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FINAL REPORT SECTION 6 ENDANGERED SPECIES ACT



FEDERAL AID PROJECT E-5

STEVEY AND SPECIES DETERMINATION OF CAVE CRAYFISH IN OKLAHOMA

SEPTEMBER 23, 1991 - SEPTEMBER 30, 1992

FINAL REPORT

STATE: Oklahoma

PROJECT NO: E-5

PROJECT TITLE: Survey and species determination of cave crayfish (Cambarus spp.) in Oklahoma.

SEGMENT DATES: 23 September 1991 - 30 September 1992

I. PROGRAM NARRATIVE OBJECTIVE: To determine the identification, distribution and abundance of troglobytic *Cambarus* in the Ozark region of Oklahoma.

JOB NUMBER: 2

JOB TITLE: Taxonomy of Ozark cave crayfish based on genetic divergence.

JOB OBJECTIVE: To collect voucher specimens of cave crayfish from Ozark caves to clarify the taxonomic relationship of *Cambarus* in northeast Oklahoma.

II. JOB PROCEDURES

- 1. Collect voucher Male-I crayfish from caves DL-91, DL-38, DL-39, and DL-74. Send vouchers to a recognized expert on troglobytic crayfish systematics for morphological examination.
- 2. Search caves DL-49 and DL-64 and collect voucher males if present. Send vouchers to the crayfish systematist.
- 3. Remove one claw from all male crayfish collected. Send fresh tissue samples on dry ice to contract molecular biologist for electrophoretic analysis.

III. SUMMARY OF PROGRESS

A. Methods

During the fall of 1991 and the spring of 1992 we searched caves DL-38, DL-39, DL-74, DL-91, DL-49, and DL-64 for male cave crayfish. Caves were surveyed by visiting all accessible aquatic habitats within each cave and surveying along the substrate and under larger rocks. Crayfish were collected either by hand or with a long-handled dip net. Specimens were sexed in the field by examining the morphology of the first gonopod and males were immediately placed in a water-filled container and transported, alive, back to a laboratory at the Oklahoma Natural Heritage Inventory.

In addition to the six caves mentioned above, we surveyed two recently re-discovered caves:

DL-119 and DL-148. Both caves contain cave crayfish in substantial numbers.

Collected Male-I crayfish were prepared for shipping by removing a single chela from each crayfish and storing it in an ultrafreeze to halt protein degradation in the tissue. The remainder of each specimen was preserved in alcohol. Male-II individuals were held in an aquarium until they molted to Form I. Frozen chelae were shipped to Jeffrey B. Koppelman at the Missouri Department of Conservation for electrophoretic analysis. Specimens preserved in alcohol were sent to Dr. Horton Hobbs III at Wittenberg University for morphological analysis.

B. Results

Cave crayfish were observed in all caves surveyed except for DL-64. Crayfish were recorded only if they could be captured, measured, and sexed. The following are the number and sex of crayfish captured in each cave: (DL-38)1-M; (DL-39)2-M, 4-F; (DL-49)1-F; (DL-74)1-M, 2-F; (DL-91)2-M, 2-F; (DL-119)1-Mjuv.; and (DL-148)2-F.

A single Male-I crayfish was collected in each of caves DL-39, DL-74, and DL-91. A single Male-II crayfish was collected from DL-38 and successfully molted to Form I in the laboratory. A juvenile male crayfish was captured in DL-119 but released due to its age and small size. We also captured and released female crayfish in DL-49 and DL-148. We found no troglobytic crayfish in DL-64.

The results of the electrophoretic analysis are shown in Table 1 and Figure 1. These results are part of a larger study conducted by Koppelman (Koppelman 1990, 1992 manuscript in review). Table 1 combines the results from all previously analyzed specimens from the Oklahoma caves along with specimens taken from nearby caves in Missouri and Arkansas. Specimens from DL-38, DL-74, and DL-91 were all genetically similar based on the use of Roger's (1972) distance measure and were closer to Cambarus aculabrum than to the C. setosus from caves in Missouri (Figure 1). Koppelman has tentatively called the crayfish from the three Oklahoma caves C. setosus based on genetic, distributional, and historical information. However, he believes that these individuals are genetically dissimilar enough from C. setosus of Missouri to be considered a different and, as yet, undescribed species (Koppelman, pers. comm.). The crayfish from DL-39 clustered closely with several of the Missouri C. setosus sites but remains the only known site for C. tartarus.

Hobbs morphological analysis supports the results of the protein analysis (Appendix A). Crayfish taken from DL-38, DL-74, and DL-91 show enough unique morphological features to be considered a separate, undescribed species. Dr. Hobbs is in the process of describing the species. The specimen from DL-39 was identified as *C. tartarus*.

C. Conclusions

Results of both the protein and the morphologial analysis support the conclusion that caves DL-38, DL-74, and DL-91 contain a new, undescribed species of troglobytic crayfish. Cave DL-39 remains the only known site in the world for *C. tartarus*.

D. Management Recommendations

Caves DL-38, DL-74, and DL-91 contain an undescribed species which is undoubtedly rare and which may merit special protection. These caves should receive greater protection at least until the species is described and its status determined. DL-38 is by far the most accessible of the three caves and appears to receive the most human traffic and disturbance. The pool containing both cave crayfish and the threatened cavefish (Amblyopsis rosae) is near the cave entrance. This pool has had trash dumped in it over the years and continues to degrade. This cave should be gated. The entrance is suited to a gate and a gate would effectively stop most disturbances to this site. DL-74 is also unprotected but is much less accessible due to the well camouflaged and dangerous entrance. This cave shows little sign of human traffic and will likely remain relatively undisturbed as long as the landowners are aware of its importance. DL-91 is owned by The Nature Conservancy and is receiving adequate protection in the form of a locked fence surrounding the site and a gate on the cave entrance. The single C. tartarus site (DL-39) is also currently relatively well protected by the landowners who carefully control access to this cave.

IV. ACKNOWLEDGEMENTS

We greatly appreciate the work of Jeff Koppelman and Horton Hobbs III in making clear and timely identifications of all specimens. Thanks to Nora Jones of The Nature Conservancy, members of the Tulsa Grotto, and all the landowners for help in gaining access to the caves. Special thanks to Bill Puckette who led us into most of the caves and without whose help this project would not have been completed. Thanks, Bill.

V. LITERATURE CITED

Koppelman, J.B. 1990. A biochemical genetic analysis of troglobitic crayfish (<u>Cambarus</u> spp.) in Missouri, Oklahoma, and Arkansas. Unpubl. report for the Missouri Dept. of Conservation, Oklahoma Natural Heritage Inventory, and Arkansas Game and Fish Commission. Missouri Department of Conservation.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, University of Texas Publication 7213:145-153.

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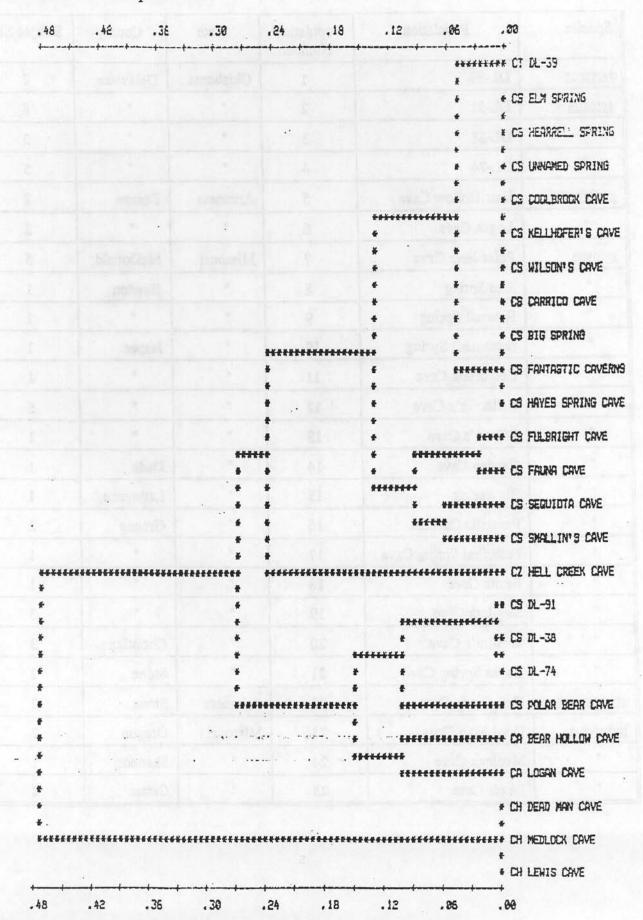
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Table 1.— Cave crayfish samples electrophoretically analyzed. Species assignations are based on morphological, geographical, and/or historical information. Unpublished data from Jeffrey Koppelman, Missouri Department of Conservation and used with his permission.

Species	Population	Population Number	State	County	Sample Size
tartarus	DL-39	1	Oklahoma	Delaware	7
setosus	DL-91	2	*		8
BILL OF THE STATE	DL-38	3	*	60	3
antigo castell	DL-74	4	. " "	7	5
aculabrum	Bear Hollow Cave	5	Arkansas	Benton	2
***************************************	Logan Cave	6	н	N	2
setosus	Polar Bear Cave	7	Missouri	McDonald	5
**	Elm Spring	8	и	Newton	1 /
W ALLES	Hearrell Spring	. 9			1
	(unnamed) Spring	10	н	Jasper	1
Parket Station	Coolbrook Cave	11	п	*	1
MI MIN BI	Kellhofer's Cave	. 12		11	5
nati monina	Wilson's Cave	13		"	1
3303 860	Carrico Cave	14	и	Dade	1
"	Big Spring	15	н	Lawrence	1
21	Fantastic Caverns	16	п	Greene	2
н	Fulbright Spring Cave	17	п	*	1
A STATE OF THE	Fauna Cave	18	m	M	1
и П.	Sequiota Cave	19	н	н .	3
н Ба	Smallin's Cave	20	и	Christian	3
• (5)	Hayes Spring Cave	21	N	Stone	2
cophonastes	Hell Creek Cave	22	Arkansas	Stone	2
ubrichti	Dead Man Cave	23	Missouri	Oregon	2
	Medlock Cave	24	н	Shannon	1
**	Lewis Cave	25	er .	Carter	2

Figure 1.—Rogers (1972) genetic distance results for cave crayfish populations described in Table 1. Unpublished data from Jeffrey Koppelman, Missouri Department of Conservation and used with his permission.



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Oklahowa Biological Survey 2001 Prisotly Avenue, Bldg. 605 "

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Thank you for your inversel, enthusiasm, and help in the ongoing sindy of the cave orayilehes of the Ozarks, Have a hippy

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Dr. Caryn C. Vaughn
Oklahoma Biological Survey
2001 Priestly Avenue, Bldg. 605
Norman, Oklahoma 73019-0543

November 18, 1992

Dear Dr. Vaughn:

After examining the troglobitic crayfish specimens from Twin, Star, and Jail caves in Delaware County, Oklahoma I have decided that they represent an undescribed species of Cambarus (Jugicambarus). I have looked at a limited number of cave crayfishes from Oklahoma, Missouri, and Arkansas that are housed in the Natural Museum of Natural History, Smithsonian Institution and have compared the Delaware County cave specimens with them and feel confident that those populations in Twin, Star, and Jail caves represent a new species.

As I indicated to you in a recent phone conversation, I am winding down the fall term and that is to be followed by a field trip to do some work in Tennessee and Alabama caves during early December. I shall also be going to Washington, D. C. in mid-December and will do some more work in the Smithsonian. Hopefully I shall have the description of the crayfish completed by the new year.

Thank you for your interest, enthusiasm, and help in the ongoing study of the cave crayfishes of the Ozarks. Have a happy Thanksgiving.

Sincerely,

Horton H. Hobbs III Professor of Biology

