

INTERIM REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-78-R

Endangered and Threatened Species Conservation

Population Genetic Analysis of the Texas Blind Salamander, *Eurycea rathbuni*

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12 November 2008

INTERIM REPORT

STATE: Texas GRANT NUMBER: E - 78

GRANT TITLE: Population Genetic Analysis of the Texas Blind Salamander, *Eurycea rathbuni*

REPORTING PERIOD: 1 Aug 07 to 31 Aug 08

OBJECTIVE(S):

To estimate migration rates, effective population size, cohesion, and genetic variation in *Eurycea rathbuni*, assess possible threats, and provide a genetic baseline for continued monitoring and management of this species.

Segment Objectives:

Task 1. Tissue samples will be obtained from live animals in the field as part of a mark-recapture study focusing on three putative populations (localities) of *E. rathbuni* (Project Statement: Attachment A, Appendix A). Year 1 and 2.

Task 2. Tissue samples from the other currently accessible localities (San Marcos Springs and possibly Fish Hatchery Well) will be obtained from salvaged specimens, captive populations, and museum collections (Texas State University, USFWS Fish Hatchery, and TNHC, respectively). Year 1 and 2.

Task 3. Genomic DNA will be extracted and numerous markers will be screened for appropriate levels of variation and utility in phylogenetic, phylogeographic, and population genetic analyses. Year 1 and 2.

Task 4. We will conduct phylogenetic and analytical analyses, and will explicitly assess metapopulation dynamics and test specific predictions regarding extinction, colonization, and migration using gene genealogies and the published models. Year 2.

Significant Deviation: None.


Summary Of Progress: Please see Attachment A.

Location: Hays County, TX

Cost: Costs were not available at time of this report.

Prepared by: Craig Farquhar

Date: 12 November 2008

Approved by: 
C. Craig Farquhar

Date: 12 November 2008

ATTACHMENT A

Population Genetic analyses of the Texas blind salamander (*Eurycea rathbuni*)

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October 2008

Introduction

Here I present a very brief interim summary of research on population structure and genetic variation in the Texas blind salamander, *Eurycea* (formerly *Typhlomolge*) *rathbuni*. The conclusions are preliminary; together with my colleague on these projects, Dr. Andrew Gluesenkamp, and my graduate student assistants, we are adding many more samples and data encompassing a wide range of mitochondrial and nuclear markers. Here I include only results based on data that are complete or near complete and have been thoroughly checked. These represent only a small fraction of the data that will be available at the conclusion of this project.

The goals of this study are to determine levels of genetic variation, genetic cohesiveness, and population size of this enigmatic blind salamander. Since its discovery in 1896, *E. rathbuni* has been the subject of intensive interest, yet very little is known of population structure or life history despite its decades long listing as a Federally Endangered Species. Understanding of the distribution and status of *E. rathbuni* has been complicated by its subterranean occurrence in the San Marcos Pool of the Edwards Aquifer, making it difficult to study. The existence of a second recognized species in the group (*E. robusta*, represented by a single specimen collected in 1951 very close to the known distribution of *E. rathbuni*), the recent discovery of the closely related but clearly distinct Austin blind salamander (*E. waterlooensis*) in the Barton Springs segment of the Aquifer, and the even more recent discovery of a putatively new species of blind salamander associated with the subterranean system that feeds Comal Springs in New Braunfels, Comal County indicate that this group is diverse and widespread and further complicates taxonomic and population studies. No substantial molecular studies of *E. rathbuni* have been conducted, although I demonstrated in my dissertation work and subsequent publications (e.g., Chippindale, Price, Wiens, and Hillis 2000, Herpetological Monographs) that based on allozyme and limited mitochondrial data, it clearly is nested within the Texas *Eurycea* group as sister to all other species that occur south of the Colorado River. This was confirmed by further studies (Hillis, Chamberlain, Wilcox, and Chippindale 2001, Herpetologica) that showed the newly described species *E. waterlooensis* to be sister to *E. rathbuni* (molecular data for *E. robusta* unobtainable), with the placement of these species among the Texas *Eurycea* the same as previously

inferred. Mark-recapture studies of *E. rathbuni* recently have been conducted; Gluesenkamp and Krejca finished the project and Krejca and Gluesenkamp have submitted a manuscript for publication in the Pearce-Sellard Series of the Texas Memorial Museum. Results presented here and work in progress reveal high levels of genetic variation within *E. rathbuni* and the possibility that this species may exhibit substantial subdivision and possibly even a wider geographic range than previously suspected.

Methods

DNA was obtained from dozens of samples plus phylogenetic outgroups using standard methods. Most samples represent animals that died in captivity at the Federal fish hatchery in San Marcos and although localities of capture are known, dates of capture are uncertain in most cases. Additional samples were obtained by Gluesenkamp and colleagues from Rattlesnake and Ezell's Caves, and several samples obtained from Rattlesnake Cave by Chippindale and colleagues in the early 1990s also are being studied. The range of quality of DNA for *E. rathbuni* is tremendous, from excellent for several dozen specimens to unusable for some that were dead for extended periods prior to preservation or were preserved in formalin, which renders DNA extraction extremely difficult or impossible. Many samples have proven problematic for molecular studies and have required extensive precautions and repeated PCR amplification to guard against contamination. However, we have obtained excellent data for roughly 45-50 individuals and are filling in data gaps as well as adding new markers.

Briefly, numerous individuals of *E. rathbuni* and close relatives have been examined for several mitochondrial DNA (mtDNA) regions. The mt genome represents a single genetic linkage group, and as expected, conclusions based on different portions of the mt genome yield the same major results with respect to relationships and diversification. Thus, here we focus on the mt cytochrome b gene (1+ kilobases, kb), which has proven most tractable and informative at a variety of scales and is representative of the mt genome as a whole, although data for limited numbers of individuals for additional mt loci will be included in the final report.

In addition, currently we are screening individuals for variation at several nuclear protein-coding loci and their introns (where applicable). These include portions of the recombination-activating gene (RAG-1), three exons and two introns of the ornithine decarboxylase gene (ODC), several exons and introns of the triosephosphate isomerase gene (TPI), an exon of the pro-opiomelanocortin gene (POMC), three exons and two introns of the rhodopsin gene, and the single-exon melanocortin receptor 1 gene. Results of these studies are very preliminary, but encouraging for some of these nuclear genomic regions. However, POMC is very conserved evolutionarily and show little variation in this species, although it has proven informative in other species and subgroups of Texas *Eurycea*; thus, we will not pursue its use further. Although extremely conserved, RAG-1 does provide information on differentiation in *E. rathbuni* (substantial variation occurs at third codon positions given enough sequence data), and we will obtain data for all

possible individuals for this species. I will elaborate on the results of analyses of nuclear sequence data in subsequent reports.

The most informative results for very fine-scale study are being obtained from nuclear microsatellite (msat) loci, extremely fast-evolving regions consisting of varying numbers of adjacent tri- or tetranucleotide repeats (we have avoided the artifact-prone mono- and dinucleotide repeats although generally they are much more abundant in vertebrate genomes). Based on our screening so far, approximately 10-12 loci of about 60 examined are variable and interpretable for *E. rathbuni*, and we are in the midst of a more comprehensive screening of this species that we expect will add several more informative loci.

Preliminary results and discussion

The preliminary phylogenetic tree for *E. rathbuni* based on cytochrome b sequences reveals a high level of mt variation (interestingly, my early work with allozymes in this species revealed very high levels of protein-level variation relative to those in nearly all other species of Texas *Eurycea*, completely consistent with these results). One possible interpretation is that population size is very large, although this requires much more rigorous analysis. Furthermore, there are two very distinct and well-supported mt haplotype clades (Fig. 1). The uncorrected sequence divergences between haplotypes in the two clades range from roughly 2.8 to 3.1%, and intraclade divergences peak at about 0.6% within the more variable of the two clades and about 0.4% in the less variable clade (see discussion of sequence divergence and species boundaries below). *Eurycea waterlooensis* appears in the very preliminary tree as distinct from but sister to the less variable of the two haplotype clades (although there is no substantial bootstrap support for this placement), and the levels of divergence between members of the two clades within *E. rathbuni* are in the same range as the divergence of *E. waterlooensis* from the clade to which it appears sister. There is no apparent geographic pattern to the distribution of haplotypes in *E. rathbuni*: specimens from the three key sampling sites (Diversion Spring, Ezell's Cave, and Rattlesnake Cave) appear in both clades, and the single available individual from the newly discovered Sessom's Creek locality (not shown) also is nested within one of the two clades. Gluesenkamp has repeatedly attempted to gain access to two additional sites in San Marcos known to harbor *E. rathbuni*, Primer's Well and Johnson's Well, but still is awaiting landowner permission (we hope that this will be facilitated by USF&W personnel in the near future). We also have very limited, degraded material from the Artesian Well at Texas State University for which we may be able to obtain data, and will add more samples from Rattlesnake Cave and likely Ezell's Cave as well.

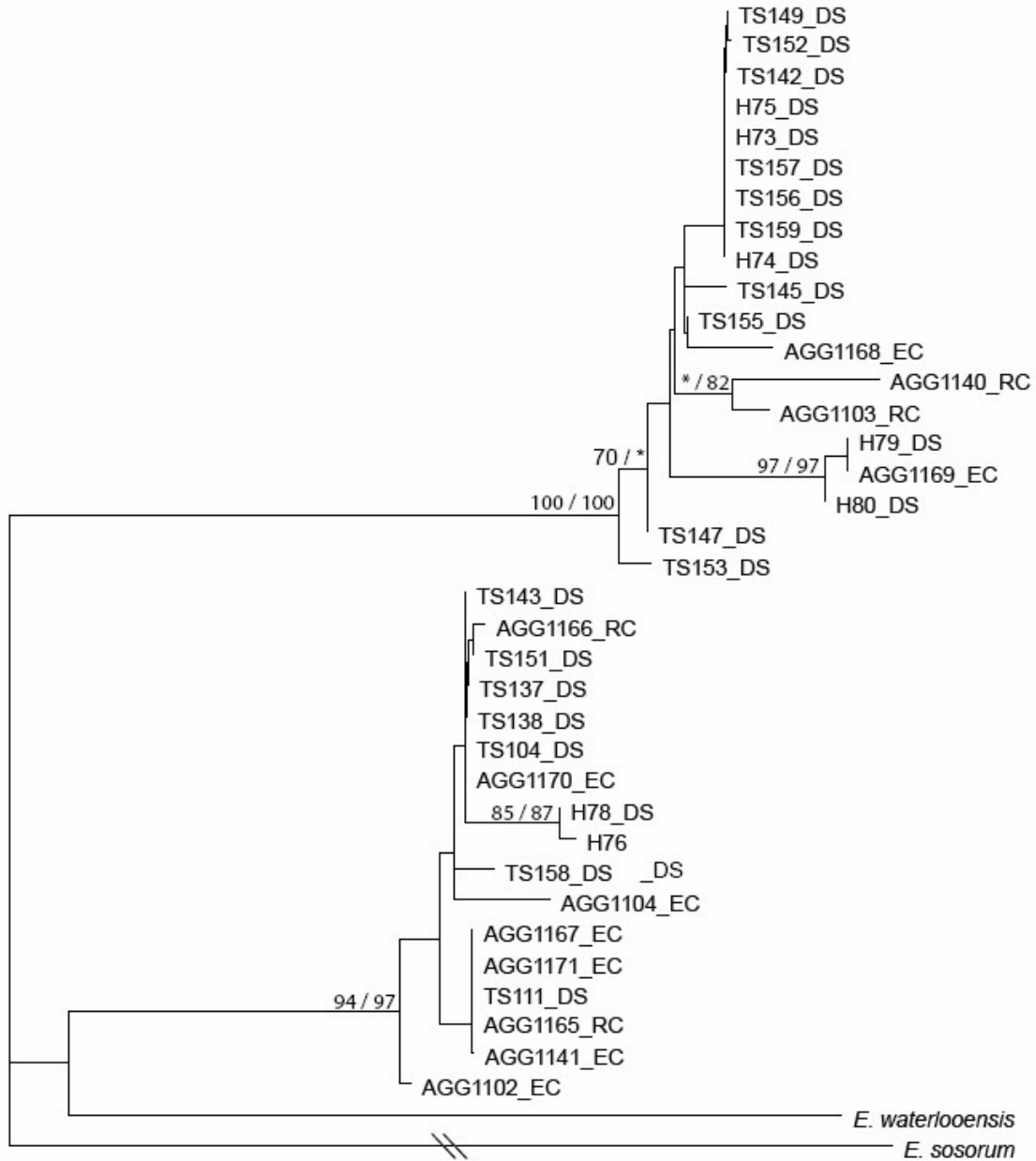
Although assessment of species boundaries based on mt data alone can be difficult, the mitochondrial pattern is striking and suggests the possibility that two distinct units occur within *E. rathbuni*. Alternatively, this may simply represent retention of ancestral mt polymorphisms, but even if this is the case, the haplotype diversity between and to a much lesser extent within clades is striking. It will be extremely important to compare

the patterns of variation shown by nuclear data (especially msats) to those based on mtDNA. In addition (data not shown), the new blind salamander associated with the portion of the aquifer that feeds Comal Springs also appears (mitochondrially) to be sister to one of the haplotype clades within *E. rathbuni*, raising the intriguing possibility that *E. rathbuni* has a much broader geographic range than previously suspected. Like *E. waterlooensis*, the Comal population appears to be morphologically distinct from *E. rathbuni* and we anticipate that it will prove to be a distinct species. Obviously further study is necessary, and the data that we are collecting from fast-evolving nuclear loci will be critical to resolving these issues.

Although not a component of this project, sampling of the numerous accessible test wells in the region between and near San Marcos and New Braunfels is central to understanding relationships and diversity in the blind salamander group. Furthermore, the area of the type locality for the long-lost *E. robusta* (last sampled in 1951; molecular data unobtainable) is readily accessible, and drilling of sampling wells is absolutely essential to unravel the complexity of genetic variation in the group. The technology is readily available and relatively inexpensive, and this recognized yet barely known species must be sampled in future work, especially given a previous (rejected due to insufficient information) attempt at Endangered Species listing. Increased knowledge of relationships in the blind salamander group is critical to understanding of the entire aquifer ecosystem in the region, and protection of water resources that are highly susceptible to degradation and eventual loss.

We have strong reason to question whether a species listed as Federally Endangered for 31 years is a single entity, and whether its range is as limited as previously thought. There is no question of the need for protection of the blind salamander group, unique to Texas. The key questions are: 1) How many species exist; and 2) How do we preserve the Texas blind salamanders and the water resources that support them?

The results presented here represent a small fraction of the data that we have collected for *E. rathbuni*. We will provide and publish more conclusive results once we finish our current studies, but our findings to date highlight the need for future work that encompasses a wide range of approaches, and in particular more extensive sampling of the subterranean systems inhabited by the blind salamander group to delineate species boundaries and geographic distribution.



0.001 substitutions/site

Figure 1. Relationships of individuals of *Eurycea rathbuni* and outgroups based on 1096 bp of the mt cytochrome b gene. Tree topology and branch lengths are based on neighbor joining using the Kimura 3-parameter distance correction; confidence values at major nodes are bootstrap support numbers based on 1000 pseudoreplicates from NJ analysis and maximum parsimony analysis, respectively. Asterisks indicate values below 50% for nodes supported by only one type of analysis. Branch length for *E. sosorum* (outgroup) is truncated to roughly 1/3 actual length. Topological results are nearly identical to those of a separate Bayesian analysis, except that support values here are more conservative. DS = Diversion Spring, EC = Ezell's Cave, and RC = Rattlesnake Cave.