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The Crayfish Identity Crisis: A Morphological and Genetic Assessment of Biodiversity

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Among the most endangered animal groups of North America is the freshwater crayfish. Their survival has been hindered by rapid growth in the human population, water pollution, and habitat degradation. The American Fisheries Society has estimated that 50% of freshwater crayfishes are in need of conservation measures (Taylor et al. 1996). However, only 1% is currently protected under the Endangered Species Act of 1973. Because crayfish are at the center of invaluable research as top predators, nutrient recyclers, and bio-indicators of our water quality, it is important that conservation measures are taken.

The lack of government listing of the crayfish, which has prevented their conservation and study, is due largely to the difficulties in species identification. Despite much debate on names and classification, current identification methods remain focused on highly variable anatomical features. The outer appendages of breeding males have been used traditionally rendering females and juveniles useless. Such difficulties have prevented important studies on life history and ecology.

Modern DNA technology has shed new light on organism identification techniques. Problems in species recognition can be resolved through genetics and DNA sequencing. The goal of this research project was to assess the genetic diversity of crayfish specific to the Tennessee and Cumberland River systems. Specifically, sequences of the 16s mitochondrial gene were to be collected from 25 different species of the genus *Orconectes,* and used to examine evolutionary relationships and intra-specific genetic variation between these species.

Work on this project began in January of 2003 and is still in progress. The current status is as follows:
Of the 4000 samples that were collected from 180 different sites, over 1500 of them have been identified and DNA extracted. The DNA was extracted using a cell lysis protocol. More than 600 of those have been sequenced and data organized for analysis using several bioinformatics computer programs. Some of the species that have been selected for intra specific analysis include: *O. forceps, O. putnami, O. placidus, O. compressus, O. durelli, O. rusticus, O. australis australis, and O. australis packardi*.

Future work and analysis:
It has been somewhat of a struggle to successfully PCR each gene for all species. With already obtained sequence data, however, new primers may be designed specifically for each species and should result in more successful PCRs. Because this is the first genetic assessment of the Tennessee and Cumberland River systems, recognition of several new species is anticipated. Once an adequate amount of sequence data has been obtained, the computer program PAUP* will be used to further examine the evolutionary relationships between the different *Orconectes* species. Evolutionary models of the obtained nucleotide sequences within the *Orconectes* genus

will then be determined using the computer program ModelTest version 3.0 (Posada and Crandall, 1998). The bootstrap analysis using 1000 pseudo replications will be performed to test the stability of the phylogeny. From this data a molecular phylogeny will be constructed to assess the differences and similarities of the 25 *Orconectes* species. Any significant geographical associations between species will be determined using nested clade analysis. Distributions of the species will be organized onto a range map.

This study represents the first step in exploring the genetic resources of crayfish from the Tennessee and Cumberland Rivers, and is the first large-scale genetic and morphological analysis of this very complex group of crayfish. Obtained data will be used to help answer questions regarding preservation of the possibly endangered *Orconectes* species. All results of investigations will be provided to federal and state biologists for future aquatic surveys. Such a thorough examination will provide needed information to discover more accurate identification techniques and further areas of biological investigations such as life history, habitat requirements, reproduction, and tolerance to pollution. New species of crayfish will be published along with their genetic analyses in systematic and molecular phylogenetic journals.

References

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