

Arkansas Game and Fish Commission Final Report:  
Descriptions and Taxonomic  
Determinations of New Cave Invertebrates



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David Kampwerth, Karst Biologist, USFWS  
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**Abstract**

*Cambarus zophanastes* is a cave crayfish endemic to a single cave in north Arkansas and listed as endangered by the U. S. Fish and Wildlife Service. While it has been suspected to occur in other nearby caves, this has not been documented. A crayfish collected from one of these caves in 2002 was believed upon examination to be a new species and not *C. zophanastes*. After repeated attempts, cave divers observed nine cave crayfish in this cave and captured five thereof. Tissue samples were collected from these crayfish for analysis and four survived to be released back into the cave. The fifth crayfish expired in handling and was preserved to be deposited in a museum as reference material. Genetic analyses suggest that these crayfish may be *C. zophanastes*. Additional tissue should be collected from *C. zophanastes* for comparison, since the analyses only included one specimen for *C. zophanastes*.

**Acknowledgements**

A debt of gratitude goes out to Howard Nesbitt and the Nesbitt family for allowing us access to this cave on their private property – without their permission, the status and identity of this cave crayfish would still be in the dark. We would like to thank Geo Graening and Charles Brickey for their assistance in the field. Shannon Wallace and the Ozark Cave Diving Alliance were instrumental in obtaining the necessary specimens for this study. Horton Hobbs, III gave invaluable input on the taxonomic issues involved, the possible identity of specimens collected, and the interpretation of the genetic results. Jeff Koppleman, Casey Dillman, Keith Crandall, and Jen Buhay contributed to genetic analyses and interpretations thereof. Finally, I would like to thank my wife, Diane Wagner, and our children, Tiger and Logan, for tolerating inconvenient trips to north Arkansas, even when water conditions resulted in cancellation of diving activities.

**Introduction & Background**

*Cambarus zophanastes* (Hobbs and Bedinger 1964) is a cave crayfish endemic to Arkansas (Robison and Allen 1995). It has been listed as endangered by the United States Fish and Wildlife Service (USFWS 1988). The only confirmed population of this cave crayfish is in Hell Creek Cave (McDaniel and Smith 1976, McDaniel et al. 1979,

Youngsteadt and Youngsteadt 1978, Graening et al., in progress). The species has, however, been conjectured to occur in some other caves in the vicinity of Mountain View, Arkansas (Graening and Brown 2000, Graening et al. 2001). Crayfish previously collected from Cave River Cave and Nesbit Springs Cave were thought to be this species (Rex Roberg, personal communication). However, the specimens collected were not Form-I males, so they could not be identified to species (Horton Hobbs, III, personal communication). The aquatic habitats in these two caves are largely inaccessible to our survey methods. Funds were secured through Section 6 of the Endangered Species Act (approved effective October 1, 2001) in order to contract professional cave divers to collect crayfishes from these two caves for identification purposes.

The owner of Cave River Cave refused to allow access for the collection of cave crayfishes, although researchers were on one occasion allowed to visit the cave and visually confirmed the presence of stygobitic crayfish (G.O. Graening, personal communication). As for Nesbitt Spring Cave, the cave dive crew we were working with proved unreliable, canceling all planned dive trips due to weather or medical issues, even though the project was extended through June 2003.

On 30 March 2002, G. O. Graening (The Nature Conservancy), Slay, Wagner, and Charles Brickey (volunteer caver) conducted an extensive bioinventory of Nesbitt Spring Cave. The cave divers had cancelled because of injuries unrelated to this study. Census of the non-submerged portions of the aquatic habitat revealed only one stygobitic crayfish. This crayfish was a male and was collected from the cave stream by Brickey and Slay. Because the specimen was not in breeding condition (Form I), which is necessary for taxonomic separation from related species, the specimen was held in an environmental chamber under similar conditions to its habitat (native water held at 14°C, complete darkness, limestone substrate). When the specimen molted into reproductive form, it was euthanized by lowering body temperature in a freezer to 3°C, and subsequently preserved in 70% ethanol and sent to Horton Hobbs III at Whittenburg State University for examination. Dr. Hobbs compared the crayfish to type specimens from the Smithsonian of other stygobitic cambarids to aid in determinations. The specimen is currently in the personal collection of Dr. Hobbs, and will be deposited in the Smithsonian for permanent curation. Dr. Hobbs determined this specimen to be a new species of the genus *Cambarus*, but additional specimens would be needed to publish a description.

The primary goal of the current project was to complete a formal description of this crayfish, so it would be "official" as a new species and could be a valid conservation target. A secondary objective of this project was to determine general abundance of this crayfish in Nesbitt Spring Cave, if possible.

## **Methods**

Typically, a series of several preserved specimens is used to write a species description. Due to the potential small population size, we were not comfortable with removing

additional specimens. Certified cave divers were contracted to make visual counts of cave crayfish in the cave to better determine population of this crayfish. After much discussion, Dr. Hobbs agreed to describe the species based on the single specimen if it was supported by genetic analyses of tissue samples from 5 additional specimens. He felt that this would be sufficient to develop a species description that would stand up to peer review.

In order to allow these analyses, a pair of walking legs were removed from each subject crayfish and preserved in 95% ethanol. The crayfish were then be released alive and the legs would regenerate in time. The samples were sent to Jeff Koppleman, geneticist with the Missouri Department of Conservation, who in turn sent them to Saint Louis University for DNA amplification and gene sequencing. The results for these samples could then be compared to archive material for *Cambarus zophonastes*, *C. aculabrum*, *C. setosus*, and other stygobitic crayfishes. The full results will be incorporated into a larger study of relationships among cave crayfishes– for the purposes of this study, we simply needed confirmation of whether these specimens represented a different species or not.

## **Results and Discussion**

On 4 September 2004 divers made it back to just past the third sump in Nesbitt Spring Cave and found no stygobitic crayfish. In cave time was four hours with four divers. The water temperature was 56 degrees Fahrenheit. They did observe approximately 75 to 100 “jumbo-sized” sculpins, as well a few six to eight-inch rock bass. Also observed were two grotto salamanders (*Typhlotriton spelaeus*), one cave salamander (*Eurycea lucifuga*), one surface crayfish (likely *Orconectes neglectes chaenodactylus*), six central stonerollers (*Campostoma pullum*), and three unidentified bats.

On 18 December 2004 a second cave dive was planned. This dive was cancelled due to poor water clarity.

On 5 March 2005 another dive was conducted in the cave, with extended penetration past the third sump. No sculpins were observed during this dive. Divers observed nine cave crayfish, all beyond 200 feet into the third sump, and were successful in capturing five specimens. Kampwerth removed and preserved walking legs from the five crayfish and four of them survived for return by the divers to the third sump. The fifth, a small form II male, was preserved whole and provided to Dr. Hobbs as additional reference material. The legs were shipped to Koppleman, and gill tissue from the deceased specimen was shipped to Dr. Keith Crandall of Brigham Young University, to allow for independent confirmation of results.

Koppleman reported results of the genetics work to us. Based on the five Nesbitt samples and the one *Cambarus zophonastes* sample they have DNA from, he concluded that they all represent the same species. It is highly unlikely that there are more than one species represented in the Nesbitt Spring Cave samples, even though we observed some diversity

among the samples. Results of the Brigham Young University confirmation have yet to be reported.

### **Conclusions**

The population of cave crayfish in Nesbitt Spring Cave appears to persist in very small numbers at a great distance into the cave. We hypothesize that the initial specimen that we collected and sent to Dr. Hobbs was washed further out in the cave by a high flow event. This small population is of great conservation importance and efforts should be made to promote its persistence.

Genetic results currently indicate that this is a second population of the rare endangered crayfish, *Cambarus zophonastes*. We will continue to treat this result cautiously until additional *C. zophonastes* tissue can be secured and analyzed. However, this tentative identification is bright news for the conservation of *C. zophonastes*, which has been believed to be a single-cave endemic confined in a perched water table to Hell Creek Cave. If additional analysis of *C. zophonastes* tissues supports this result, it will significantly increase the opportunity for recovery of this species.

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