Genetic identification of putative *Lampsilis virescens* specimens collected in the Emory River, TN

Report generated by USFWS Conservation Genetics Lab

21 June 2011

Principle Investigator: Dr. Gregory R. Moyer

Co-PI: Dr. Edgardo Diaz-Ferguson

5151 Spring Street
Warm Springs, GA 31830
Email: Greg_Moyer@fws.gov
Phone: 706.655.3382 ext 1231

Summary of major findings

Samples of putative *Lampsilis virescens* from the Emory River appear genetically more similar to *L. virescens* from the Paint Rock River than other species of *Lampsilis* and *Lasmigona* based on 407 nucleotides of the mtDNA *COI* gene. Also, these specimens are probably not a new species because they do not group together in a well-resolved clade apart from Paint Rock River *L. virescens*.

GENETIC IDENTIFICATION OF PUTATIVE *LAMPSILIS VIRESCENS* SPECIMENS COLLECTED IN THE EMORY RIVER, TN

Purpose

The Warms Springs Conservation Genetics Lab (CGL) was asked by USFWS Ecological Services (Cookeville, TN Office) to provide genetic identification of three putative *Lampsilis virescens* specimens collected from the Emory River (11May2011).

Introduction

The Alabama lampmussel *L. virescens* is considered endangered under the Endangered Species Act (USFWS 1976) impart due to its limited current distribution -- the Paint Rock river basin. Historically, it was also found in the Emory river basin; however, specimens (dead or alive) have not been observed in the Emory River since 1974. To the surprise of state and federal biologist, several specimens (Fig. 1) appearing morphologically similar to *L. virescens* were collected on a recent sampling trip to the Emory River. To confirm the identification of these specimens, CGL compared the mtDNA for these three specimens with DNA obtained from two hatchery propagated *L. virescens* of Paint Rock River origin. Our null hypothesis was that the putative *L. virescens* from the Emory River should be genetically more similar to *L. virescens* found in the Paint Rock River than other morphologically similar species found in the Emory River (i.e., *L. ovata*, *L. fasciola*, and *Lasmigona costata*, and *Lasmigona subviridis*).

Methods

Taxon sampling and DNA extraction

CGL was provided a non-invasive tissue swab sample from each of the three collected specimens (here in designated USFWS #001, 002, 003, Fig. 1). DNA was extracted from the tissue swabs (Boca Scientific) using standard organic extraction procedures (Sambrook et al. 1989). DNA was re-suspended in 50uL AE buffer (QIAGEN, Inc., Valencia, California). We also obtained two whole specimens of *L. virescens* (Paint Rock River origin) that were propagated at the Alabama Aquatic Biodiversity Center (Alabama Department of Conservation and Natural Resources). DNA was extracted from mantel tissue of these whole specimens using the DNeasy Blood and Tissue kit (QIAGEN, Inc.) protocol. After DNA extraction, the concentration of DNA for each specimen was estimated using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). We also included samples of *L. ovata*, *L. fasciola*, *Lasmigona costata*, *Lasmigona complanata*, and *Lasmigona subviridis* for analysis. Justification for inclusion of these taxa

was because they are morpholigcally similar to *L. virescens* and also found in the Tennessee/Cumberland river system. Sequence data for *L. ovata*, *L. fasciola*, and *Lasmigona* spp. were obtained from Genbank (an annotated collection of all publicly available DNA sequences).

We targeted a partial coding segment of the mitochondrial (mt) DNA gene *cytochrome c oxidase* (*COI*). Specifically, a 650 base pair (bp) segment of *COI* was amplified via the polymerase chain reaction (PCR) using universal heavy (HCO) and light (LCO) strand primers (Folmer et al. 1994). PCR amplifications (20 µL reaction volume) were performed in triplicate and contained 30-80ng/uL DNA, 0.5 × *Taq* reaction buffer (GoTaq Flexi, Promega, Madison, WI), 3.0 mM $MgCl_2$, 0.25 mM of each dNTP, 0.50 uM of each primer, and 0.05 U *Taq* DNA polymerase (GoTaq Flexi, Promega). Optimized thermal cycle conditions for *COI* were an initial 94 ˚C (2 min) denaturation followed by 35 cycles of 95 ˚C (1 min), 45˚C (1min), and 72 ˚C (1.3 min) and a final 72 ˚C (7 min) extension.

PCR products were cleaned using the QIAquick Purification Kit (QIAGEN, Inc.). Cycle sequencing was conducted following the Big Dye Terminator v3.1 protocol (Applied Biosystems, Inc., Foster City, CA) using the LCO primer under the following thermal cycle parameters: 25 cycles of 96˚C for 10 s, 50˚C for 5s and 60˚C for 4 min. Cycle sequencing PCR products were purified using the BigDye XTerminator Purification kit (Applied Biosystems, Inc.) and then run on an ABI PRISM 3130 genetic analyzer (Applied Biosystems, Inc.). All sequences were imported into BioEdit Sequence Alignment Editor (Hall 1999), ends trimmed, and the remaining sequence aligned by eye.

We estimated species relationships using maximum parsimony (Fitch 1971) and Bayesian (Larget and Simon 1999) methods. Maximum parsimony analysis was conducted with PAUP* v.4.0b10 (Swofford 1998) and Bayesian analyses were performed using MrBayes v.3.1 (Ronquist and Huelsenbeck 2003). Maximum parsimony searches were heuristic and employed 1,000 random addition sequences and tree bisection-reconnection branch swapping. Resolution was assessed by bootstrap re-sampling with 10,000 pseudo-replicates using the same heuristic search strategy. We chose to employ a general time reversible model of sequence evolution with a proportion of invariable sites and a gamma shaped distribution of rates across sites (Felsenstein 2004) for Bayesian analyses. Bayesian analysis is contingent upon sampling trees from their posterior distribution. The algorithm implemented in MrBayes heuristically obtains this posterior using a Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method (Metropolis et al. 1953). To assess parameter stationarity, the dataset was extended for 500,000 generations using four simultaneous MCMCMC chains. Bayesian analyses may be starting point dependent; to check for more than one local optimum, we assessed the standard

deviation of split frequencies for two trees that differed in their starting points (Ronquist and Huelsenbeck 2003). As added confirmation of parameter stationarity we confirmed that the potential scale reduction factor for each parameter estimate was reasonably close to a value of 1.0 (Ronquist and Huelsenbeck 2003). In order to ensure that we were examining trees from the approximated posterior distribution, the initial 12,500 topologies were discarded prior to final estimation of posterior probabilities. Resolution was assessed by sampling trees every 10 generations from the approximated posterior distribution and summarizing these on a 50% majority-rule consensus tree. Node values on the interior branches of the consensus tree represent the percentage occurrences of each clade among sampled trees (Larget and Simon 1999).

Results

We were unable to obtain reliable sequence data for sample 003; however, DNA sequence data were successfully acquired from three independent PCR amplifications of samples 001 and 002. Alignment and trimming of sequencing data resulted in a data set of 26 sequences all of 407 bp in length (see Appendix). Maximum parsimony and Bayesian methods produced identical topologies revealing strong support (i.e., 100% bootstrap or posterior probabilities) for a clade consisting of putative Emory River *L. virescens* samples and known *L. virescens* (Paint Rock River origin). This clade was sister to other *Lampsilis* and *Lasmigona* spp. (Fig. 2). Note that putative *L. virescens* sequence was identical to *L. virescens* from the Paint Rock except for minor sequence ambiguities (i.e., one or two differing base pair calls; Appendix) among the various PCR amplification products.

Discussion

We set out to test the null hypothesis that putative *L. virescens* from the Emory River was genetically similar to those found in the Paint Rock River and more distantly related to other morphologically similar species found in the Emory River (i.e., *L. ovata*, *L. fasciola*, and *Lasmigona costata*, and *Lasmigona subviridis*). Our analysis supported this hypothesis using both maximum parsimony and Bayesian analyses. We found that all putative *L. virescens* did not group with other *Lampsilis* spp. and *Lasmigona* spp., instead they grouped with known *L. virescens* from the Paint Rock River. Furthermore, we would expect that if these samples were an undescribed species, then they should group together in a well resolved clade and be sister to *L. virescens* from the Paint Rock River. Just the opposite was observed, suggesting indeed that the putative samples of *L. virescens* found in the Emory River can be identified as *Lampsilis virescens* (Lea, 1858) base on mtDNA *COI* sequence data.

Literature Cited

Felsenstein, J. 2004. Inferring Phylogenies. Sinauer Associates, Sunderland, Massachusetts.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3:294-297

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.

Larget, B., Simon, D.L. 1999. Markov chain Monte Carlo algorithms for Bayesian analysis of phylogenetic trees. Mol. Biol. and Evol. 16:750-759.

Fitch, W.M., 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. 20, 406-416.

Metropolis,N., Rosenbluth, A., Rosenbluth, M., Teller, A. Teller, E. 1953. Equation of state calculations by fast computing machines. J. Chem. Phys.21:1087-1092.

Ronquist, F., Huelsenbeck , J. P. 2003. MRBAYES 3, Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Sambrook, F., Fritsch, E.F., Maniatas, T. 1989. Purification of nucleic acids. In: Nolan, C. (Ed.), Molecular cloning, a laboratory manual, 2nd ed., Cold Spring Harbor Press, New York.

Swofford, D. L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Massachusetts.

USFWS (United States Fish and Wildlife Service). 1976. Endangered and threatened wildlife and plants -- endangered status for 159 taxa of animals. Federal Register 41:24062-24067.

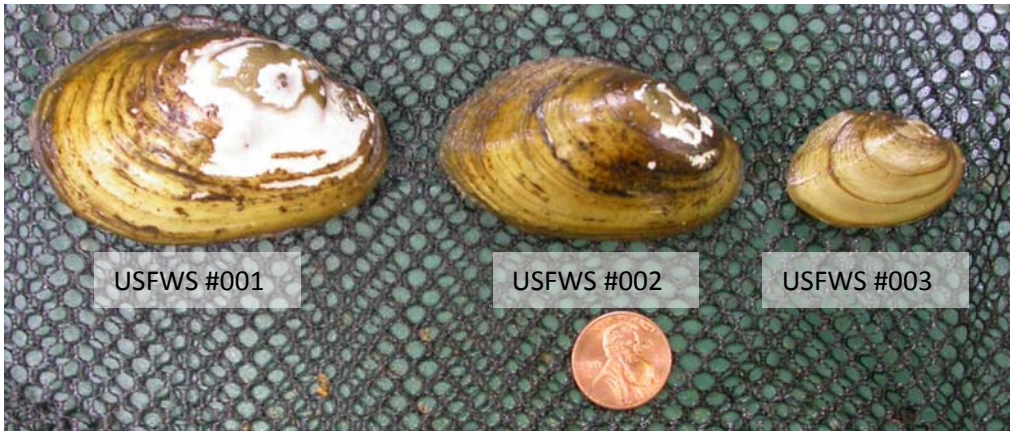

Figure 1. Photo voucher of putative *L. virescens* for the Emory River, TN with designated tissue swab number.

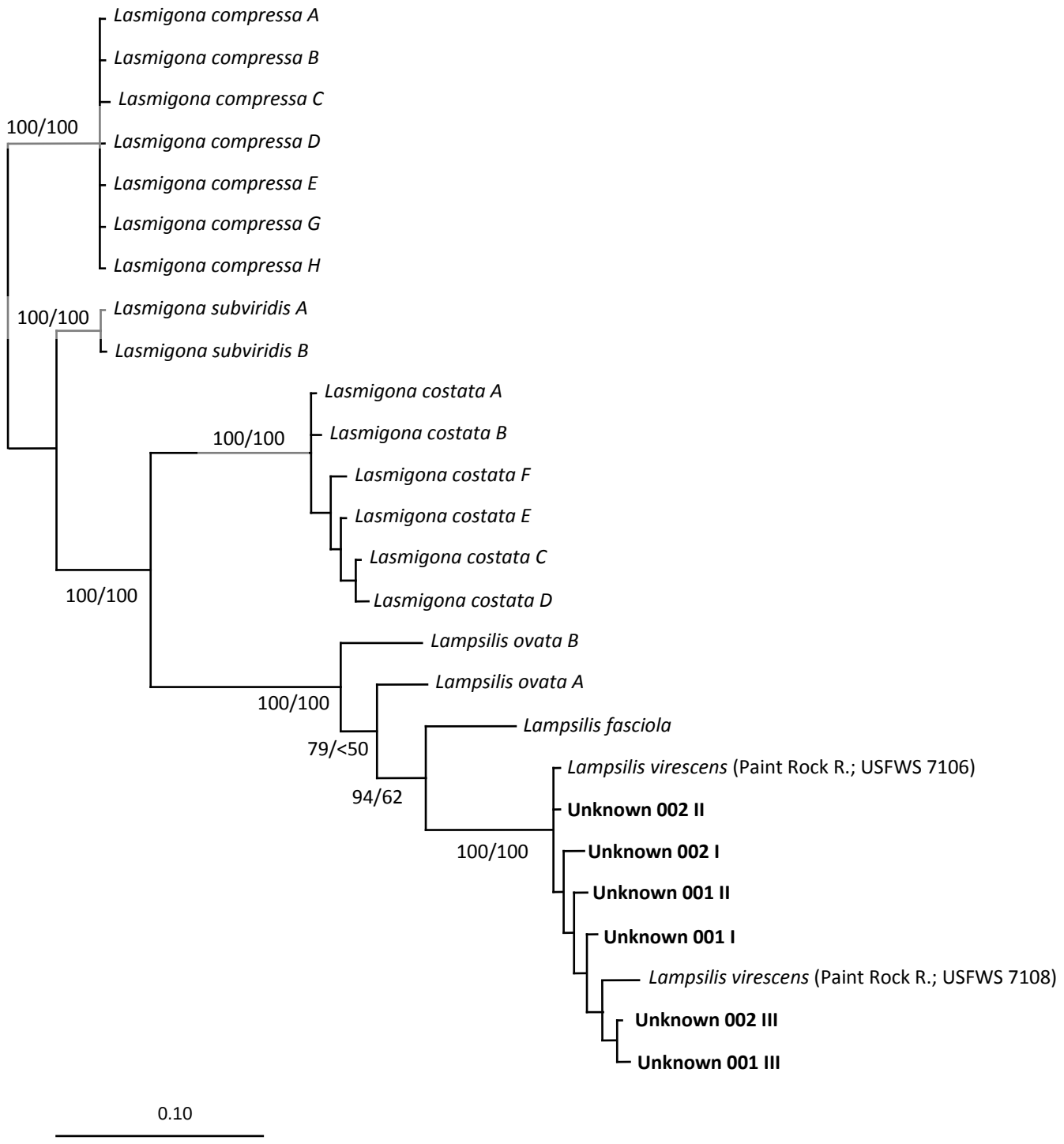

Figure 2. Mitochondrial DNA *COI* phylogeny of *Lampsilis* and *Lasmigona* spp. from Bayesian analysis. Unknown samples refer to putative *L. virescens* collected in the Emory River, TN. Numbers 001 and 002 denote USFWS # designated to Emory River specimens (see Fig. 1). Letters behind taxa indicate different haplotypes and Roman numerals refer to differing PCR amplifications. Numbers at nodes represent Bayesian posterior probability percentages and bootstrap (10,000 pseudoreplicates) values. Nodes without numbers indicated support <60%

Appendix. Sequence alignment for phylogenetic analyses. Unknown samples refer to putative *L. virescens* collected in the Emory River, TN. Numbers 001 and 002 denote USFWS # designated to Emory River specimens (see Fig. 1). Letters behind taxa indicate different haplotypes with Roman numerals referring to differing PCR amplifications.

```
Lampsilis_virescens_PR_7106   TTGGGAGATGATCAGCTATATAATGTGATTGTGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Lampsilis_virescens_PR_7108   TTGGGAGATGATCAGCTATATAAAGAAAATGAGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_001_I                 TTGGGAGATGATCAGCTATATAACGAGAATGTGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_001_II                TTGGGAGATGATCAGCTATATAACGAGATTGGGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_001_III               TTGGGAGATGATCAGCTATATAACGTGAAAGAGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_002_I                 TTGGGAGATGATCAGCTATATAATGAGATTGTGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_002_II                TTGGGAGATGATCAGCTATATAATGTGATTGTGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Unknown_002_III               TTGGGAGATGATCAGCTATATAAAGTGATTGAGACAGCACATGCTTTTATAATAATTTTCTTTTTGGTTATGCCAATAATGATTGGTGGTTTTGGGAATT
Lampsilis_ovata_A             TTGGGGGATGATCAACTATATAATGTTATTGTGACAGCACATGCTTTTATGATAATTTTCTTTTTAGTTATACCTATAATGATTGGTGGTTTTGGAAATT
Lampsilis_ovata_B             TTGGGGGATGATCAACTATATAATGTGATTGTAACAGCGCATGCTTTTATAATAATTTTCTTTTTGGTNATACCAATAATGATTGGTGGTTTTGGAAATT
Lampsilis_fasciola            TTGGGAGATGATCAGCTATATAATGTGATTGTGACAGCACATGCTTTTATAATGATTTTCTTTTTAGTTATACCAATAATGATTGGTGGTTTTGGGAATT
Lasmigona_costata_A           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGATTTGGTAATT
Lasmigona_costata_B           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGATTTGGTAATT
Lasmigona_subviridis_A        TTAAGAGATGATCAGTTATATAATGTGATTGTTACGGCTCATGCTTTTATAATAATTTTCTTTTTGGTGATGCCTATAATAATTGGTGGATTTGGTAATT
Lasmigona_subviridis_B        TTAAGAGATGATCAGTTATATAATGTGATTGTTACGGCTCATGCTTTTATAATAATTTTCTTTTTGGTGATGCCTATAATAATTGGTGGATTTGGTAATT
Lasmigona_costata_C           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGGTTTGGTAATT
Lasmigona_costata_D           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGGTTTGGTAATT
Lasmigona_costata_E           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGGTTTGGTAATT
Lasmigona_costata_F           CTAGGGGATGATCAGTTGTATAATGTTATTGTTACAGCTCATGCTTTTATAATAATTTTTTTTTTGGTAATACCTATAATAATTGGTGGGTTTGGTAATT
Lasmigona_compressa_A         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_B         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_C         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_D         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_E         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_G         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT
Lasmigona_compressa_H         TTAGGGGATGATCAATTATATAATGTGATTGTTACGGCTCACGCTTTTATAATAATTTTCTTTTTGGTAATGCCTATGATAATTGGTGGATTTGGTAATT

Lampsilis_virescens_PR_7106   GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Lampsilis_virescens_PR_7108   GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_001_I                 GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_001_II                GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_001_III               GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCgATTAAATAATTTAAgCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_002_I                 GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_002_II                GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Unknown_002_III               GACTTATTCCGCTTATGGTTGGGGCCCCTGATATAGCTTTTCCTCGATTAAATAATTTAAGCTTTTGGTTGTTAGTGCCGGCCTTATTTTTATTGTTGAG
Lampsilis_ovata_A             GGCTTATTCCACTTATGATTGGGGCTCCTGATATGGCTTTTCCTCGGTTGAATAATTTGAGTTTTTGGTTATTGGTGCCGGCTTTATTTTTATTATTGAG
Lampsilis_ovata_B             GACTTATTCCCCTCATGATTGGGGCTCCTGATATGGCTTTCCCTCGGTTGAATAATCTGAGTTTTTGGTTACTGGTGCCGGCTTTATTTTTACTATTGAG
Lampsilis_fasciola            GACTTATTCCTCTTATGATTGGGGCTCCTGATATGGCTTTTCCTCGGTTAAATAATTTGAGTTTTTGGTTATTGGTACCGGCTTTGTTTTTATTGTTGAG
Lasmigona_costata_A           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGACTTAATAATTTAAGATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
Lasmigona_costata_B           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGACTTAATAATTTAAGATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
Lasmigona_subviridis_A        GGCTTATTCCCTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGGTTTTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_subviridis_B        GGCTTATTCCCTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGGTTTTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_costata_C           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGGCTTAATAATTTAAGATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
Lasmigona_costata_D           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGGCTTAATAATTTAAAATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
Lasmigona_costata_E           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGGCTTAATAATTTAAGATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
```

```
Lasmigona_costata_F           GGCTTATTCCTTTGATGATTGGTGCTCCTGACATAGCCTTTCCTCGGCTTAATAATTTAAAATTTTGGCTTCTTGTGCCTGCTTTGTTTTTATTATTGAC
Lasmigona_compressa_A         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_B         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_C         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_D         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_E         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_G         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG
Lasmigona_compressa_H         GGCTTATTCCTTTAATAATTGGCGCTCCTGATATGGCTTTTCCTCGATTAAATAATTTAAGATTCTGGCTCCTTGTACCAGCTTTATTTTTACTATTAAG

Lampsilis_virescens_PR_7106   TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Lampsilis_virescens_PR_7108   TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_001_I                 TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_001_II                TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_001_III               TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCtCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_002_I                 TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGtTTATCCACCATTGTCTGGGAATGTACCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_002_II                TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Unknown_002_III               TTCTTCTTTGGTGGAGAGTGGTGTTGGAACCGGATGGACGGTTTATCCACCATTGTCTGGGAATGTAGCTCATTCTGGGGCTTCGGTGGATTTGGCCATT
Lampsilis_ovata_A             TTCTTCTTTGGTAGAGAGTGGTGTTGGGACTGGGTGGACGGTTTATCCACCTTTGTCTGGAAATGTGGCTCATTCTGGTGCTTCGGTGGATTTGGCTATT
Lampsilis_ovata_B             TTCTTCTTTGGTAGAGAGTGGTGTTGGGACTGGGTGGACGGATTATCCTCCTTTGTCTGGAAATGTGGCTCATTCTGGGGCCTCGGTGGATTTGGCTATT
Lampsilis_fasciola            TTCTTCTTTGGTAGAGAGTGGTGTTGGGACTGGGTGAACGGTCTACCCCCCATTGTCTGGAAATGTGGCTCATTCTGGGGCCTCGGTGGATTTGGCCATT
Lasmigona_costata_A           GTCTTCATTGGTGGAGAGGGGTGTTGGTACTGGTTGGACAGTGTATCCTCCCTTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_costata_B           GTCTTCATTGGTGGAGAGGGGTGTTGGTACTGGTTGGACAGTGTATCCTCCCCTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_subviridis_A        GTCTTCATTAGTGGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCAGGAAATGTTGCTCATTCTGGGGCTTCGGTGGATTTAGCTATT
Lasmigona_subviridis_B        GTCTTCATTAGTGGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCAGGAAATGTTGCTCATTCTGGGGCTTCGGTGGATTTAGCTATT
Lasmigona_costata_C           GTCTTCATTGGTGGAGAGGGGTGTTGGTACCGGTTGGACAGTGTATCCCCCCTTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_costata_D           GTCTTCATTGGTGGAGAGGGGTGTTGGTACCGGTTGGACAGTGTATCCCCCCTTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_costata_E           GTCTTCATTGGTGGAGAGGGGTGTTGGTACCGGTTGGACAGTGTATCCTCCCTTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_costata_F           GTCTTCATTGGTGGAGAGGGGTGTTGGTACCGGTTGGACAGTGTATCCTCCCTTATCAGGGAATGTTGCTCATTCTGGGGCTTCGGTGGATTTGGCTATT
Lasmigona_compressa_A         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_B         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_C         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_D         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_E         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_G         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lasmigona_compressa_H         ATCTTCATTGGTAGAAAGAGGTGTAGGAACTGGGTGAACAGTGTATCCTCCTTTGTCCGGAAATGTTGCTCATTCTGGGGCTTCGGTAGATTTAGCTATT
Lampsilis_virescens_PR_7106   TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAGCGTA
Lampsilis_virescens_PR_7108   TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTTGGTGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGACCGTA
Unknown_001_I                 TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAACGTA
Unknown_001_II                TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAGCGTA
Unknown_001_III               TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAACGTA
Unknown_002_I                 TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAGCGTA
Unknown_002_II                TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCGCTCTCCTGGATTAGTTGCTGAGCGTA
Unknown_002_III               TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTGGGGGCTATTAATTTTATTTCCACTGTAGGGAATATGCgCTCTCCTGGATTAGTTGCTGAACGTA
Lampsilis_ovata_A             TTTTCTTTACATCTTGCTGGTGCTTCTTCTATTTTAGGGGCTATTAATTTTATTTCTACTGTTGGGAATATACGATCTCCTGGATTAGTTGCTGAGCGGA
Lampsilis_ovata_B             TTTTCTTTACATCTTGCTGGTGCTTCTTCTATTTTAGGGGCTATTAATTTTATTCCTACTGTTGGGAATATGCGATCTCCTGGGTTAGTTGCTGACCGGA
Lampsilis_fasciola            TTTTCTTTGCATCTTGCTGGTGCTTCTTCTATTTTAGGGGCTATTAACTTTATTTCTACTGTTGGGAATATACGATCCCCTGGATTGGTTGCTGAGCGGA
Lasmigona_costata_A           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGNATATGCGATCTCCGGGTTTAATCGCTGAACGNA
Lasmigona_costata_B           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGAATATGCGATCTCCGGGTTTAATCGCTGAACGAA
Lasmigona_subviridis_A        TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAACTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_subviridis_B        TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAACTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_costata_C           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGAATATGCGATCTCCAGGCTTAATCGCTGAACGAA
```

```
Lasmigona_costata_D           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGAATATGCGATCTCCAGGCTTAATCGCTGAACGAA
Lasmigona_costata_E           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGAATATGCGATCTCCAGGTTTAATCGCTGAACGAA
Lasmigona_costata_F           TTTTCTTTGCATCTTGCTGGTGCCTCATCAATTTTAGGCGCTGTTAATTTTATTTCTACTGTTGGGGATATGCGATCTCCGGGTTTAATCGCTGAACGAA
Lasmigona_compressa_A         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_B         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_C         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGAAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_D         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_E         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_G         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA
Lasmigona_compressa_H         TTCTCTTTACATCTTGCTGGTGCTTCATCAATTTTAGGTGCTATTAATTTTATTTCTACTGTTGGGAATATGCGGTCTCCTGGTTTGGTTGCTGAGCGAA

Lampsilis_virescens_PR_7106   TTCCGTT
Lampsilis_virescens_PR_7108   TTCCGTT
Unknown_001_I                 TTCCGTT
Unknown_001_II                TTCCGTT
Unknown_001_III               TTCCGTT
Unknown_002_I                 TTCCGTT
Unknown_002_II                TTCCGTT
Unknown_002_III               TTCCGTT
Lampsilis_ovata_A             TTCCGTT
Lampsilis_ovata_B             TTCCGTT
Lampsilis_fasciola            TTCCGTT
Lasmigona_costata_A           TTCCTTT
Lasmigona_costata_B           TTCCTTT
Lasmigona_subviridis_A        TTCCTTT
Lasmigona_subviridis_B        TTCCTTT
Lasmigona_costata_C           TTCCTTT
Lasmigona_costata_D           TTCCTTT
Lasmigona_costata_E           TTCCTTT
Lasmigona_costata_F           TTCCTTT
Lasmigona_compressa_A         TTCCTTT
Lasmigona_compressa_B         TTCCTTT
Lasmigona_compressa_C         TTCCTTT
Lasmigona_compressa_D         TTCCTTT
Lasmigona_compressa_E         TTCCTTT
Lasmigona_compressa_G         TTCCTTT
Lasmigona_compressa_H         TTCCTTT
```