Identification of Motile Aeromonads Isolated From Study Number AEH-09-MAS-02

Administrative Report: Study Number AEH-11-MAS-03

Test facility:
Upper Midwest Environmental Sciences Center (UMESC)
U.S. Geological Survey, Department of the Interior
2630 Fanta Reed Road
La Crosse, Wisconsin 54603
Phone: (608) 783-6451
Fax: (608) 783-6066

Report Authors

Christopher B. Rees[1], Christopher M. Merkes[1], Maren T. Tuttle-Lau[2]

Study Director: Mark P. Gaikowski
Principal Investigator: Christopher B. Rees

Experimental Start Date: December 5, 2013
Experimental Termination Date: April 2, 2014

[1] U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603, Phone: (608) 783-6451, Fax: (608) 783-6066

[2] U.S. Fish & Wildlife Service, Whitney Genetics Laboratory, 555 Lester Avenue, Onalaska, Wisconsin 54650, Phone: (608) 783-8403, Fax: (608) 783-8450

U.S. Department of the Interior
SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2015

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Introduction

The unstable taxonomic position of motile aeromonads led to the transfer of many organisms that were associated with hemorrhagic septicemias in fish within the genera *Bacillus*, *Pseudomonas*, *Proteus*, and *Aerobacter* into the new genus *Aeromonas* (Cipriano, 2001). Cipriano (2001) reported that Snieszko suggested dividing the genus into three species: *A. hydrophila*, *A. punctata*, and *A. liquefaciens*. In this scheme, *A. liquefaciens* contained most of the fish pathogens. Schubert (1967) confirmed that there was enough biochemical similarity to establish the genus *Aeromonas* but invalidated species-specific considerations. Later, Popoff and Vernon (1976) demonstrated that motile aeromonads could be classified into two distinct species: *A. hydrophila* (composed of the organisms previously described as *A. punctata* and *A. liquefaciens*) and a new species named *A. sobria*.

Four species were originally described in the genus *Aeromonas*: *A. hydrophila*, *A. caviae*, *A. sobria*, and *A. salmonicida* (Popoff, 1984). The four species defined in the genus *Aeromonas* are separated into two well-defined groups. The psychrophilic and nonmotile aeromonads are clustered in the first group, which contain *Aeromonas salmonicida*. A second group is also recognized, consisting of mesophilic and motile bacteria divided into three species: *A. hydrophila*, *A. caviae*, and *A. sobria* (Popoff and Vernon, 1976; Popoff, 1984). Only five species of *Aeromonas* were originally recognized, three of which (*A. hydrophila*, *A. sobria*, and *A. caviae*) contain multiple DNA groups; hence, some members cannot be distinguished from one another by simple biochemical characteristics (Abbott and others, 2003). At present, the genus *Aeromonas* is reported to be represented by at least 14 genetically distinct species, including *A. hydrophila*, *A. bestarium*, *A. salmonicida* (non-motile psychrophilic and motile mesophilic biogroups), *A. caviae*, *A. media*, *A. eucrenophila*, *A. sobria*, *A. veronii* (with biotypes sobria and veronii), *A. jandaei*, *A. trota*, *A. schubertii*, *A. encheleia*, *A. allosaccharophila*, and *A. popoffii* (Janda, 1991; Martínez-Murcia and others, 1992a; Joseph and Carnahan, 1994; Esteve and others, 1995; Borrell and others, 1997; Huys and others, 1997a, 1997b; Orozova and others, 2009). Of these, *A. hydrophila*, *A. bestarium*, *A. salmonicida*, *A. veronii* biotype sobria, *A. caviae*, and *A. jandaei* have been reported as fish pathogens in the United States and throughout the world (Torres and others, 1993; Esteve and others, 1995; Ogara and others, 1998; Nielsen and others, 2001; Kozinska and others, 2002; Rahman and others, 2002).

Two clinical trials evaluating medicated feed effectiveness were performed at Spirit Lake Fish Hatchery in Spirit Lake, Iowa, as part of the study AEH-09-MAS-02 titled "Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 for Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to motile aeromonad infections." To determine the etiological agents of the infections that occurred for those trials, bacteria were isolated from necropsied fish and identified using biochemical and genetic methods.

Materials and Methods

Good Clinical Practices Compliance Statement

All sample handling and data collection, storage, and retrieval procedures for this study were conducted in compliance with Food & Drug Administration (FDA) regulations for Good Clinical Practices (GCP; 21 CFR).

Pipette Verification

All pipettes used in this study were verified for accuracy by following the "Procedure for Evaluating Accuracy and Precision of RAININ Pipettes: Factory-Approved Method for Using Gravimetric Analysis" as outlined in Mettler-Toledo Document AB-15 prior to working with samples (RAININ, 2009). Briefly, 2-, 10-, 20-, 200-, and 1000-µL pipettes were calibrated by adding selected amounts of deionized (DI) water at a temperature of 18 ± 0.2 °C in an appropriate weighing vessel to a zeroed Sartorius Balance Model:200D (S/N: 20903620, Sartorius Corporation, Bohemia, NY). Calibration limits for relative standard deviation (RSD, between replicate aspirations) and mean mass followed guidelines as outlined in Mettler-Toledo Document AB-15.

Acquisition of Samples

During necropsy of selected mortalities from study number AEH-09-MAS-02, kidney stabs were streaked onto Tryptic-Soy Agar (TSA) plates that were incubated at 30 °C for 24 hours. Colonies with morphology characteristic of motile aeromonads (cream to gray color, shiny, smooth, round, and raised) were subcultured on new TSA plates. Single colonies from the subcultured plates were grown on Rimler-Schotts (RS) selective media plates. A total of 75 pure bacterial isolates with yellow growth from RS media plates were stored in a microbank system (Pro-Lab Diagnostics, Austin, TX) at −80 °C (So-Low Ultra-Low model #PR120-17, S/N: 06071105; So-Low Environmental Equipment Company, Cincinnati, OH). Isolates were dated and named for the fish they were collected from in the format of "TANK-FISH." The tanks containing the fish were numbered A1–A9 and B1–B9. The fish were numbered sequentially as mortalities were removed from tanks throughout the study. When multiple colonies were isolated from the same fish, an "a" or "b" was added to the isolate name to differentiate between them. The year that bacteria were isolated was added to isolate names electronically to distinguish between those of the first or second trial. Two cultures were labeled as B7-15b-2012, so red or yellow were added to those isolate names, based on the microbank bead colors for those isolates to differentiate them.

Recovery of DNA from Archived Isolates

Samples were handled with aseptic technique. After thawing on ice, an individual Microbank bead (Pro-Lab Diagnostics, Austin, TX) was removed from each tube using sterile forceps (flame sterilized) and transferred to a new 1.75-mL tube containing 100 µL 10 mM tris (pH 8.0; Amresco, Solon, OH). Immediately after transfer, original isolates were placed back in archive storage. Bacteria were lysed by incubating the tubes containing a single Microbank bead for 5 minutes at 95 °C. Resulting lysates were then frozen and stored at −80 °C (Panasonic Ultra Low model #MDF-U76VC-PA, S/N: 12057J0201; Panasonic Healthcare Corporation, Wood Dale, IL).

Amplification of PCR products

Of the 75 individual lysates obtained, preliminary PCR amplification on 5 random lysates was performed to confirm that bacterial DNA could be amplified from the lysates without further culturing or DNA purification. Random samples were selected by using the "sample" command in R statistical package, version 3.0.0. Two markers were chosen for species identification in this study, 16S ribosomal RNA and RNA polymerase sigma D factor (rpoD). Amplification primers

for 16S rRNA and rpoD were synthesized (Integrated DNA Technologies, Coralville, IA) based on those used by Puthucheary and others (2012), designed by Borrell and others (1997) and Yamamoto and Harayama (1998). These primers are listed in Table 1. Primers were diluted with Tris-EDTA buffer (pH 8.0; Amresco, Solon, OH) to a 100-µM stock. Forward and reverse primer pairs were mixed and diluted to a 10-µM working solution with molecular biology grade water. PCR reactions with a 25-µL total volume were set up as follows for 16S rRNA and rpoD amplification: 12.5 µL 2x MangoMix (Syngenta Bioline, Oxnard, CA), 2.5 µL of 10 µM forward and reverse primer mixture, 9 µL deionized H_2O, and 1 µL template. The PCR cycling temperature profile was the same for both genes, other than the annealing temperatures (16S rRNA: 56 °C, rpoD: 59 °C). The temperature cycling profile consisted of an initial incubation at 95 °C for 2:00 minutes, followed by 35 cycles of 95 °C for 30 seconds, annealing temperature for 30 seconds, and 72 °C for 1 minute, an additional extension incubation at 72 °C for 5 minutes, and an indefinite hold at 4 °C.

Table 1. Primers used for PCR amplification of sequencing products

[Table showing the oligonucleotide sequences used for PCR amplification. Abbreviations: Adenine (A), Cytosine (C), Guanine (G), Thymine (T), Forward (Fwd), Reverse (Rev)]

Gene	Primer Name	Primer Sequence
16S Ribosomal RNA	16S-Fwd	AGAGTTTGATCATGGCTCAG
	16S-Rev	GGTTACCTTGTTACGACTT
RNA Polymerase Sigma D Factor	rpoD-Fwd	ACGACTGACCCGGTACGCATGTA
	rpoD-Rev	ATAGAAATAACCAGACGTAAGTT

Agarose Gel Electrophoresis and Product Purification

All PCR products following amplification were analyzed by electrophoresis on 1% agarose (IBI #13F4004) in Tris Acetate EDTA (TAE) gel at 10 V cm^{-1}. Product size confirmation was accomplished by co-migrating a GeneRuler 100-bp DNA ladder (Thermo Scientific, Waltham, MA). Reactions that result in a single sharp band of appropriate size after gel analysis were cleaned up using the Qiaquick PCR Purification Kit (Qiagen Inc., Valencia, CA). In the event that multiple products were detected (excluding primer dimers), the remaining product was loaded onto a separate gel and electrophoresed, and the appropriate sized band was excised and purified using the Qiaquick Gel Extraction Kit (Qiagen Inc., Valencia, CA). These gel-purified products were then verified by PCR and gel electrophoresis to ensure the band of the predicted size was harvested. Products detected of the incorrect size due to off-target amplification were disregarded.

Sequencing of purified products

Cycle sequencing reactions were carried out using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems [now Life Technologies], Valencia, CA). Briefly, reactions were assembled as follows: 1 µL of 1 µM forward or reverse sequencing primer mix (these were gene-specific primers based on 16S rRNA and rpoD sequences), 0.75 µL BigDye Terminator, 1.5 µL 5x buffer, 2.25 µL deionized H_2O, 5 µL DNA template for a total reaction volume of 10.5 µL. The cycling parameters administered included an initial 96 °C incubation for 2 minutes, followed by 35 cycles of 96 °C for 10 seconds, annealing temperature (16S rRNA: 52 °C, rpoD: 59 °C) for 15 seconds, and 60 °C for 3 minutes, an additional extension step at 72 °C

for 1 minute, and then incubation at 4 °C indefinitely. All cycle-sequenced plates were then shipped on ice to the University of Wisconsin-Madison biotechnology Center (UWBC) for Sanger sequencing. UWBC staff are recognized as experts in Sanger sequencing, so they followed standard procedures to generate reliable data. Raw sequencing data were extracted from UWBC servers and then were quality trimmed and aligned to known motile aeromonad 16S rRNA and rpoD sequences at UMESC using BioEdit, version 7.2.0 (Hall, 1999).

Species Determination

After alignment to known aeromonad 16S rRNA and rpoD sequences, unknown sequences from specific isolates were carefully compared to characterize polymorphisms. Methods used to speciate isolates followed that of Wakabongo and others, 1992; Borrell and others, 1997; Yamamoto and Harayama, 1998; Soler and others, 2004; Alperi and others, 2008; Beaz-Hidalgo and others, 2010; and Puthucheary and others, 2012. Systematic comparison of polymorphisms was used to determine species of all aeromonad isolates. Non-aeromonad isolate sequences obtained were blasted against all known DNA sequences in GenBank to determine identity at least to genus and to species if possible.

Study Records and Archiving

All raw data, including the laboratory notebooks, electronic files, test material information, and other records pertaining to the study prepared by the Study Director or study staff are retained in the UMESC archives.

Results

The majority (58 of 75) of isolated bacteria were found to be a mixture of different species within the *Aeromonas* genus. Among the 58 *Aeromonas* spp. isolates, 40 were identified as *A. allosaccharophila*, 14 as *A. veronii*, 2 as *A. sobria*, 1 as *A. media*, and 1 as *A. salmonicida*. The 17 isolates outside the *Aeromonas* genus were determined to be *Acinetobacter sp.*, *Klebsiella oxytoca*, *Plesiomonas shigelloides*, or *Pseudomonas sp.* A summary of the isolate identification is in Table 2. The accession numbers of the sequences used for comparison to make species determinations are in Table 3. The 16S sequences obtained are in Table 4, and the rpoD sequences obtained are in Table 5 in accompanying files Table4.xlsx and Table5.xlsx.

Table 2. Speciation results of selected isolates

[Table showing how many and which bacterial isolates identified to each species or genus (n = 75 isolates total). Abbreviations: *Aeromonas* (*A.*)]

Species	Count	Isolates
Aeromonads		
A. allosaccharophila	40	A1-6-2012, A2-2-2012, A2-29-2011, A3-3-2012, A3-4-2012, A3-5-2012, A4-25-2012, A4-27-2012, A5-38-2012, A5-5-2011, A7-1-2012, A7-26-2012, A7-3-2012, A7-5-2012, A7-6-2012, A8-22b-2012, A8-3-2012, A8-45-2011, B1-23-2012, B1-3-2012, B1-5-2012 , B1-9a-2012, B2-8-2012, B3-2-2012, B3-3-2012, B3-5-2012, B4-25a-2012, B4-25b-2012, B5-14-2012, B5-3a-2012, B5-4-2012, B5-5-2012, B5-8-2012, B7-2-2012, B8-1-2012, B8-12-2012, B8-3a-2012, B8-6-2012, B9-1b-2012, B9-6b-2012
A. media	1	A9-10-2011
A. salmonicida	1	A9-01-2011
A. sobria	2	B6-15-2012, B9-1a-2012
A. veronii	14	A2-25-2012, A4-31-2011, A7-22-2011, B1-20a-2011, B1-42-2011, B1-46-2011, B2-25- 2011, B2-26-2011, B3-41-2011, B4-21-2011, B5-13-2012, B6-33-2011, B6-35-2011, B7-37-2011
Non-Aeromonads		
Acinetobacter sp.	1	B6-10-2011
Klebsiella oxytoca	1	A4-02-2011
Plesiomonas shigelloides	12	A1-10a-2011, A4-21-2012, A8-21a-2012, A8-21b-2012, A8-25-2012, A8-4-2012, B2-12-012, B7-14b-2012, B7-15b-red-2012, B7-15b-yellow-2012, B8-11-2012, B8-38-2012
Pseudomonas sp.	3	A1-086-2011, A1-8a-2011, B3-1-2011

Table 3. Accession numbers used for species determination

Species	Accession Numbers Used for Speciation – 16S rRNA	Accession Numbers Used for Speciation – rpoD
A. allosaccharophila	NR_025945, GU205199, GU205192, FJ940841, S39232, GU722154, KC816585, KC884672, KC884670, KC130967, KC130961, FJ464571, FJ464569, JF920535, JF920534, JF920533, FJ233858, KF625182, KC914539	AY169348, JN412629, FN773344, FN773342, KC865055, JN663976, HQ442836, HQ442834, HQ442828, HQ442827, HQ442826, FN808214, AY185586, AY185585, DQ411507, HQ442825
A. aquariorum	EU085557, JX308270, JQ034592, JQ034591, JF920491, JF920490, JF920489,JF920488, JF313412, KF307776, KC840875, KC840857	EU268457, EU268461, EU268460, EU268459, EU268458, EU268456, AB765402, AB765401, JF972632, JF972631, HQ442798, FJ936132, HE965647, HE965646, HE965645, HE965644, FR682782, FR681596, FR681595, FR681594, FR681593, FR681592, FR681591, FR681590, FR681589, FR675894, FR675893, FR675892, FR675891, FR675890
A. bestiarum	NR_026089, X60406, AB034759, AY987757, AY987756, AY987755, AY987750, AY987749, FM999977, FM999976, FM999975, KF625176, KF625168	AY987694, AY987693, AY987692, AY987686, AY987687, AY169326, HQ442854, FN773343, FN773317, AY129000, JN712346, JN712345, JN712344, JN712343, JN712342, JN712341, JN712340, JN712339, JN712338, JN712337, JN712336, JN712335, JN712334, JN712333, JN712332, JN712331, JN712330, JN712329, JN712328, JN712327
A. bivalvium	NR_043885, DQ504429, DQ504430	EF465512, HQ442817, JN412628, FN773318, EF465511, HQ442818
A. cavernicola	N/A	HQ442864
A. caviae	X60408 S4286, AB626132, AB034760, KF158411, KC776584, X60409 S42862, KC776583	KC601657, JF738015, JF738017, JF738016, JF738014, JF738013, JF738012, JF738011, JF738010, JF738008, JF738005, HQ442790, JF738021
A. culicicola	AF170914, AY347680, AY130991, AY130992, AY347682, AY347681, AY347679, Y347677, AY347678	N/A
A. diversa	N/A	HQ442806, HQ442805
A. encheleia	HQ832414, NR_041962, AJ458416, AJ458415, AJ458414, AJ458409, AJ458408, AJ224309, KC884669	AY169346, HQ442778, AY169343, AY249197, FN773320, AY129003, HQ442780, HQ442779, HQ442777, HQ442776, HQ442775, FN398051
A. enteropelogenes	AY987726, FJ940845, EF465529, NR_044846, FJ940844, AY987742, FJ940843, FJ940842, FJ940838, KC122705, GQ304779, JF920494, JX188075, JN644602	AY987679, AY987663, AY169344, EF465508, HQ442824, HQ442823, HQ442822, HQ442821, HQ442820, HQ442819, AY249198, JN544576, FN773339, AB828767, KC601658
A. eucrenophila	AJ458411, JF920496, X60411 S42864	AY169339, HQ442770, FN773321, AY129002, HQ442774, HQ442773, HQ442772, HQ442771, FN645422
A. fluvialis	NR_116586, FJ230078	FJ603453
A. guangheii	AB028881	N/A
A. hydrophila	NR_043638, AY538658, KC210773, KC210804, KC210763, KC210762, JX029046, KC150866, JN561162, GU205194, GU205191, GU205190, X60404 S42857, GU563992, FJ940839, FJ940826, FJ940823, FJ940799, FJ940795, EU932956	AY987691, AY987678, AY987676, AY987674, AY987673, AY987672, AY987671, AY987670, AY987669, AY169325, AB526565, HQ442791, HE965651, HE965650, HE965649, HE965648, JN182269, JN182266, FR681892, KC601655
A. hydrophila decolorationis	AY686711	N/A

Species	Accession Numbers Used for Speciation – 16S rRNA	Accession Numbers Used for Speciation – rpoD
A. hydrophila dhakensis	NR_042155, AM262157, AJ508765	EF465510, HE965643, HE965642, HE965641, HE965640, HE965639, HE965638, HE965637, HE965636, HE965635, KC601656
A. hydrophila hydrophila	AB626121, AB472951, NR_074841	KC601654
A. hydrophila ranae	AJ508766, NR_042518, AM262151	EF465509
A. jandaei	NR_037013, X60413 S42867, FJ940832, FJ940830, FJ940821, FJ940816, FJ940814, FJ940805, FJ940804, JQ040105, JF920538, JF920537, JF920536, JF313413, FJ233860, AY987745, KF358439, KF358438, KF358437, KC906261, KC906260, KC906259	AY987682, AY169341, HQ442840, JN182268, FR682798, FN773327, FN773326, FN773323, AB828758, HQ442842, HQ442841, HQ442839, HQ442838, HQ442837, FN773329
A. kambli	HM452127	N/A
A. media	NR_036911, KC210759, KC210758, GU205201, FJ940840, FJ940831, X60410 S42863, FJ940809, FJ168773, FJ940794, EU488684, EU488683, EU488682, EU488681, AF418219, AF418218, AF418217, AF418216, AF418215, AF418214, GU174505, GU174504, GU174503, AM262152, AM262150, AM262149, AY928481, KC130966, KC130965, FJ464587	AY987710, AY987684, AY169338, HQ442785, FR682800, FR682799, FN773332, FN773324, KC601660, KC601659, JN663948, JN663941, JN663940, JN663938, JN663936, JN663931, JN663922, JN663918, JN663915, JN663911, JN663910, JN663907, JN663906, JN663905, JN663903, HQ442784, HQ442783, HQ442782, HQ442781, EU488680
A. molluscorum	NR_025807, AY532690, AY532692, AY532691, AY532689, AY532688, JX103508, AY987772	AY987709, EF465515, HQ442812, DQ411504, FN773325, EF465517, EF465516, EF465514, EF465513, HQ442816, HQ442815, HQ442814, HQ442813
A. piscicola	HQ832417, FM999973, KC130962, FM999974, FM999972, FM999971, FM999970	HQ442859, JN712349, JN712348, JN712347, FM999071, JN215538, HQ442863, HQ442862, HQ442861, HQ442860
A. popoffii	HQ832415, NR_025317, GU205196, AJ223181, AJ223180, AJ224308, AY987777, FM999979, FM999978, AY534350, KF578025	AY987721, AY169347, JN712350, HQ442853, FN773336, JN712356, JN712355, JN712354, JN712353, JN712352, JN712351, HQ442852, HQ442851, HQ442850, HQ442849, AY185584, AY185583, AY185582, AY185581, AY169367, AY169349
A. punctata	GU205197, GU205198, GU205200, AB472939, NR_029252, AB472999, AB472940, AB473012, AB473000, AB473007, AB473002, AB473015, EU770300, FJ940829, FJ940815, FJ940812, FJ940803, FJ940800, FJ940806, FJ940797	AY987720, AY987698, AY987697, AY987696, AY987695, AY987665, AY987664, AY987662, AY987660, AY169337, AB526579, AB526576, AB526571, AB526566, AB526563, AB526505, AB526504, AY129001, EU488678
A. rivuli	FJ976900, FJ976899	FJ969433, FJ969437
A. salmonicida	FJ936134, AM931169, KC210807, EU932930, KC210757, KF478208, KF478201, KF364949, KC884674, KC884668, KC884667, KC884666	FJ936138, HQ442843, JN388922, JN388921, JN388920, JN388919, JN388918, JN388917, FN773331, FN773330, JN712431, JN712430, JN712429, JN712428, JN712427, JN712426, JN712425, JN712424, JN712423, JN712422, JN712421, JN712420, JN712419, JN712418, JN712417, JN712416, JN712415
A. salmonicida achromogenes	AY910844,NR_037011, HQ283362, X60407 S42860, AB027543	AY169329
A. salmonicida flounderacida	AY786177, AY786178	N/A
A. salmonicida masoucida	AM296506, AB680514, AB027542	JN712432

Species	Accession Numbers Used for Speciation – 16S rRNA	Accession Numbers Used for Speciation – rpoD
A. salmonicida pectinolytica	NR_025001	N/A
A. salmonicida salmonicida	NR_074844, NR_043324, X60405 S42858, EF502001, AB027541	AY987688, AB504902, AY169327, GU734699
A. salmonicida smithia	NR_025295, AB027544	AY169331, JN712433
A. sanarellii	FJ230076, JF920493, JF920492	FJ807275, FJ472929, JN664018, JN664016, JN664013, JN663995, JN663993, JN663967, JN663953, JN663952, AB844711, AB844709
A. schubertii	NR_037014, GQ845453, GQ845452, GQ845451, GQ845450, GQ844302, GQ844303, GQ844301, GQ844300, X60416 S42870, HQ541165, HQ541164, HQ407423, JQ319029, FJ233866, AY987730, KF307773	AY987667, AY169336, HQ442809, FR865967, JN663954, HQ442808, HQ442807, AY169345
A. sharmana	NR_043470, DQ013306	EF465518
A. simiae	NR_025585, GQ860945, GQ860944, AJ536821, AJ536820	HQ442811, DQ411508, HQ442810, GQ860943
A. sobria	NR_037012, AB472942, KC776587, KC210798, KC776588, KC210785, KC210786, KC210761, GU187060, X60412 S42865, KC816586, JN120266, JN120310, JN120309, JN120308, JN120306, JN120305, JN120304, JN120303, JN120301, JN120300, JN120299, JN120298, JN120297, JN120296, JN120295, JN120294, JN120293, JN120292, JN120291	AY169340, HQ442867, JN412625, HQ442868, HQ442866, HQ442865, FN645424, FN773338, FN398050, FN398049, FN398048, FN398047, FN398046, AB526584, AB526581
A. taiwanensis	FJ230077,JF920495	FJ807271, FJ472928, JN663951, JN663939, JN663898, AB844712, AB844710
A. tecta	HQ832416	HQ442762, FJ936133, FN773337, FJ847838, HQ442764, HQ442763, HQ442761, HQ442760, FN398052
A. trota	X60415 S42871	N/A
A. veronii	EU770295, KC660990, AB472950, JQ013893, KC776586, KC210805, KC660987, KC210797, KC210803, KC210796, KC210795, KC210794, KC210793, KC210792, KC210791, KC210789, KC210790, KC210788, KC210787, KC210784, KC210783	AY987712, AY987711, AY987685, AY987683, DQ411505, HQ442833, HQ442831, DQ411513, DQ411512, DQ411510, DQ411511, FR682797, FR682796, FR682794, FR682793, FR682792, FR682791, FR682790, FR682789, FR682788, FR682787, , FR682786, FR682785, FR682784, FR682783, FR682781, FR682780, FR682779, FR682778 ,FR682777, FR682776

Trade Statement

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Acknowledgments

The authors thank Dr. Jon Amberg and Mr. Mark Gaikowski for assistance in study design and project implementation, Ms. Bridget Ladell for assistance with sequence quality trimming, and Mr. Aaron Cupp and Mr. Chris Glodowski for assistance with performing fish necropsies and initial bacterial isolations. This study was funded through a combination of the Food and Drug Administration (FDA) and U.S. Geological Survey (USGS) appropriated funds.

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