Extracting Genetic Information from Alabama Mollusks

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BACKGROUND

Alabama is known for its incredible diversity of freshwater animals. Alabama has the highest diversity of freshwater mussels, freshwater fishes, freshwater snails, crayfish, and freshwater turtles compared to all other states (Duncan 2013). Thanks to the wealth and age of its river systems, Alabama's diversity of freshwater mussels is greater than anywhere else in the world (Garner 2014). However, the genetic diversity within many species of mussels is not well known, and many freshwater mussels are endangered or have already become extinct. In addition, some freshwater mussels contain cryptic species, which are genetically distinct species despite having the appearance of another.

The genus *Amblema* is a widespread group of freshwater mussels, and currently has three described species: *Amblema plicata*, *Amblema elliottii*, and *Amblema neislerii*. While *A. plicata* and *A. elliottii* are commonly found in Alabama's rivers, *A. neislerii* is currently listed as an endangered species and is known to only be found in Georgia and Florida's rivers.

RESEARCH QUESTIONS

How genetically diverse is *Amblema plicata*?

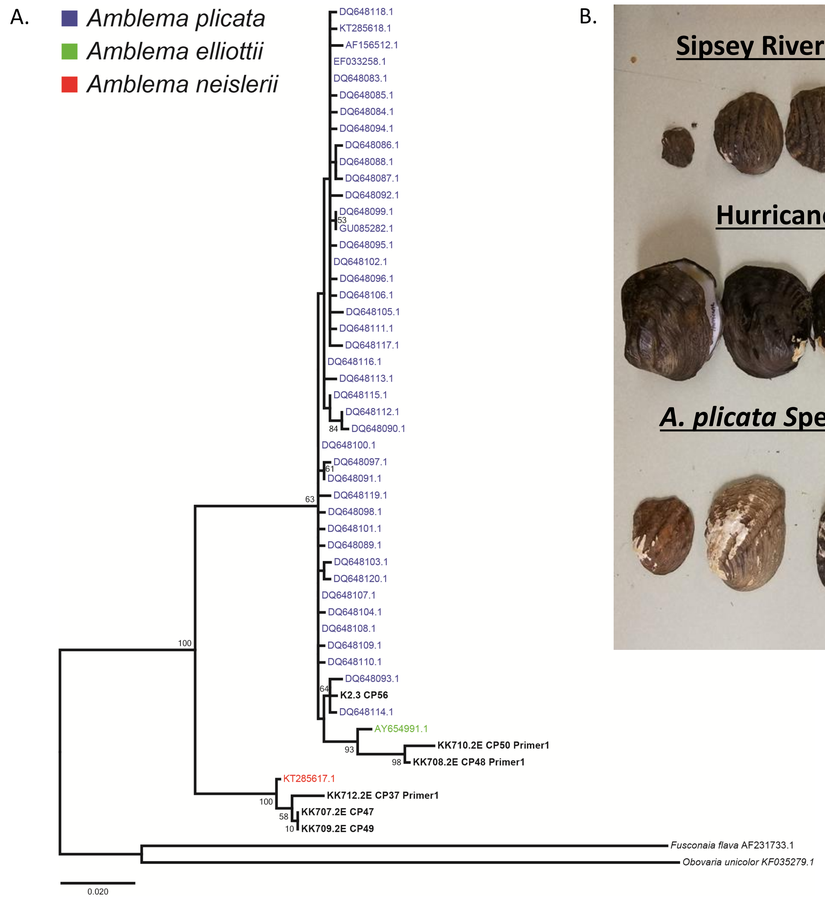
Are populations of *Amblema plicata* genetically structured with respect to geographical boundaries (e.g., different river drainages)?

Are there cryptic species of *Amblema*?

METHODS

We extracted DNA from the mussel samples using the Omega Bio-Tek E.Z.N.A. Tissue DNA Extraction Kit. We amplified a fragment of the mitochondrial COI gene using polymerase chain reaction (PCR) with mollusc-specific primers (Meyer 2003). Next, agarose gel electrophoresis was conducted on the PCR Product to separate the DNA fragments by size. Then we extracted the correct DNA fragment and purified it using the Omega Bio-Tek Gel Purification Kit before finally sending the fragment off to be Sanger sequenced at the University of Arizona Genetics core.

Once the results were sent back, the program Sequencher was used to assemble the forward and reverse reads into contigs. Subsequently, sequences were aligned in MEGA 7 (Sudhir et al. 2015) using MUSCLE (Edgar 2004). A phylogenetic analysis was conducted in RAxML 8 (Stamatakis et al. 20??) using the GTR+GAMMA model with the optimal number of bootstrap replicates determined using the AUTOMRE criterion. *Fusconaia* plus *Obovaria* were used to root the tree.



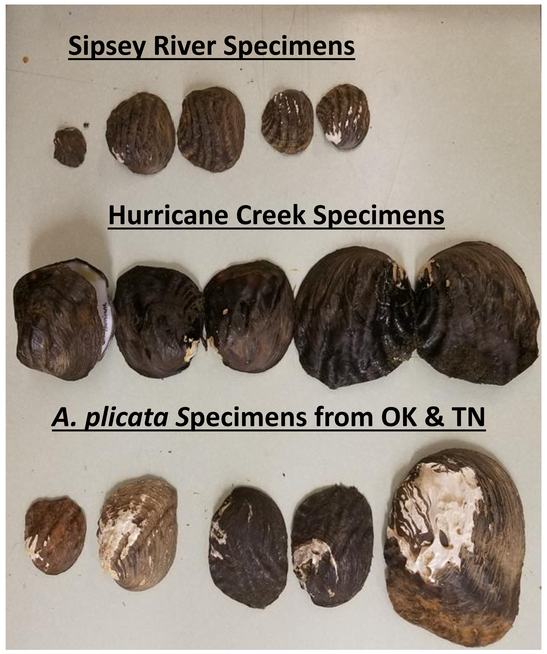


Figure 1. A. Phylogenetic analysis of *Amblema* COI sequences. Bootstrap support values above 50 are shown at each node. Expert identified taxa are color coded. *Fusconaia flava* and *Obovaria unicolor* were used as outgroups. B. Photograph of *Amblema* shell specimens.

CURRENT RESULTS

After an initial run of DNA barcoding, we were able to successfully obtain 6 COI sequences of *Amblema.* Three sequences matched up with *A. neislerii,* two others matched up with *A. plicata,* and one matched up with *A. elliottii.*, but *A. elliottii* was nested within *A. plicata*. When the sequences that matched up with *A. neislerii* were isolated and tested on, the number of base substitutions per site from averaging over all sequence pairs shown was 0.006. In addition, all sequences except the outgroups underwent the same testing and produced a result of 0.016.

CONCLUSION

The initial results were quite unexpected. The samples that matched up with *A. neislerii* were taken from Hurricane Creek, a body of water in Alabama that is outside of *A. neislerii*'s habitat. **This suggests we may have found a new locality for an endangered species.** Having such a low number of base substitutions per site also helps support this conclusion of us correctly identifying the species as *A. neislerii* and not some other species of *Amblema*.

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