

# Genetic Structure/Phylogeography of Sheepnose Mussel

**Principal Investigator:** Kevin J. Roe  
**Student Investigator:** n/a  
**Collaborators:** n/a  
**Duration:** August 2007 to June 2010  
**Funding Source(s):** Iowa Department of Natural Resources, Endangered Species  
**Goals and Objectives:**

The objective of the project is to document genetic diversity, population structure, the extent of gene flow, and historical connections between populations of the sheepnose (*Plethobasus cyphus*). This information would be used in identifying unique or genetically distinct populations of the sheepnose and serve as guidelines for reintroduction population augmentation aimed at reversing declines in population size and number of populations of this species throughout the U.S.

Goals for this project have been separated into three phases as follows:

Phase I – Generate species-specific microsatellites/ Mt DNA sequences for the sheepnose. Obtain specimens from across the range of the species.

- A genomic DNA library will be constructed from a single individual of the sheepnose. The genomic library will be probed for stretches of nucleotide repeats, such as CA(n) or CGTT(n). These nucleotide repeats are the microsatellite markers. This phase will include primer design of potential microsatellite sites. Tissue samples will be obtained via contacts in various state and federal agencies as well as through collecting by the Principal Investigator.
- Obtain Mt DNA sequence data for the ND1 gene from available specimens – use as a guide to identify “deep” genetic differences within the species. Subgroups identified at this level will guide collecting efforts and further investigations using microsatellites.

Phase II – Screen potential microsatellites

- Species-specific microsatellite primers will be tested in a subset of the individual sheepnose specimens. The goal of this phase is to identify 20-25 microsatellite regions that have variation both within a population as well as between populations. Individuals will be screened with all available primer sets. Primer sets that amplify variable microsatellite regions will be used in the Phase III.

Phase III – Determine genetic diversity of the sheepnose

- Populations identified using the Mt DNA sequence data will be screened with microsatellite primers. The number of individuals genotyped from each population will depend on the availability of samples. Microsatellite data will be analyzed for genetic variation and population structure.

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## Progress:

To date, substantial progress has been made on the project. Samples for ~80+ individual mussels have been obtained representing > 12 localities across the species range of the Sheepnose, and DNA has been extracted from these samples. Mitochondrial DNA sequences for the first subunit of the NADH dehydrogenase gene have been generated for all individuals on hand. These sequences have been edited and are in an aligned data matrix. A species specific microsatellite library has been developed and ~12 polymorphic microsatellite markers have been identified. Currently, generating microsatellite genotypes for all samples on hand are in progress for the markers mentioned above.

## Future Plans:

Principal Investigator has hired a technician to assist with genotyping mussel specimens on hand and expects this portion of the project to be completed by June 2009. Additional samples from approximately three additional localities will be collected during summer of 2009 and will generate mitochondrial sequences and genotype data during the Fall and Winter of 2009/2010. Following the completion of this portion of the project the data will be analyzed and the final report will be written.