	•		pseudomycoides			
17B	Speers Ferry	A. pectorosa	Novosphingobium	lindaniclasticum	99.9	816	MK318596.1
17C	Speers Ferry	A. pectorosa	Bacillus	pseudomycoides	100	1398	MG905900.1
17D	Speers Ferry	A. pectorosa	Pseudomonas	putida	98.6	808	MK064155.1
26A	Sycamore Island	A. pectorosa	Gordonia	hongkongensis/terrae	100	1139	MK277458.1/MH518251.1
26B	Sycamore Island	A. pectorosa	Pontibacter	sp.	95.6	158	KY814729.1
32A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.9	808	LC383918.1
36A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.8	939	LC383918.1
37A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	100	882	LC383918.1
37B	Sycamore Island	A. pectorosa	Gordonia	hongkongensis/terrae	100	1163	MK277458.1/MH518251.1
37C	Sycamore Island	A. pectorosa	Yokenella	regensburgei	100	872	LC383918.1
44A	Wallen's Bend	A. pectorosa	Hannaella	oryzae	99.3	425	NG_063522.1
44B	Wallen's Bend	A. pectorosa	Sphingobium	sp.	99.8	1345	DQ413165.1
44C	Wallen's Bend	A. pectorosa	Microbacterium	chocolatum	100	639	MH748626.1
44D	Wallen's Bend	A. pectorosa	Sphingobium	yanoikuyae	100	603	CP033230.1
46A	Wallen's Bend	A. pectorosa	Bacillus	toyonensis	99.8	1225	KU179349.1
46B	Wallen's Bend	A. pectorosa	Microbacterium	flavenscens	100	692	JQ958839.1
46C	Wallen's Bend	A. pectorosa	Bacillus	cereus/wiedmannii/thuringiensis	100	718	MK696545.1/MK696254.1/MK696253.1
47A	Wallen's Bend	A. pectorosa	Yokenella	regensburgei	99.8	794	LC383918.1
49A	Wallen's Bend	A. pectorosa	Oceanobacillus	massiliensis	99.9	887	HQ586893.1
49B	Wallen's Bend	A. pectorosa	Microbacterium	oxydans/foliorum	99.5	894	MK696389.1/MK696388.1
49C	Wallen's Bend	A. pectorosa	Pseudomonas	putida/guariconensis	100	794	MK534000.1/MK318649.1
50A	Wallen's Bend	A. pectorosa	Yokenella	regensburgei	100	694	LC383918.1
50B	Wallen's Bend	A. pectorosa	Oceanobacillus	massiliensis	100	766	KU727149.1
50C	Wallen's Bend	A. pectorosa	Pseudomonas	cichorii	99.9	878	KU923374.1
59A	Kyle's Ford	A. pectorosa	Yokenella	regensburgei	99.3	947	KR537290.1
59B	Kyle's Ford	A. pectorosa	Chromobacterium	haemolyticum	99.4	869	LT628074.1
63A	Kyle's Ford	A. pectorosa	Pseudomonas	monteilii/plecoglossicida/putida	100	863	MH997643.1/MH997642.1/MH997641.1
63B	Kyle's Ford	A. pectorosa	Acinetobacter	schindleri	100	1399	CP025618.2
63C	Kyle's Ford	A. pectorosa	Pseudomonas	parafulva	99.9	1398	CP009747.1
63D	Kyle's Ford	A. pectorosa	Pseudomonas	monteilii/putida	100	1384	KU862315.1/KT259326.1
64A	Kyle's Ford	A. pectorosa	Acinetobacter	schindleri	100	1201	MG461629.1
64B	Kyle's Ford	A. pectorosa	Acinetobacter	sp.	99.8	829	MG517420.1
64C	Kyle's Ford	A. pectorosa	Serinibacter	sp.	98.9	368	LC203065.1
66A	Kyle's Ford	A. pectorosa	Yokenella	regensburgei	99.9	891	KT260720.1
66B	Kyle's Ford	A. pectorosa	Rhizobium/Agrobacterium	sp./larrymoorei	99.2	1351	JQ660121.1/JN084151.1
67A	Kyle's Ford	A. pectorosa	Microbacterium	proteolyticum	99.9	903	MK281612.1
70A	Kyle's Ford	A. pectorosa	Acinetobacter	sp.	99.9	1385	MG517420.1
70B	Kyle's Ford	A. pectorosa	Pseudomonas	sp.	99.6	1333	MK680062.1
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006A	Artrip	A. pectorosa	Knoellia	flava	100	640	MG205530.1
016A	Speers Ferry	A. pectorosa	Cellulosimicrobium	cellulans	99.9	915	MH718838.1
016B	Speers Ferry	A. pectorosa	Massilia	timonae	100	901	MF592282.1
016C	Speers Ferry	A. pectorosa	Bacillus	pumilus/aerius/stratosphericus/	99.9	885	MH261170.1/MK629822.1/MH261171.1
0			D :	altitudinis	100		/MH261156.1
016D	Speers Ferry	A. pectorosa	Bacillus	safensis/altitudinis/pumilus	100	1412	MK285608.1/MK241863.1/MG597491.1
021A		A. ligamentina	Cutibacterium	acnes	100	418	CP033842.1
023A		A. pectorosa	Bacillus	zhangzhouensis/pumilus	100	881	MK583949.1/MH819709.1
027A		A. pectorosa	Actinotalea	solisilvae	100	832	NR_159882.1
028A	Speers Ferry	A. pectorosa	Yokenella	regensburgei	100	850	LC383918.1
028B	Speers Ferry	A. pectorosa	Bacillus	aerius/altitudinis/pumilus	100	839	MG996801.1/MK521068.1/MK521063.1

## Appendix A1, continued.

Icolata	Location	Mussel	Ganus	Species	% Similarity	Lanath	Accession No(s)
Isolate	Location	Species	Genus	Species	Similarity	Length	Accession No(s).
028gA	Speers Ferry	A. pectorosa	Yokenella	regensburgei	100	831	LC383918.1
044A	Sycamore Island	A. pectorosa	Pseudomonas	kribbensis/protegens	100	768	MK240439.1/LT628144.1
044B	Sycamore Island	A. pectorosa	Pseudomonas	kribbensis/reinekei/syringae/ fluorescens	100	819	MK240439.1/MK392138.1/CP024646.1/ CP022313.1
049A	Sycamore Island	A. ligamentina	Unidentifiable				
049B	Sycamore Island	A. ligamentina	Knoellia	flava	99.9	845	MG205530.1
049C	Sycamore Island	A. ligamentina	Pseudarthrobacter	oxydans/siccitolerans	100	812	MG694475.1/MF681913.1
049D 049E	Sycamore Island Sycamore Island	A. ligamentina	Microbacterium Bacillus	phyllosphaerae pumilus	98.7 99	832 799	KC355288.1 MH261008.1
051A	Sycamore Island	A. ligamentina A. pectorosa	Bacillus	cereus/bingmayongensis/ pseudomycoides/mycoides/ subtilis	100	793	MK285635.1/MK120869.1/MH578628.1 MH422001.1/MH422001.1
051B	Sycamore Island	A. pectorosa	Pseudomonas	putida	99.6	550	MH298490.1
051C	Sycamore Island	A. pectorosa	Pseudomonas	taiwanensis/putida/parafulva	100	686	MK598329.1/CP030750.1/MH304303.1
057A	Sycamore Island	A. pectorosa	Pseudomonas	helmanticensis	100	664	MK070159.1
057B	Sycamore Island	A. pectorosa	Bacillus	gibsonii	100	759	MH910172.1
068A	Kyle's Ford	A. pectorosa	Knoellia	flava	99.7	770	MG205530.1
068B	Kyle's Ford	A. pectorosa	Rhodococcus	sp.	100	790	KY922741.1
068C	Kyle's Ford	A. pectorosa	Arthrobacter	halodurans/aurescens	100	705	MK424278.1/KR611860.1
068D	Kyle's Ford	A. pectorosa	Rhodococcus	gingshengii/erythropolis	100	886	MH938043.1/MK371078.1
068E	Kyle's Ford	A. pectorosa	Microbacterium	sp.	99.4	1392	JQ977333.1
069A	Kyle's Ford	A. pectorosa	Microbacterium	aureliae	99.7	992	MK226317.1
069B	Kyle's Ford	A. pectorosa	Terrabacter	terrigena/koreensis/tumescens	100	732	MF681978.1/NR_134212.1/JQ342911.1
073A	Kyle's Ford	A. pectorosa	Curtobacterium	oceanosedimentum/luteum/	100	760	MK618608.1/MK618607.1/MK618606.1
				herbarum/citreum			MK618605.1
073B	Kyle's Ford	A. pectorosa	Microbacterium	lemovicicum	100	721	CP031423.1
074A	Kyle's Ford	A. pectorosa	Leucobacter	tardus	99.7	770	NR_042694.1
077A	Kyle's Ford	A. pectorosa	Yokenella	regensburgei	100	824	LC383918.1
077B	Kyle's Ford	A. pectorosa	Plantibacter	flavus	99.9	1230	LN774386.1
October 4,		D C . 1 .	r · · · · · · · · · · · · · · · · · · ·	C . C	00.0	017	MECO5500 1
16A	Sycamore Island	P. fasciolaris	Lysinibacillus	fusiformis	99.9	917	MT605500.1
19A	Sycamore Island	L. fasciola	Curtobacterium	ammoniigenes	99.9	809	KP296215.1
21A 26A	Sycamore Island Sycamore Island	A. ligamentina A. plicata	Falsirhodobacter Bacillus	halotolerans	100 100	701 390	LN774250.1 MT647568.1
26B	Sycamore Island	A. plicata	Bacillus	paramycoides cereus	100	934	LR215149.1
28A	Sycamore Island	A. pectorosa	Oerskovia	paurometabola	100	1266	KX034798.1
28B	Sycamore Island	A. pectorosa	Bacillus	aryabhattai	99	1336	MK519060.1
28C	Sycamore Island	A. pectorosa	Agrococcus	terreus	99.9	856	MH934923.1
28D	Sycamore Island	A. pectorosa	Microbacterium	paludicola	99.8	536	MT733951.1
29A	Sycamore Island	A. pectorosa	Acinetobacter	sp.	75	803	AY922105.1
29B	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.6	1312	MT505131.1
29C	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.8	1345	MT505131.1
31A	Sycamore Island	L. ovata	Staphylococcus	hominis subsp. novobiosepticus	100	1262	MT585539.1
33A	Sycamore Island	A. plicata	Micrococcus	yunnanensis	100	855	MT033093.1
33B	Sycamore Island	A. plicata	Staphylococcus	hominis subsp. novobiosepticus	100	840	MT544813.1
33C	Sycamore Island	A. plicata	Agrococcus	terreus	99.5	913	JN585711.1
33D	Sycamore Island	A. plicata	Micrococcus	yunnanensis	100	836	LT978429.1
33E	Sycamore Island	A. plicata	Staphylococcus	epidermidis	99.4	883	KM972394.1
35A	Sycamore Island	A. ligamentina	Micrococcus	yunnanensis	99.9	828	KF758820.1
40A	Sycamore Island	A. ligamentina	Exiguobacterium	aurantiacum	99.4	837	MT225757.1
40B	Sycamore Island	A. ligamentina	Staphylococcus	epidermidis	100	1348	MT613456.1
40C	Sycamore Island	A. ligamentina	Citricoccus	zhacaiensis	100	1139	MG025801.1
40D	Sycamore Island	A. ligamentina	Massilia	oculi	99.9	1308	CP029343.1
October 7,							
1A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.8	1335	MT505131.1
1B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Kocuria	rhizophila	100	847	MT377849.1
2A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Aeromonas	veronii	100	910	MT384380.1
2B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Aeromonas	allosaccharophila	100	887	MN216261.1

# Appendix A1, continued.

		Mussel			%		
Isolate	Location	Species	Genus	Species	Similarity	Length	Accession No(s).
3A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.9	1372	MT505131.1
3B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	100	819	MT505139.1
4A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.9	1342	MT505131.1
4B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Aeromonas	veronii	100	1375	MT384380.1
5A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Chryseobacterium	sp.	96.8	704	KU360139.1
5B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.8	1372	MT505131.1
5C	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	100	904	MT505139.1
6A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.9	1362	MT505131.1
6B	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.7	1362	MT505131.1
7A	Speers Ferry/Sycamore Island/Clinchport	A. pectorosa	Yokenella	regensburgei	99.7	1375	MT505131.1
October 20,	-						
ALI-1A	Sycamore Island	A. ligamentina	Yokenella	regensburgei	99.9	698	MT505139.1
ALI-1B	Sycamore Island	A. ligamentina	Aeromonas	hydrophila	100	387	MK880338.1
APE-1A	Sycamore Island	A. pectorosa	Aeromonas	hydrophila	100	549	MK880338.1
APE-1B	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.9	791	MT505139.1
APE-2A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	100	636	MT505139.1
APE-2B	Sycamore Island	A. pectorosa	Pseudomonas	poae	100	758	MT631989.1
APE-2C	Sycamore Island	A. pectorosa	Aeromonas	veronii	100	760	MT384380.1
APE-3A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	100	700	MT505139.1
APE-3B	Sycamore Island	A. pectorosa	Aeromonas	caviae	100	578	MN481052.1
APE-4A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.7	762	MT505131.1
APE-4B	Sycamore Island	A. pectorosa	Aeromonas	veronii	97.7	303	CP012504.1
APE-6A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.7	653	MT505131.1
APE-6B	Sycamore Island	A. pectorosa	Stentrophomonas	pavanii	95.6	484	MH810095.1
APE-6C	Sycamore Island	A. pectorosa	Pseudomonas	putida	100	811	CP050951.1
APE-7A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.8	1063	MT505131.1
APE-7B	Sycamore Island	A. pectorosa	Aeromonas	hydrophila	99.8	493	MK880338.1
APE-8A	Sycamore Island	A. pectorosa	Yokenella	regensburgei	100	751	MT505139.1
APE-8B	Sycamore Island	A. pectorosa	Aeromonas	aquatilis	100	765	LT630765.1
APE-9A	Sycamore Island	A. pectorosa	Aeromonas	hydrophila	100	452	MK880338.1
APE-9B	Sycamore Island	A. pectorosa	Yokenella	regensburgei	99.9	794	MT505139.1
EBR-1A	Sycamore Island	E. brevidens	Yokenella	regensburgei	88	673	MT505131.1
EBR-1B	Sycamore Island	E. brevidens	Aeromonas	caviae	100	467	MN481052.1
EBR-1C	Sycamore Island	E. brevidens	Flavobacterium	tructae	99.9	759	NR_133749.1