

Project Title: Genetic examination of the Ringed Crayfish species group, with special emphasis on the endemic Gapped Ringed Crayfish (*Orconectes neglectus chaenodactylus*)

Project Summary: Morphological and genetic research suggests that the Ringed Crayfish species complex (*Orconectes neglectus* ssp.) is comprised of multiple taxonomic groups, however additional genetic analyses are needed to further clarify taxonomic status of this species complex and refine conservation priorities in the Arkansas State Wildlife Action Plan. The goal of this study is to further elucidate the taxonomic relationships within the Ringed Crayfish species complex by extending phylogenetic molecular techniques. Our specific objectives are 1) Clarify taxonomic status and phylogenetic relationships within the species complex and 2) Identify management units (MUs) and evolutionary significant units (ESUs) within the White River Basin. Results from this work help guide conservation rankings and prioritize efforts to maintain biodiversity within Arkansas including the biological integrity of the Ozark Highlands Ecoregion.

Project Leader: Whitney J.B. Anthonysamy, Post-Doctoral Fellow, University of Arkansas, Department of Biological Sciences, Fayetteville, AR 72701, wjba@uark.edu, 217-343-0603

Project Partners:

Brian K. Wagner, Nongame Aquatics Biologist, Arkansas Game and Fish Commission, 915 E. Sevier Street, Benton, AR 72015, bkwagner@agfc.state.ar.us, 877-847-2690

Marlis R. Douglas, Bruker Professor in Life Sciences, University of Arkansas, Department of Biological Sciences, Fayetteville, AR 72701, mrd1@uark.edu, 479-575-4176

Michael E. Douglas, 21st Century Chair in Global Change Biology, University of Arkansas, Department of Biological Sciences, Fayetteville, AR 72701, med1@uark.edu, 479-575-6343

Project Budget:

State Wildlife Grant Requested: \$16,412
Project Match (UARK in-kind services): \$5,744
Total Project Cost: \$22,156

Budget Category	Year 1	Year 2	Total
Postdoc Salary/Benefits (1 mo)	\$4,710	\$4,710	\$15,164
Supplies/Sequencing	\$4000	-	\$4,000
Travel	-	\$1,500	\$1,500
Indirect Costs (10%)	\$871	\$621	\$1,492
Subtotal	\$9,581	\$6,831	\$16,412
35% Match (UARK in-kind services)	\$3,354	\$2,391	\$5,744
Total	\$9,581	\$6,831	\$22,156

Proposal Statement

Need

Morphological and genetic research suggests that the Ringed Crayfish species complex (*Orconectes neglectus* ssp.) is comprised of multiple taxonomic groups (Crandall & Fitzpatrick 1996, Crandall 1998), however level of species status and number of distinct genetic lineages remains unclear (Dillman et al. 2007). Formerly, two subspecies were designated based on morphological characteristics (Williams 1952). *Orconectes neglectus neglectus* has a widespread distribution throughout the White River Basin and naturally occurs in disjunct populations in other states (Pflieger 1996). In contrast, the Gapped Ringed Crayfish, *O. n. chaenodactylus* is uncommon in Arkansas, endemic to the Ozarks, and maintains a restricted distribution primarily in the North Fork White River and Sylamore Creek basins (Wagner et al. 2010). In addition, Ringed Crayfish have become established outside of their native range, including the Spring River drainage in Arkansas (Rabalais & Magoulick 2006). Such invasions have been associated with declines in native crayfish species (Magoulick & DiStefano 2007, Imhoff et al. 2012).

More recently, genetic work based on mitochondrial DNA sequence data suggested that considerable fine-scale geographic variation exists in the Ringed Crayfish species complex within the White River Basin (Dillman et al. 2007). This work indicated that as many as “three deeply divergent lineages” that correspond to each of the previously described subspecies as well as a third undescribed taxon and the authors argued results warranted elevation of each lineage to species status (Dillman et al. 2007). In addition, each lineage showed further subdivision corresponding to historical connectivity and isolation among tributaries in the basin (Dillman et al. 2007). Additional genetic analyses are needed to further clarify taxonomic status of this species complex, identify potential cryptic species, and improve efforts to develop conservation rankings and refine goals of the Arkansas State Wildlife Action Plan.

Priority actions addressed

1. Gapped Ringed Crayfish (*Orconectes neglectus chaenodactylus*) – additional genetic examination of this subspecies and other *O. neglectus* forms to identify cryptic species and develop appropriate conservation targets.
2. Newly Described Species – Several new species have recently been described in the state. Determining distribution and population status is necessary to determine their need to be added to the AWAP as SGNC.

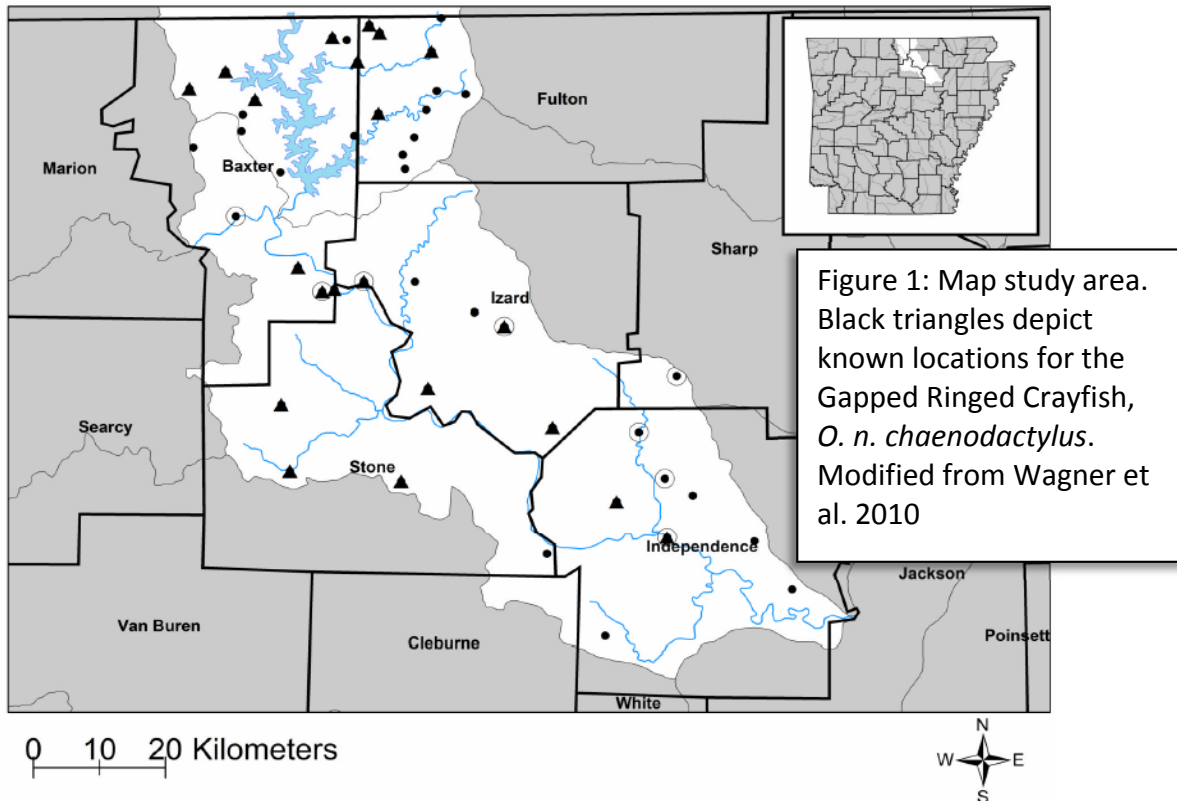
Purpose and Objectives

The goal of this study is to further elucidate the taxonomic relationships within the Ringed Crayfish species complex. Here, we build on existing genetic research and test taxonomic hypotheses derived from previous sequence data by examining additional genetic markers to identify congruent patterns that confirm lineages. Such data are needed to support taxonomic reclassification, recognition of distinct species and identification of diversity and population structure within each species. Our specific objectives are:

1. Genotype ~100 samples from the White River Basin to clarify taxonomic status and phylogenetic relationships within the *Orconectes neglectus* species complex.
2. Identify management units (MUs) and evolutionary significant units (ESUs) within the North Fork White and Middle White River Basins.

Location

This project will be conducted at the Arkansas Conservation and Molecular Ecology Laboratory (aCaMEL) located at the University of Arkansas, Fayetteville, and will target specimens collected from the North Fork White River and Middle White River basins in the Ozark Highlands Ecoregion (Fig 1).



Approach

DNA Extraction

Material for genetic analysis will be obtained from approximately 100 tissue samples (e.g., gill, tail muscle) either from voucher specimens stored at the AGFC Nongame Aquatics Program reference collection or from live specimens collected by BKW. Sampling procedures and locations will follow Wagner et al. (2010). Whole genomic DNA will be extracted using a modified DNeasy Blood and Tissue Kit (QIAGEN Inc.) protocol.

DNA Sequencing

We will analyze three distinct genes (markers) to confirm consistent patterns of genetic distinct entities within and among recognized subspecies. We will sequence two mitochondrial DNA genes (16S and COI) and one nuclear gene (GAPDH) following the methods of Dillman et al. (2007) and Mathews et al. (2008). Analyzing the same genes as used in previous studies will allow us to directly compare our data to existing results (available from public databases such as GenBank). Briefly, we will amplify and sequence each gene for each sample using dideoxy terminator chemistry (Sanger sequencing) and analyze nucleotide sequences on an automated DNA sequencer (ABI PRISM 3730). We then will manually align sequences using SEQUENCHER (Gene Codes).

Data Analyses

We will analyze genetic data at two levels: first we will identify genetic lineages corresponding to distinct species and then examine population substructure within each species. Distinct lineages will be identified using standard phylogenetic (clustering) approaches including Bayesian and maximum-likelihood methods implemented in the programs MrBayes 3.2 and MEGA 5. We then will characterize genetic diversity within among distinct lineages by estimating standard phylogeographic and population genetic parameters such as percent sequence divergences, number of haplotypes and nucleotide diversities using DNAsp v.5. To visualize geographic distributions of unique genetic lineages within and among taxonomic groups a haplotype network (tree) will be constructed.

This project is anticipated to take two years. Sampling, DNA extraction, and DNA sequencing will be completed during the first year. Analyses and writing (final report, manuscripts) will be completed during the second year.

Expected Results and Benefits

This work would clarify taxonomic relationships (i.e., new species) within the Ringed Crayfish species complex and facilitate identification management units (MUs) and evolutionary significant units (ESUs). This valuable information would help guide conservation rankings and prioritize efforts to maintain biodiversity within Arkansas including the biological integrity of the Ozark Highlands Ecoregion.

In addition, the PIs are currently exploring other funding sources to investigate the development of genomic tools that can be applied to assess population-level questions. This approach provides increased genetic resolution compared to more traditional genetic markers (mtDNA) and could also be used to track the invasion ecology (sources and routes of invasion) of *O. neglectus* spp.

Results will be summarized in a final report submitted to the Arkansas Game and Fish Commission and will also be disseminated in presentations at local, regional and national professional meetings and submitted for publication to peer-reviewed journals.

Budget

State Wildlife Grant Requested: **\$16,412**

Project Match (UARK in-kind services): **\$5,744**

Total Project Cost: **\$22,156**

Budget Category	Year 1	Year 2	Total
Postdoc Salary/Benefits (1 mo)	\$4,710	\$4,710	\$15,164
Supplies/Sequencing	\$4000	-	\$4,000
Travel	-	\$1,500	\$1,500
Indirect Costs (10%)	\$871	\$621	\$1,492
Subtotal	\$9,581	\$6,831	\$16,412
35% Match (UARK in-kind services)	\$3,354	\$2,391	\$5,744
Total	\$9,581	\$6,831	\$22,156

Qualifications

Dr. Whitney J. B. Anthonysamy (Project Lead) is a Post Doctoral Fellow in the Department of Biological Sciences at the University of Arkansas, Fayetteville. She obtained her Ph.D. in Natural Resources and Environmental Sciences at the University of Illinois in 2012 and spent three years as a postdoctoral Research Associate at the Illinois Natural History Survey prior to her current position at UARK. Dr. Anthonysamy uses field and genetic techniques to understand how species adapt and persist in anthropogenically disturbed landscapes and has worked extensively with aquatic species including reptiles, non-game fish, macro-invertebrates, and invasive plants.

Mr. Brian K. Wagner (Project Partner) is the Interim Assistant Chief/Nongame Aquatics Biologist at the Arkansas Game and Fish Commission. He has over 15 years of research experience in aquatic conservation and received his M.S. in Fisheries from Virginia Tech in 1991. Mr. Wagner is a certified Fisheries Scientist through American Fisheries Society and leads the Arkansas Wildlife Action Plan Crayfish Taxa Team, and is active on the Fish, Cave, and Invertebrate Taxa Teams.

Dr. Marlis R. Douglas (Project Partner) is the Bruker Professor in Life Sciences in the Department of Biological Sciences at the University of Arkansas, Fayetteville. Dr. Douglas established and is co-directing the Arkansas Conservation and Molecular Ecology Laboratory (aCaMEL) UARK in BISC and has over 20 years of experience in conservation genetics research involving rare, threatened or endangered, as well as invasive, aquatic species. Prior she held faculty positions and co-directed CaMEL laboratories at Colorado State University in the Department of Fish, Wildlife and Conservation Biology and at the Illinois Natural History at the University of Illinois. She has a long history working closely with state and federal agencies, as well as NGOs and tribal groups, to address information needs and generate scientifically sound data that inform management and help refine conservation efforts. She also serves as liaison between the American Fisheries Society and the American Society for Ichthyologists and Herpetologists, the two primary scientific societies focusing on fish and fisheries.

Dr. Michael E. Douglas (Project Partner) is the 21st Century Chair in Global Change Biology in the Department of Biological Sciences at the University of Arkansas, Fayetteville. Dr. Douglas is co-director of aCaMEL and held previous faculty positions at Oklahoma State (Biology), Arizona State University (Life Sciences), and Colorado State University (Fish, Wildlife and Conservation Biology), and was Director of the Division of Ecology and Conservation Science and Associate Director for Research at the Illinois Natural History Survey at the University of Illinois. He has over 30 years experience in conducting conservation and ecology oriented research in a wide variety of disciplines including taxonomy, phylogenetics, quantitative ecology, morphometrics, statistical analyses, primarily involving aquatic organisms, as well as wildlife species. He served as president of the American Society of Ichthyologists and Herpetologists and the Desert Fishes Council, and was for 15 years editor of the journal *Copeia*.