

Title: Status, Distribution, and Genetics of Three Arkansas Crayfish: *Cambarus causeyi*, *Procambarus parasimulans*, and *Orconectes meeki brevis*

Summary: This study investigates the conservation status, distribution, and genetics of three Arkansas crayfish taxa, *Cambarus causeyi*, *Procambarus parasimulans*, and *Orconectes meeki brevis*. All three of these are poorly known taxa inhabiting the Ozark and/or Ouachita Mountains in western Arkansas. The study will clarify the conservation status of these crayfish, their distributions within Arkansas, and their taxonomic status within their respective genera. This study will also extend recent systematic sampling of stream crayfish faunas in the Ozarks.

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SWG Funds Requested: \$30,500

Amount and Source of Matching Funds: \$31,023 (50%+) from Brigham Young University and Arkansas Game and Fish Commission

Total Project Cost: \$61,523

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Duration: 1 July 2007 to 30 June 2009

Need:

Cambarus causeyi is an Arkansas endemic crayfish occurring in the Ozark Mountains Ecoregion of northwestern Arkansas (Robison and Allen, 1995; Robison and Leeds, 1996). It was originally described by Reimer (1966) from Pope County, Arkansas. This species is a primary burrowing crayfish that inhabits complex burrows in seeps and springs in the southern Ozarks (Hobbs, 1969; Robison and Leeds, 1996). The specialized habitat of this species and the required sampling methods make general surveys ineffective for this species. Specific searches of springs and seepage areas are necessary to obtain specimens of this rare crayfish. Robison and Leeds (1996) reported 40 new locations for this crayfish in five new counties following a study of the Ozark National Forest areas north of the Arkansas River. The previous collecting experience of Dr. Henry Robison with this rare crayfish will be most helpful in locating new populations of this species in the Ozark Mountains Ecoregion. Taylor et al. (1996) considered *Cambarus causeyi* to be of “Special Concern” and The Nature Conservancy ranks it as G1S1.

Procambarus parasimulans is an Arkansas endemic crayfish occurring in the Arkansas Valley Ecoregion and the Ouachita Mountains Ecoregion (Hobbs and Robison, 1988; Robison and Allen, 1995). This species is a primary burrowing crayfish that inhabits roadside seepage areas and pastures. This crayfish was originally described from near Bismarck, Arkansas by Hobbs and Robison (1982). Later, Hobbs and Robison (1988) provided additional collection localities. Since the original description by Hobbs and Robison (1982) and a paper on the Arkansas members of the subgenus *Girardiella* which provided a few additional locations for *P. parasimulans* (Hobbs and Robison, 1988), there has been virtually nothing else published concerning this species. Taylor et al. (1996) considered it to be of “Special Concern” and The Nature Conservancy ranks it as G4S2.

Orconectes meeki brevis is a stream crayfish occurring in the Ozark Mountains of extreme northwestern Arkansas and eastern Oklahoma (Hobbs, 1989). This subspecies inhabits medium and small size streams in the southern Ozarks (Williams, 1952; Williams, 1954). Taylor et al. (1996) considered it to be “Threatened” and The Nature Conservancy ranks it as G4T3S1. This crayfish is contained within the subgenus *Buannulifictus* of the genus *Orconectes* and was described originally by Austin Williams (1952) from Stilwell, Adair County, Oklahoma. Its relationship with the nominate subspecies, *Orconectes meeki meeki* is not known and needs to be clarified. In addition, the status of *Orconectes meeki brevis* within the genus *Orconectes* needs to be investigated. Co-Investigator Dr. Keith Crandall is an evolutionary biologist who did his doctoral dissertation research on the genus *Orconectes* and thus will be in a good position to elucidate relationships once the study is completed.

Recent collections by Co-Investigator Mr. Brian Wagner incidental to Arkansas Darter surveys in the Illinois River basin have documented 20 locations for *Orconectes meeki brevis*. Systematic stream crayfish sampling in the Robert S. Kerr Reservoir basin and Frog-Mulberry basin will help define the limit of the Arkansas range of *Orconectes meeki brevis*, as well as providing nearby *Orconectes meeki meeki* specimens for comparison. Similar sampling in the Little Red River basin would yield far-removed *Orconectes meeki meeki* specimens for comparison. These samples would also provide a useful extension of AGFC’s crayfish sampling efforts in recent years in the Beaver Lake, Bull Shoals Lake, North Fork White, Middle White, Strawberry, and Eleven Point basins.

Funding Priorities Addressed:

This project will address all priority implementation actions identified by the Crayfish Taxa Team and included in the 2007 Request for Preproposals. The project will also address critical data gaps and provide necessary information for on-the-ground conservation by making new information available to biologists and land managers with Arkansas Game and Fish Commission, U.S. Forest Service, U. S. Fish and Wildlife Service, The Nature Conservancy, and other agencies.

Location of Work:

Work will be conducted within portions of the Ozark Highlands, Boston Mountains, Arkansas Valley, and Ouachita Mountains ecoregions, within the following eco-basins: Arkansas Valley - Arkansas River, Arkansas Valley - White River, Boston Mountains - Arkansas River, Boston Mountains - White River, Ouachita Mountains - Arkansas River, Ouachita Mountains - Ouachita River, Ouachita Mountains - Red River, Ozark Highlands - Arkansas River, Ozark Highlands - White River, and Ozark Highlands – Arkansas River. A map is attached (following the Literature Cited section) to allow the reviewers to see the areas in question that will be sampled during this study.

Conservation Priorities Addressed:

This project will address all crayfish-related implementation priorities determined by the State Wildlife Action Plan Steering Committee for 2007, namely:

1. Survey for additional populations of *Cambarus causeyi*.
2. Survey for additional populations of *Orconectes meeki brevis*.
3. Establish genetic status of *Orconectes meeki brevis* relative to other subspecies.
4. Survey for additional populations of *Procambarus parasimulans* and determine its habitat requirements and related threats.

Goal:

By the end of this study, we propose to address critical gaps in our knowledge base regarding Arkansas crayfishes by (1) reevaluating the conservation status of *Cambarus causeyi*, *Procambarus parasimulans*, and *Orconectes meeki brevis* in Arkansas; (2) establishing improved baseline distributions of these three crayfishes; (3) clarifying the taxonomic status of each of these crayfishes by employing genetic analyses; and (4) extending recent systematic sampling of Ozark stream crayfish faunas to include the Robert S. Kerr Reservoir, Frog-Mulberry, and Little Red River basins.

Monitoring Methodology:

Cambarus causeyi is a primary burrowing species associated with the Ozark Mountains and Ouachita Mountains ecoregions occurring in seeps and springs (Bouchard and Robison, 1980; Robison and Leeds, 1996). Appropriate habitats will be sampled throughout the Arkansas Ozark Mountains by physically extricating burrows by hand, aquatic dip nets, and/or baited traps.

Procambarus parasimulans is a primary burrower associated with wet seepage areas along roadsides, in pastures and open fields (Hobbs and Robison, 1982; Hobbs and Robison, 1988; Robison and Allen, 1995). Appropriate habitats will be sampled throughout the Arkansas River Valley Ecoregion and the Ouachita Mountains Ecoregion by excavating by hand, using minnow seines, aquatic dip nets, and/or baited traps.

Orconectes meeki brevis is a stream species associated with Ozark streams and rivers (Williams, 1954; Hobbs, 1989). Appropriate habitats will be sampled throughout the Arkansas Ozark Mountains Ecoregion by hand, using minnow seines, dip nets, and/or baited traps.

For genetic analyses, gill tissue will be dissected from crayfish specimens and preserved in 95% EtOH. DNA will be extracted using standard protocols and a ~500 base pair highly variable segment of the 16S gene of the mitochondrial genome will be PCR amplified using the methods and primers described in Crandall and Fitzpatrick (1996). Sequences will be obtained from all known populations of *Cambarus causeyi*, *Procambarus parasimulans*, and *Orconectes meeki brevis*, plus many members of the genera *Cambarus*, *Procambarus*, and *Orconectes* and taxonomic relatives for phylogenetic comparison. Sequences will be aligned in MacClade 4.08 (Maddison and Maddison, 2000) and phylogenetic analyses will be performed in PAUP* (Swofford, 2001) and MrBayes (Huelsenbeck and Ronquist, 2001).

Phylogenetic trees using the an appropriate model of evolution (Posada and Crandall, 2001) will be estimated from the complete data set and node confidence will be assessed using the bootstrap (BS) procedure and with Bayesian posterior probabilities (PP). Significant support for a clade is 70% BS and 95% PP (Hillis and Bull, 1993; Ronquist and Huelsenbeck, 2003).

A part of this study will focus on the Robert S. Kerr Reservoir, Frog-Mulberry, and Little Red River basins as identified by USGS in northern Arkansas. The Arkansas portions of these units include parts of Van Buren, Sebastian, Franklin, Washington, Madison, Johnson, Pope, Van Buren, Cleburne, Independence, and White counties. Since these areas are largely in private ownership, road access to sampling sites is particularly important. U. S. Census Bureau data (2000 TIGER Line Files) on roads in these counties will be used to identify stream segments that intersect roads using ArcMap GIS software, and a subset of these segments will be selected randomly.

Because headwater streams are more numerous and more easily bridged than larger streams, it is anticipated that this site selection will be skewed toward headwater streams. It is likely that some of these headwater streams are intermittent and may not hold water or crayfish when visited for sampling. When this is the case, the site will be replaced with a nearby site on a larger stream that was not selected for sampling.

Sites will be intensively sampled in all available habitats using minnow seines or dip nets appropriately sized to the area being sampled, until we are confident that all crayfish species and habitats have been sampled. This will be supplemented by approximately 30 person-minutes of visual search and hand capture of crayfish by overturning rock slabs if present.

At each sample site, latitude and longitude coordinates will be recorded for the midpoint of the sample area. Other information recorded will include water temperature, typical depth and width of pool and riffle habitats, predominant substrate sizes, and notes regarding aquatic vegetation, riparian vegetation, turbidity, and flow class. Incidentally collected fish species will also be recorded. Data will be recorded separately for distinct major habitat units (pools vs. riffles) at each site, as applicable.

Crayfish will be sorted by perceived species, gender recorded, carapace length measured, and series of voucher specimens taken including males and females of each species. All voucher specimens will be preserved in 70% ethanol, identification verified, and deposited in a museum collection (Brigham Young University Monte L. Bean Museum Crustacean Collection) or the AGFC Nongame Aquatics Program reference collection.

The project methodology will be entered in the Natural Resource Monitoring Partnership database (<http://nrmp.nbj.gov>) as required by the AGFC.

In an effort to update the Comprehensive Wildlife Conservation Strategy database, Mr. Brian Wagner, AGFC, will be the main contact person and will work closely with Dr. Henry Robison, SAU, to coordinate information delivery. We are committed to updating the CWCS database by providing our research results at the conclusion of this project.

Expected Outcomes:

Data collected will be used to assess the conservation status of *Cambarus causeyi*, *Procambarus parasimulans*, and *Orconectes meeki brevis*, to define the extent of their distributions in Arkansas, and to clarify the taxonomic status of each of these three crayfish within their respective genera. A comprehensive final report and distributional data with geographic coordinates will be submitted no later than June 30, 2009. These results concerning each of the individual crayfish species will be published in state, regional, or national peer-reviewed scientific journals. In addition, in an effort to make the public

aware of this research on crayfishes, a popularized article will be written and submitted to a local (El Dorado Times) or state newspaper (Arkansas Democrat Gazette). In addition, a popularized article on state crayfishes will be prepared for publication in the *Arkansas Wildlife* magazine. Data collected will also significantly enhance our understanding of crayfish distributions in Arkansas and will be incorporated into databases maintained by the Arkansas Game and Fish Commission and the Arkansas Natural Heritage Commission. Finally, we will deposit our genetic data on the crayfishes in GenBank (<http://www.ncbi.nlm.nih.gov/>) while the specimens and tissues will be deposited in the Monte L. Bean Life Sciences Museum at Brigham Young University in Provo, Utah.

Existing Resources Utilized:

As reflected in the matching funds portion of the budget below, this project takes advantage of past cooperation between Southern Arkansas University, Brigham Young University, and Arkansas Game and Fish Commission and builds on recent Arkansas crayfish studies by the cooperators jointly and independently. All three cooperators contribute expertise in the field, systematic, and genetic areas of this project. Utilizing lab equipment and personnel resources as contributions by the cooperators directly enhances the project.

Proposed Budget:

Requested SWG Funds:	SAU	BYU	AGFC
Salary/Benefits	\$1,500	\$1,500	\$6,000 *1
Operating Expenses:			
Travel	\$7,500	\$5,000	\$0
Expendable Supplies	\$1,800	\$4,300	\$500
10 % Overhead	\$1,200	\$1,200	\$0
<i>Subtotal</i>	\$12,000	\$12,000	\$6,500
TOTAL	\$30,500		

Matching Funds:	SAU	BYU	AGFC
Salary/Benefits	\$0	\$10,023 *2	\$15,000 *3
Operating Expenses:			
Travel	\$0	\$0	\$2,000
PCR/Sequencing Equipment (prorated)	-	\$2,000	-
Publishing costs	-	\$2,000	-
<i>Subtotal</i>	\$0	\$14,023	\$17,000
TOTAL	\$31,023		

*1 – Extra Labor Technician

*2 – Crandall – 15 days effort at \$621/day plus benefits

*3 – Wagner

Qualifications of Project Partners:

Dr. Henry W. Robison is a Distinguished Professor of Biology at Southern Arkansas University where he has spent the last 35 years studying the fishes and the crayfishes of Arkansas. He is widely published (165 publications) in national, regional, and state publications and has written seven books including *Fishes of Arkansas*, *Amphibians and Reptiles of Arkansas* and *Only in Arkansas*. In the 1970s Dr. Robison teamed with Dr. Horton H. Hobbs, Jr., Smithsonian Institution and world expert on crayfishes, in studying the burrowing crayfish species of Arkansas, resulting in four joint publications. In these four publications Hobbs and Robison described six new species of crayfishes. In addition, Dr. Robison has spent the past 20 years collecting Arkansas crayfishes for an future *Crayfishes of Arkansas* book to be co-authored with molecular biologist and crayfish expert, Dr. Keith Crandall of Brigham Young University.

Dr. Keith Crandall is Professor & Chair of the Department of Integrative Biology at Brigham Young University. He has 18 years of research experience in the conservation biology, population genetics, systematics, and taxonomy of freshwater crayfishes. Dr. Crandall has published over 130 articles in peer-reviewed journals and is recognized as a leading authority on freshwater crayfish evolution. Dr. Crandall has worked closely with Dr. Robison and Mr. Wagner over the last three years developing a comprehensive understanding of the evolutionary history, conservation genetics, and natural history of the Arkansas crayfish.

Mr. Brian Wagner is Nongame Aquatics Biologist with the Arkansas Game and Fish Commission. He has a Masters Degree in Fisheries from Virginia Tech, and has been involved in aquatic conservation and research with the Commission for 18 years. For the past 9 years, he has been the Commission's Nongame Aquatics Biologist. Brian coordinates the Commission's Nongame Aquatics Program and has specific oversight of nongame fish and crayfish efforts. He is a Certified Fisheries Scientist and has authored or co-authored peer-reviewed publications on sport fish, nongame fish, crayfish, reptiles, and amphibians. Brian led the State Wildlife Grants Crayfish Taxa Team, and was also active on the Fish, Cave, and Invertebrate Taxa Teams.

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