



Proposed Listing of Austin Blind and Jollyville Plateau Salamanders

Briefing to the City of Austin Environmental Board

September 5, 2012

Chuck Lesniak, City of Austin Environmental Officer



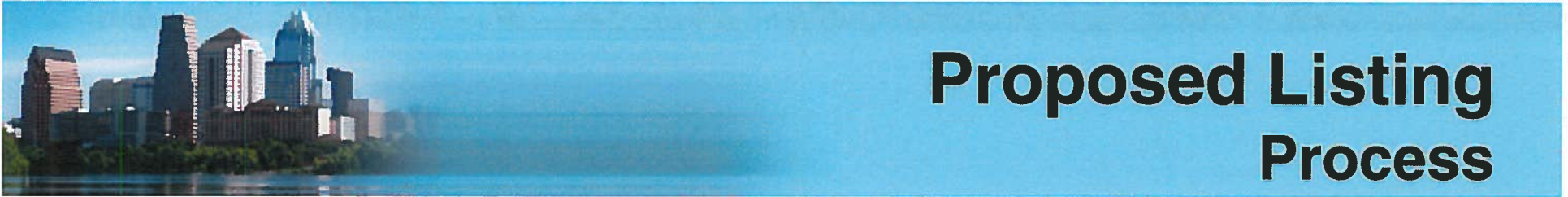
Presentation Overview

- Summary of proposed listing
- What Austin's data shows
- Quality assurance for Austin's research and data management



Background

- The Austin Blind, Jollyville Plateau, Georgetown, and Salado salamanders have been on candidate list for 5-10 years
- A 2007 review found the JPS listing to be “warranted, but precluded”
- Current review was initiated by a court settlement by USFWS with WildEarth Guardians and the Center for Biological Diversity in September 2011



Proposed Listing Process

- Proposed rule was published on August 22nd, 2012 initiating a 60 day comment period
- Includes JPS, Austin Blind, Georgetown and Salado salamanders
- Public hearings in Round Rock on September 5th and Austin on September 6th
- Comments are due by October 22nd, 2012



Proposed Listing Process

- An economic analysis is being prepared
- USFWS is seeking peer review
- The agency reviews and responds to comments, makes any changes to the rule, and publishes a final rule
- Process generally takes about 12 months from publication date



Proposed Listing Summary

- Two species found in the Austin area – Austin Blind and the Jollyville Plateau salamanders
- Austin Blind is only found at Barton Springs
- JPS is found in northwest Travis and western Williamson counties
- Designates critical habitat for all species
 - 120 acres in one unit for the Austin Blind
 - 4,460 acres in 33 units for JPS



Proposed Listing Key Findings

From USFWS proposed rule:

Basis of listing is finding that the species is endangered on 2 of the 5 listing criteria:

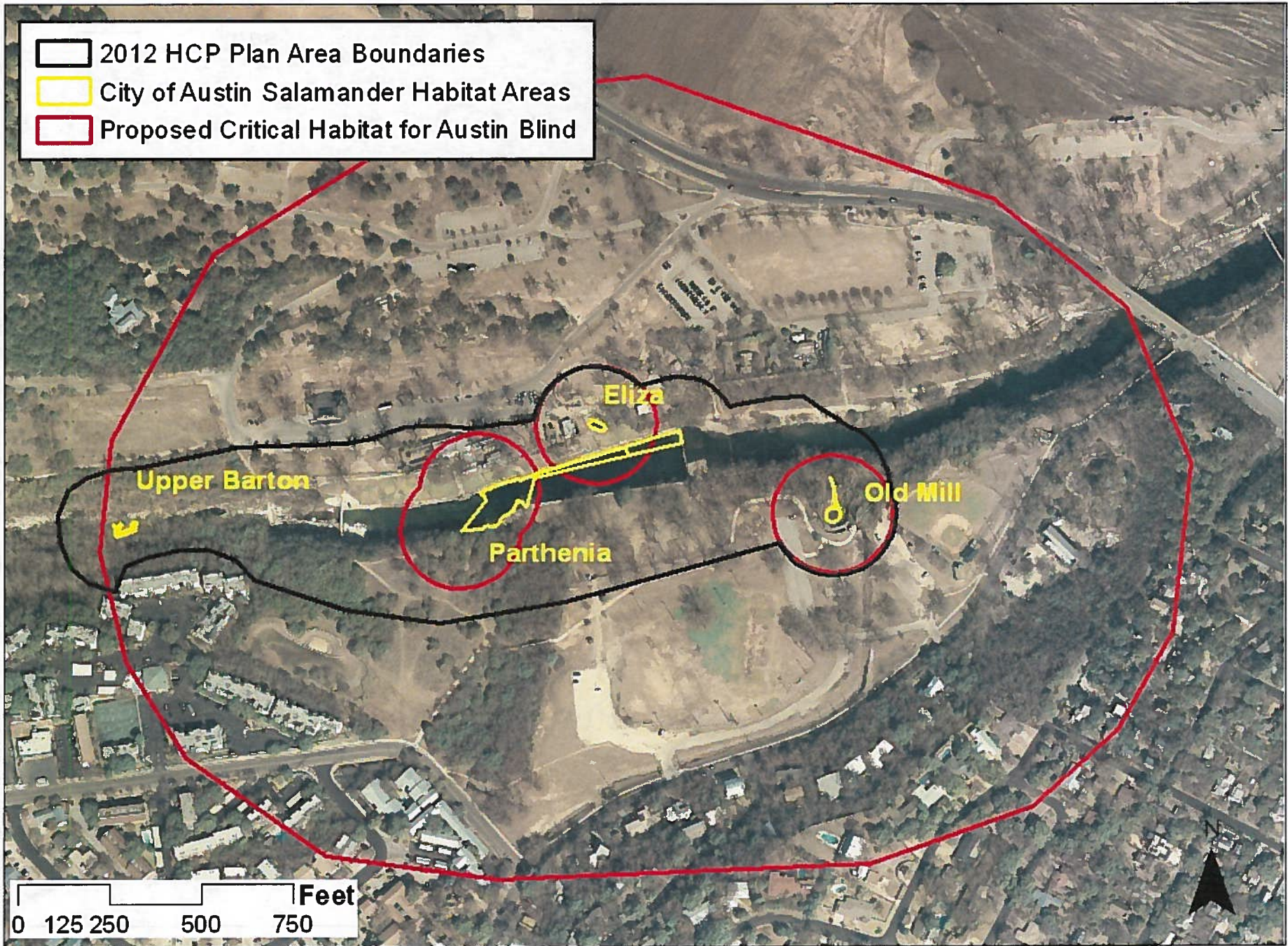
- Destruction, modification, or curtailment of its habitat or range
 - Focuses mostly on decreasing water quality due to increased impervious cover
- Inadequacy of existing regulatory mechanisms
 - Focuses decreasing water quality “despite the existence of current water quality current regulatory mechanisms”

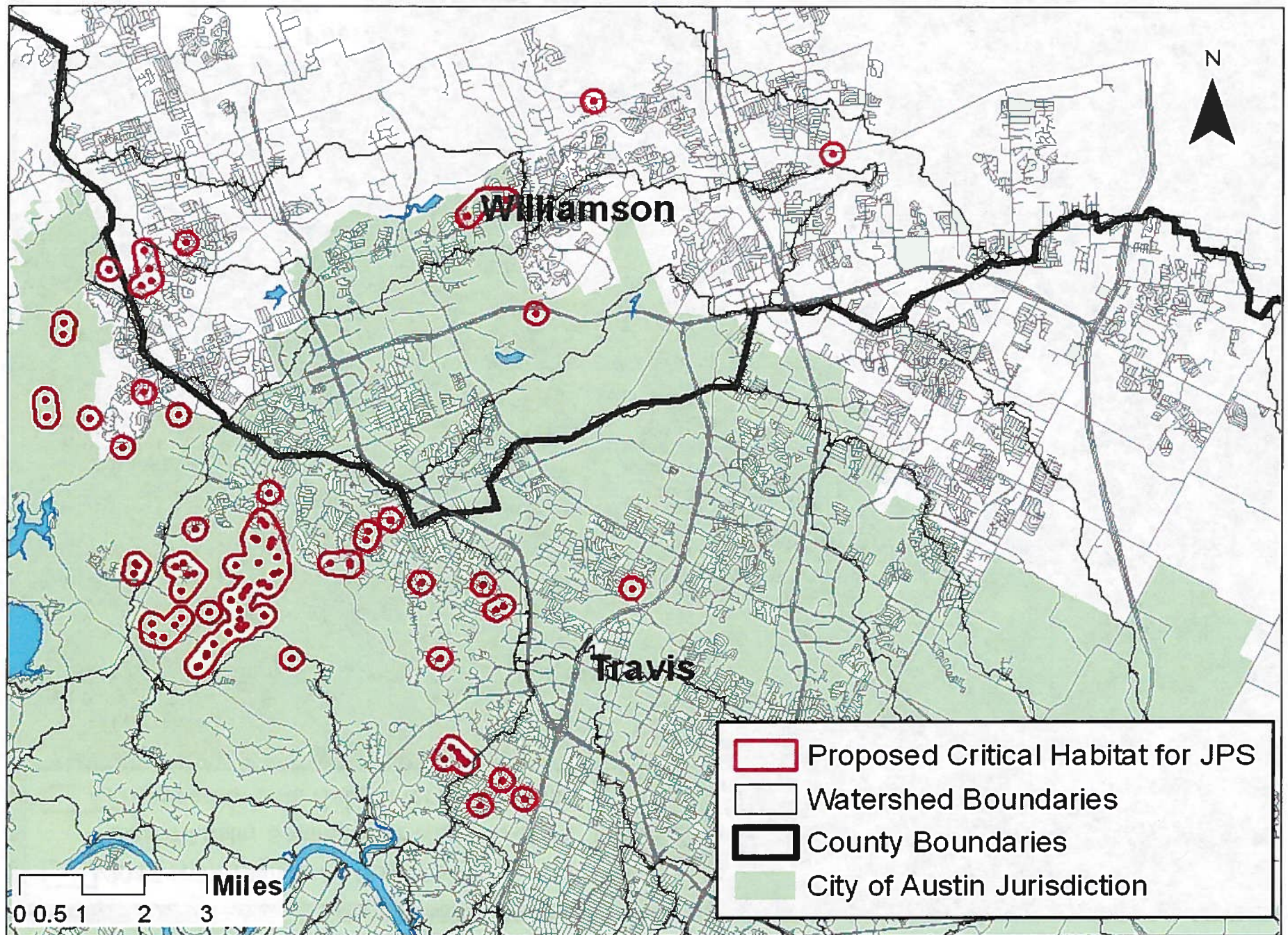


Proposed Listing Key Findings

From USFWS FAQ:

- The four salamanders “are presently in danger of extinction throughout their entire ranges, based on the immediacy, severity and scope of the threats from habitat degradation.”
- Most significant threat is from decreasing water quality/quantity and spring disturbance in part due to increasing impervious cover and inadequate regulations to protect the species







Proposed Listing Listing Implications

- Listing would require a federal permit for any activity likely to cause “take” of a listed species
- COA will have to obtain 10(a)(1)(a) permits for scientific monitoring
- USFWS will have to develop a recovery plan
- Federal agencies must consult with USFWS prior to activity in an area with listed species
- Federal agency activities may not destroy or modify critical habitat
- Critical habitat generally has no impact to non-federal activities or properties



Proposed Listing Listing Implications (cont'd)

- Listing of Austin Blind will not impact operation and maintenance of Barton Springs Pool
 - *Amended Habitat Conservation Plan including Austin Blind already in negotiation between COA and USFWS*
 - *Measures protective of Barton Springs Salamander are also protective of Austin Blind Salamander*



Proposed Listing Public Comment

Informational meetings and public hearings will be held:

- Sept. 5 - Info mtg. 5:30-7:00, public hearing 7:00-8:30 at Wingate by Wyndham, Round Rock, TX
- Sept. 6 - Info mtg. 5:30-7:00, public hearing 7:00-8:30 at the Thompson Conference Center, 2405 Robert Dedman Drive, Rm. 2.102, Austin, TX

Comments may be entered and monitored via the Federal Register website:

- <http://www.regulations.gov>
- Search for Docket ***“FWS–R2–ES–2012–0035”***
- All comments must be submitted by **October 22, 2012**

A photograph of the Austin skyline, featuring several tall buildings and a body of water in the foreground.

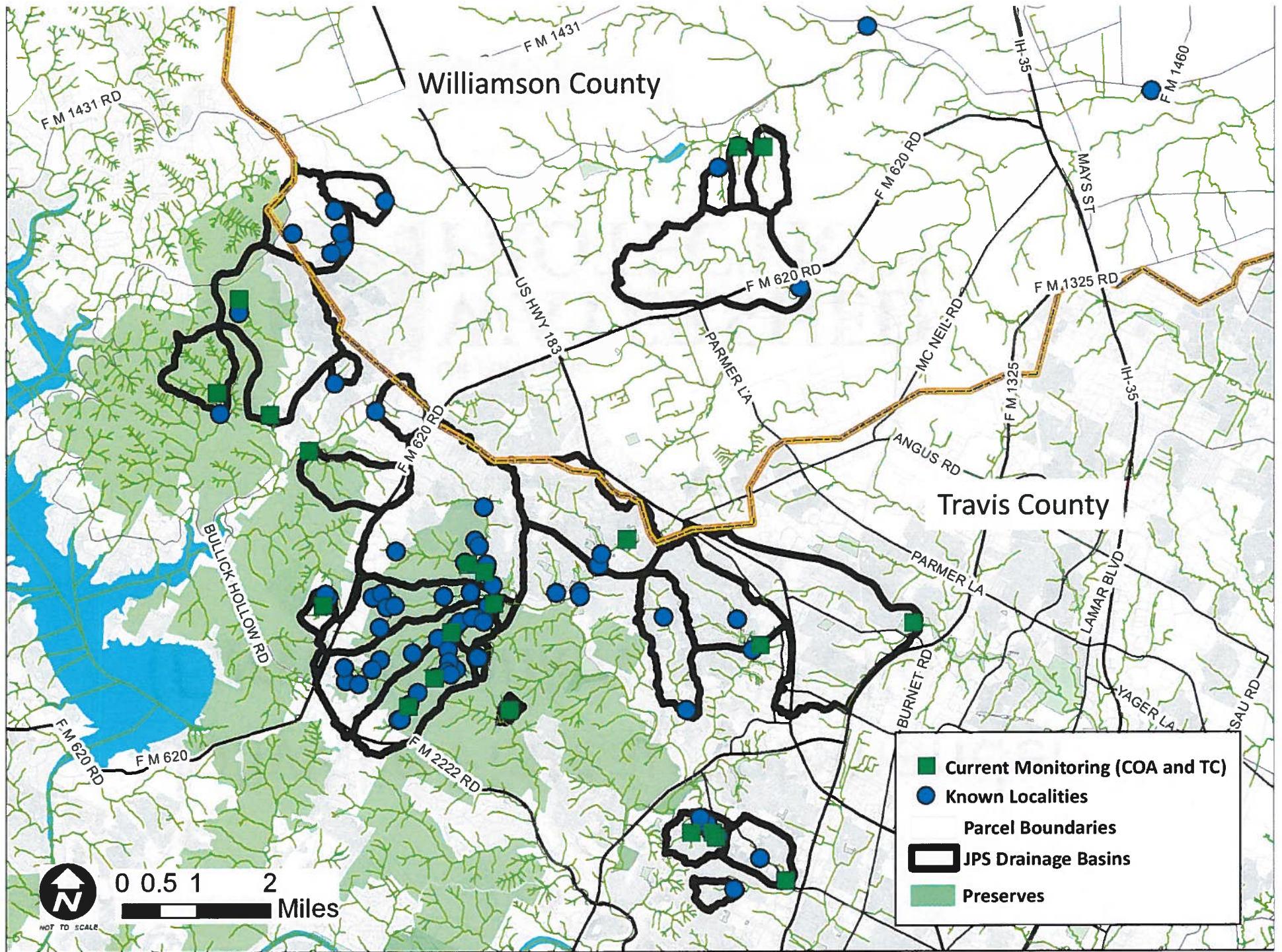
Proposed Listing More Information

More information is available on the web at:
http://www.fws.gov/southwest/es/AustinTexas/ESA_Sp_Salamanders.html

Jollyville Plateau Salamander (*Eurycea tonkawae*) Follow-up

Nathan F. Bendik
Environmental Scientist



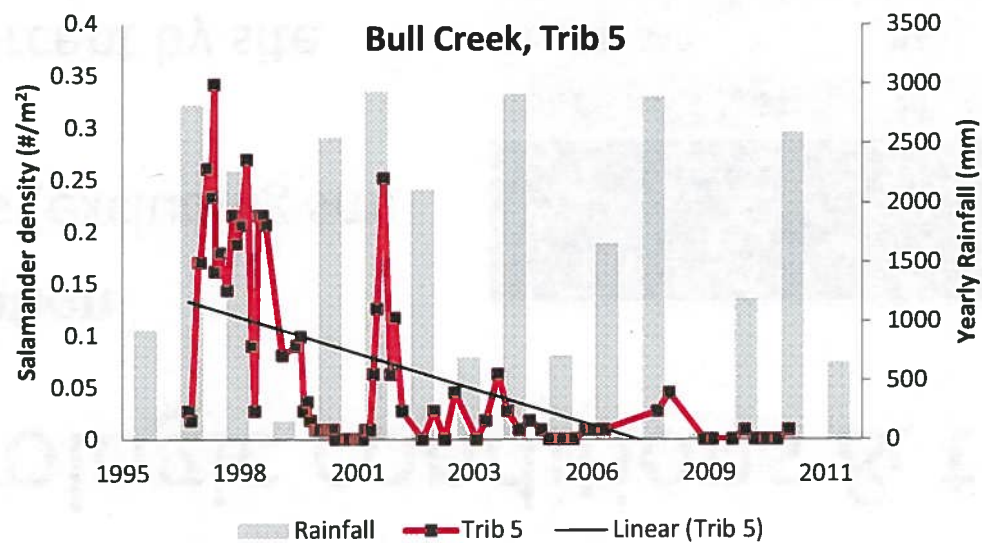
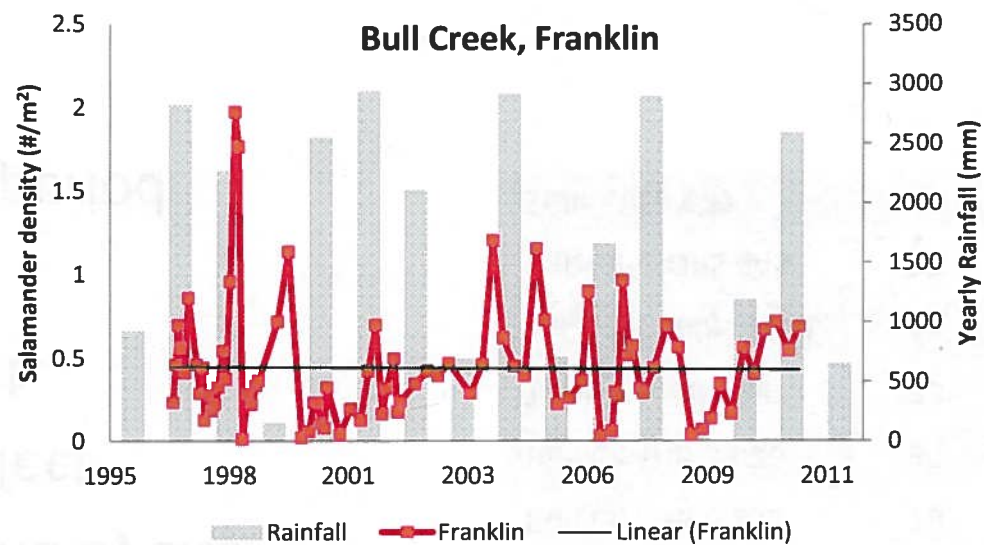


Number of sites

- What is a “salamander site”?
 - Any point location where at least one JP salamander has been observed at some point in time
 - Give us information about distribution, habitat
 - 1 “site” does not = 1 population
 - Biased
 - Some areas frequently accessed by biologists (BCP)
 - Specific surveys targeted a particular drainage (but not all drainages)
 - JPS status is poorly assessed by “# of sites”

Does rainfall explain decline and low density at urban sites?

- No



Linear model: JPS counts, hydrologic conditions & time

Model description

- Total counts (excluding sm. juv.)
- Unique intercept by site
- Unique trend by site
- Drought effect
- Flood effect
- Flow effect
- 90 day lag-period

Site	Sample Size
Bull Cr. Trib 5 1164	70
Franklin 349	83
Spicewood 930	46
Bull Cr. Trib 6 151	78
Bull Cr. Trib 3 926	30
Tanglewood 928	33
Upper Ribelin 4184	11
Balcones DPS 445	27
Stillhouse 927	76

Model Results

Site	Current Impervious Cover %	Median Structure Age	Survey Data	Category	Trend
Baker Spring 3959	<1		2006-2010	Rural	
Franklin 349	<1		1996-2011	Rural	
Upper Ribelin 4184	21	~2008	2007-2011	New Development	+
Bull Cr. Trib 3 926	25	1993	1996-2011	90's Development	-
Bull Cr. Trib 5 1164	19	1991	1997-2011	90's Development	-
Bull Cr. Trib 6 151	25	1991	1996-2011	90's Development	-
Tanglewood 928	36	1979	1996-2011	Old Development	-
Spicewood 930	48	1979	1996-2011	Old Development	
Balcones DPS 445*	41	1978	2004-2011	Old Development	
Stillhouse spring*	27	1974	1996-2011	Old Development	+

Changing hydrologic conditions do not explain away negative trends

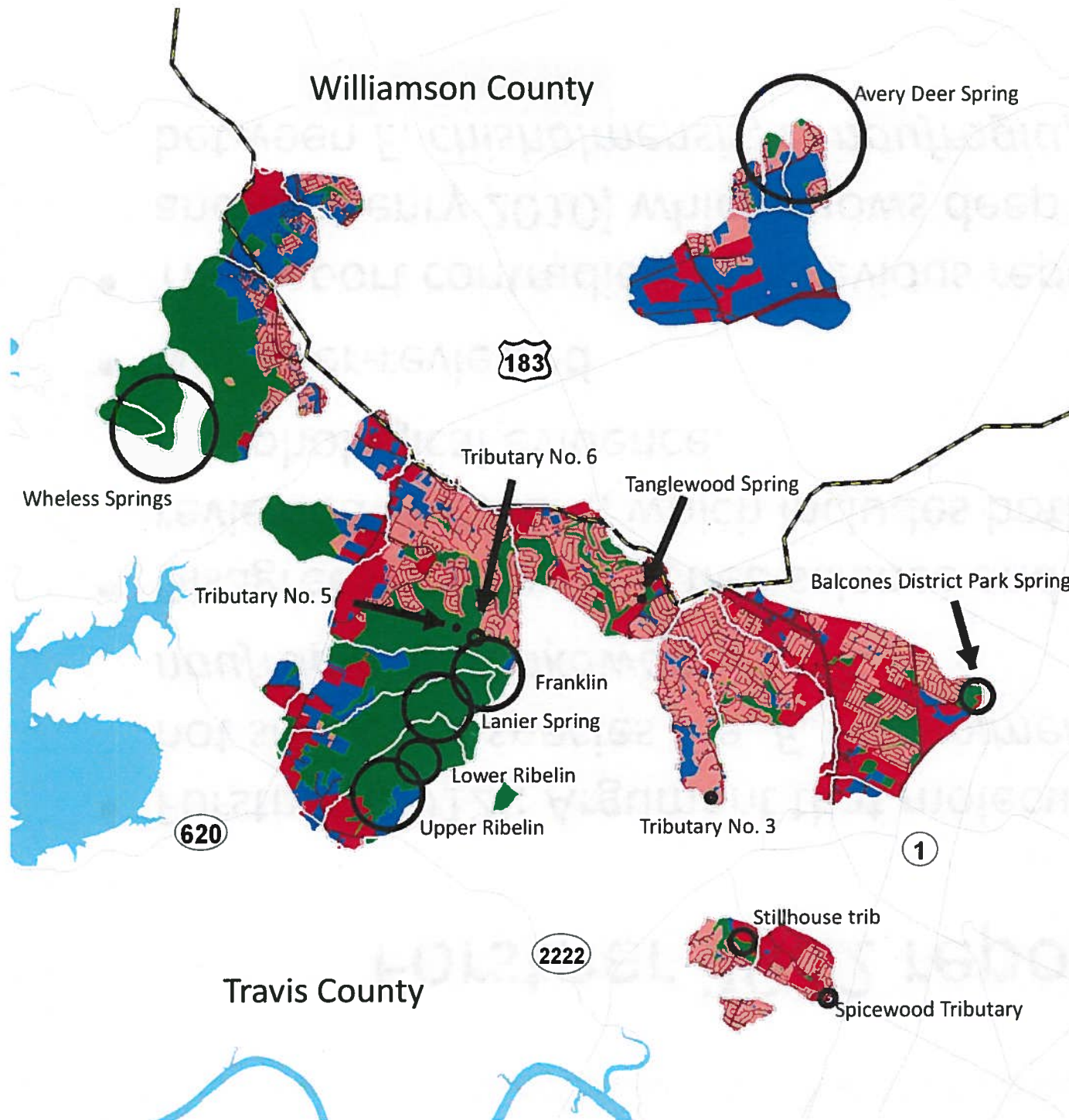
* < 3 m² survey area

Average Density (2009-2011)

Site	Current Impervious Cover %	Median Structure Age	Sample Size	Typical Area (m ²)	Avg. Density (sal/m ²)	Avg. Count
Avery Deer Spring 1355*	20	~2005	3	11	3.8	42
Wheless Spring 1045	0		3	40	2.8	112
Franklin 349	<1		9	35	1.4	49
Upper Ribelin 4184	21	~2008	7	54	1.4	76
Lanier Spring 3963	<1		5	64	1.3	86
Lower Ribelin 4035	<1		2	52	0.57	30
Balcones DPS 445*	41	1978	11	2	0.36	1
Stillhouse trib 927	27	1974	11	22	0.22	2
Spicewood 930	48	1979	11	33	0.10	3
Bull Cr. Trib 6 151	25	1991	10	140	0.08	12
Bull Cr. Trib 3 926	25	1993	3	190	0.03	5
Tanglewood 928	36	1979	10	45	0.01	1
Bull Cr. Trib 5 1164	19	1991	8	70	<0.01	1

10x-100x lower density

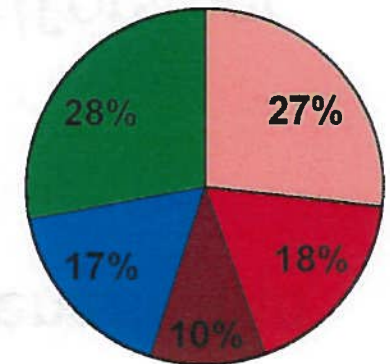
*small Edwards spring runs



Development

19% Impervious Cover
55% of Land Developed

Land Use



JPS Drainage Areas

Developed

- Single-Family Residential
- Multifamily/Commercial
- Roads

Undeveloped

- Undeveloped (as of 2006)
- Preserves and Parks



0 0.5 1 2 Miles

Forstner 2012 report

- Forstner (2012): Argument that molecular data do not support 3 species (i.e. *E. chisholmensis*, *E. naufragia*, *E. tonkawae*)
- Disagrees with established science and peer-reviewed literature, which includes both genetic and morphological evidence
- Not peer-reviewed
- This report contradicts his previous report (Forstner and McHenry 2010) which shows deep divergences between *E. chisholmensis*, *E. naufragia*, *E. tonkawae*

Summary

- JPS have declined in urbanized watersheds
 - Likely caused by chemical and physical changes of water due to urbanization
 - Impacts of urbanization do not appear immediately
 - Declines have occurred despite COA water quality regulations
- Rainfall does not explain declines in urban areas
- JPS is a distinct species based on best available science



City of Austin Data Quality

Chris Herrington, Watershed Protection Department



City of Austin Data Quality

In part, USFWS used City of Austin monitoring data listing review for the Austin Blind and Jollyville Plateau Salamander.

- *Individual Quality Assurance Project Plans (QAPP) consistent with EPA objectives implemented for all projects to document and justify sampling methods and objectives*
- *QAPPs are independently audited annually to maintain consistency*
- *Specific quality assurance/quality control procedures used to validate accuracy and precision of all data*
- *All QAPP and reports reviewed by committees of expert staff to ensure valid and appropriate scientific methods are used*
- *Reports and data available via the web*



City of Austin Data Quality

- City staff have a high level of confidence in the quality of our scientific research and analysis
- Austin's monitoring data is appropriate for use in the type of review conducted by USFWS



U.S. Fish & Wildlife Service

Critical Habitat

What is it?

When the Fish and Wildlife Service proposes a listing under the Endangered Species Act, we are required to consider whether there are geographic areas that are essential to conserve the species. If so, we may propose designating these areas as critical habitat.

Here are answers to some of the most frequently asked questions about critical habitat.

What is critical habitat?

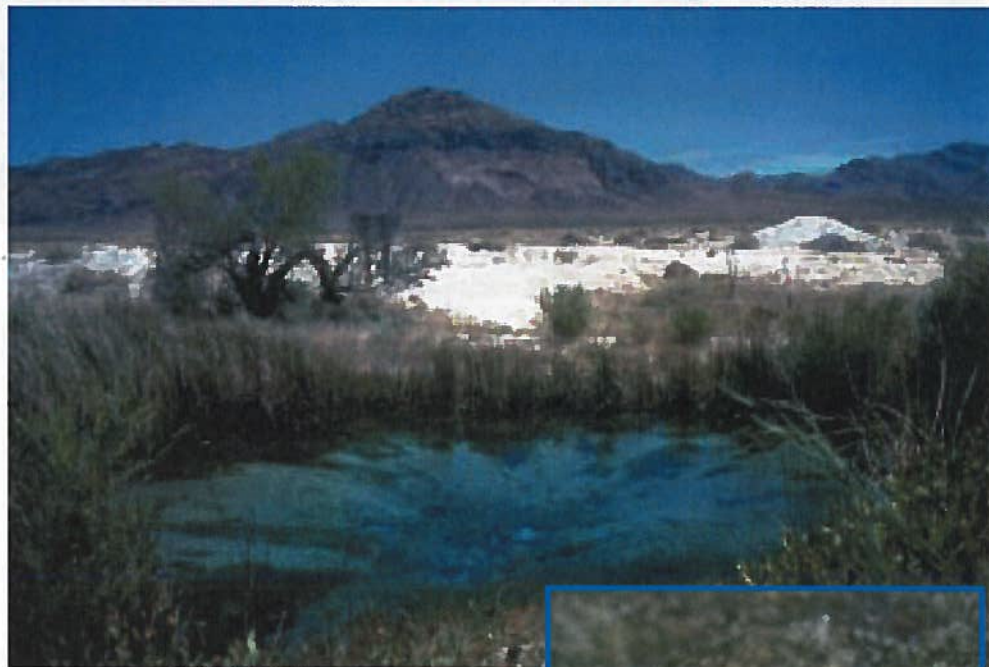
Critical habitat is the specific areas within the geographic area, occupied by the species at the time it was listed, that contain the physical or biological features that are essential to the conservation of endangered and threatened species and that may need special management or protection. Critical habitat may also include areas that were not occupied by the species at the time of listing but are essential to its conservation.

An area may be excluded from critical habitat designation based on economic impact, the impact on national security, or any other relevant impact, if we determine that the benefits of excluding it outweigh the benefits of including it, unless failure to designate the area as critical habitat may lead to extinction of the species.

Critical habitat designations affect only Federal agency actions or federally funded or permitted activities. Critical habitat designations do not affect activities by private landowners if there is no Federal “nexus”—that is, no Federal funding or authorization. Federal agencies are required to avoid “destruction” or “adverse modification” of designated critical habitat. The ESA requires the designation of “critical habitat” for listed species when “prudent and determinable.”

What provisions of the Endangered Species Act relate to critical habitat?

To protect endangered and threatened



About 90 miles northwest of Las Vegas, Nevada, Ash Meadows National Wildlife Refuge was established to protect endangered species in uplands and spring-fed wetlands. Plants and animals found nowhere else in the world are at home here, including the endangered Devil's Hole pupfish, the endangered Amargosa niterwort (a plant), and a threatened aquatic beetle species, the Ash Meadows naucorid.



Photo of Ash Meadows National Wildlife Refuge by Mike Bender, USFWS

Photo of the Devil's Hole pupfish by Olin Feurerbacher, USFWS

species, the Endangered Species Act makes unlawful a range of activities involving such species without a permit for purposes consistent with conservation goals of the Act. These activities include take, import, export, and interstate or foreign commerce. “Take” includes kill, harm, harass, pursue, hunt, capture, or collect or to attempt to engage in any such conduct.

The Act requires Federal agencies to use their authorities to conserve endangered and threatened species and to consult with the Fish and Wildlife Service about actions that they carry out, fund, or authorize to ensure that

they will not destroy or adversely modify critical habitat. The prohibition against destruction and adverse modification of critical habitat protects such areas in the interest of conservation.

How does the Fish and Wildlife Service determine areas to designate as critical habitat?

Biologists consider physical and biological features that the species needs for life processes and successful reproduction. These features include:

- space for individual and population growth and for normal behavior;

Myths & Realities

Does designating critical habitat mean no further development can occur?

No. A critical habitat designation does not necessarily restrict further development. It is a reminder to Federal agencies of their responsibility to protect the important characteristics of these areas.

Does a critical habitat designation affect all activities that occur within the designated area?

No. Only activities that involve a Federal permit, license, or funding, and are likely to destroy or adversely modify critical habitat will be affected. If this is the case, we will work with the Federal agency and landowners—including private landowners—to amend their project to enable it to proceed without adversely affecting critical habitat. Most Federal projects are likely to go forward, but some may be modified to minimize harm.

- cover or shelter;
- food, water, air, light, minerals, or other nutritional or physiological requirements;
- sites for breeding and rearing offspring, germination, or seed dispersal; and
- habitats that are protected from disturbances or are representative of the historical geographical and ecological distributions of the species.

What is the process for designating critical habitat?

The Service may propose to list a species and concurrently propose to designate critical habitat, or it can address a species' critical habitat up to a year after the date of its listing. The Service proposes a critical habitat designation, publishing it in the *Federal Register* and requesting public comments. We may modify a proposal as a result of information provided in public comments. We base our final designation of critical habitat on the best scientific data available, after taking into consideration the probable economic and other impacts of the designation. After reviewing the comments, the Service responds to them and publishes a rule, including final boundaries, in the *Federal Register*.

Are Federal agencies required to consult with the Fish and Wildlife Service outside critical habitat areas?

Yes, even when there is no critical habitat designation, Federal agencies are required fulfill their conservation responsibilities by consulting with

the Fish and Wildlife Service if their actions "may affect" listed species. The requirement helps to ensure that Federal agencies do not contribute to the decline of endangered and threatened species or their potential for recovery.

What is the purpose of designating critical habitat?

Designating areas as critical habitat does not establish a refuge or sanctuary for a species. Critical habitat is a tool to guide Federal agencies in fulfilling their conservation responsibilities by requiring them to consult with the Fish and Wildlife Service if their actions may "destroy or adversely modify" critical habitat for listed species. A critical habitat designation helps to protect areas—occupied and unoccupied—necessary to conserve a species. Critical habitat has value in requiring the Service to gather more detailed information about a species than what is required for listing, thereby increasing knowledge to share with Federal agencies—and, in turn, increasing their effectiveness.

The areas shown on critical habitat maps are often large. Are all the areas within the mapped boundaries considered critical habitat?

No. Our rules typically exclude developed areas such as buildings, roads, airports, parking lots, piers, and similar facilities. Accompanying text describes those areas.

Critical habitat is designed to protect the essential elements of physical and biological features of a landscape and essential areas in the appropriate quantity and spatial arrangement that a

species needs to survive and reproduce.

Why are large areas shown on critical habitat maps if the entire area is not actually considered critical habitat?

In some cases, precisely mapping critical habitat boundaries is impractical or impossible, because the required descriptions for these precise boundaries would be unwieldy.

Does the Act require consideration of economic impacts as part of designating critical habitat?

Yes. The Service is required to consider potential economic impacts, as well as any other benefits or impacts of designating critical habitat—and may exclude an area if the benefits of excluding it outweigh the benefits of including it unless that would result in the extinction of the species.

Do economic considerations affect decisions to list species as endangered or threatened?

No, the Act requires listing decisions to be made solely on the basis of the best available scientific and commercial information.

What is the impact of a critical habitat designation on economic development?

Most activities that require consultation by Federal agencies proceed without modification. In areas where the species is not present, some project modifications that would not have occurred without the critical habitat designation may be required. For example, the U. S. Army Corps of Engineers may schedule a beach renourishment project—that is adding sand to a beach to stabilize it—before or after the nesting season of sea turtles to avoid harm to the sea turtles, their eggs, or their hatchlings.

How many species have critical habitat designations?

As of April 1, 2011, critical habitat has been designated for 604 of the 1,372 U.S. species, subspecies, and distinct vertebrate populations listed as threatened or endangered.

**U.S. Fish & Wildlife Service
Endangered Species Program
703/358-2171
<http://www.fws.gov/endangered/>**

September 2011

**Genetic characterization of *Eurycea* salamanders from
Jacob's Well, Hays County, Texas**

**Final Report to the Coypu Foundation,
New Orleans, Louisiana**

and

**The River Systems Institute,
Texas State University,
San Marcos**

by

**Michael R.J. Forstner and Diana J. McHenry
Texas State University, Dept. of Biology**

December 1, 2010

Abstract

Introduction

The genus *Eurycea* Rafinesque, 1822 contains salamander species from across the USA, with most species occurring in cave or spring habitats. The genus is known for taxonomic uncertainty, usually associated with limited specimen availability often directly consequent of small geographic distributions and difficulty in accessing those habitats by researchers. The group also shows a high morphological variability with some forms once considered unique enough to warrant distinction as monotypic genera (i.e. *Typhlomolge* and *Haideotriton*). Recent investigations of *Eurycea* from central Texas have concluded that many species are represented where previously only a few species had been previously distinguished (Chippendale et al. 2000; Hillis et al. 2001).

The results to date from these studies and others have not resolved many questions surrounding the genetic divergence among populations, the potential for gene flow among those populations, or the applicability of assigning species rank to each spring head population. Alternative hypotheses have been tested to discern above- or below-ground connectivity (Lucas et al. 2009) with those authors concluding that the populations are isolated and should be treated as such.

The geology of central Texas is dominated by its karst topography. As the limestone has dissolved, the subterranean habitats, and springs that provide the habitats for these salamanders, have become available. The region is characterized by historically abundant surface springs, large cave systems, and dramatic rainfall events. With increased urbanization, surface water impoundment, and ground-water withdrawals, the original ecosystem structure for these salamanders has been altered significantly in the last century. Today, only the largest springs maintain outflows and many of those sites are potentially at risk from ongoing development or other anthropogenic impacts.

One well known site is Jacob's Well outside of Wimberley, Texas. This site is noted as the longest underwater cave in Texas and for its consistent outflow from the aquifer. It represents one of the primary inflows supporting the Blanco River and continued to flow throughout the drought of record during the 1950s. The spring has stopped flowing twice in recent times; the first was in 2000 and second in 2008. The site has recently been documented to contain *Eurycea*.

The purpose of this study was to characterize the mtDNA variation for salamanders collected from Jacob's Well by Zara Environmental, Inc.

Materials and Methods

Individuals were sampled across central Texas (Appendix A), nonconsumptively where possible. Forty whole specimen, 75 tail tip, 3 liver, 3 skin, 1 muscle, 1 heart, and 4 unknown tissue samples were collected and stored in 70% ethanol at -80°C. Tissues were deposited in the Michael R. J. Forstner Frozen Tissue catalog at Texas State University–San Marcos.

Eurycea were sampled under Department of the Interior, U.S. Fish and Wildlife Service, Federal Fish and Wildlife Permit Number TE676811-0 and Texas Parks and Wildlife Scientific Permit Numbers SPR-0102-191, SPR-0290-022, and SPR-0390-045 and under Institutional Animal Care and Use Committee approvals 0715_0428_07, 04-3D2AAE71, 04-046 E25 EBSA, and 1010_0501_09.

DNA was isolated from tissue (1-2 mm³) using a DNeasy® Tissue Kit (QIAGEN Inc.). A partial sequence of the mitochondrial cytochrome b gene was sequenced. Amplification was performed using the primers MVZ15 (Chippindale et al. 2000) and EURCB9 (Hillis et al. 2001) in 50 µl reactions with 4 mM MgCl₂, 0.1 mM dNTPs, 0.01 µM each primer, 2.5 units GoTaq® Flexi DNA polymerase (Promega), and pH = 8.5. PCR was performed with an initial denaturing period of 95°C for 5 min then 35 cycles, each consisting of denaturing at 95°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and a final extension period of 72°C for 5 min. PCR products were purified with an AMPure® PCR Purification System (Agencourt Bioscience Corporation), and then cycle sequenced with the above primers, using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Thermal cycling was performed with an initial denaturing period of 96°C for 1 min then 25 cycles, each consisting of 96°C for 1 min, 50°C for 5 sec, and 60°C for 4 min. Products were cleaned by ethanol precipitation and analyzed on an ABI 3500xL Genetic Analyzer (Applied Biosystems). Resultant sequences were edited and aligned in SEQUENCHER™ 4.5 (Gene Codes Corp.).

To assess the phylogenetic relationships within central Texas *Eurycea*, maximum likelihood (ML, Felsenstein 1981), neighbor joining (NJ), and Bayesian analyses using mtDNA data were performed. In addition to sequences generated by us, 22 GenBank accessions were included in analyses (Table 1). *Eurycea multiplicata* (GenBank AY014854) was used as an outgroup (Chippindale et al. 2000). Model parameters for maximum likelihood, which were estimated by hLRT and AIC using MODELTEST 3.7 (Posada & Crandall 1998), were used as input in a ML heuristic search in PAUP* 4.0b10 (Swofford 2002). Neighbor joining topologies were generated using HKY85 in PAUP* 4.0b10. Bootstrap values (Felsenstein 1985) were estimated from 1,000 replicates in a neighbor-joining search in PAUP* 4.0b10 for NJ analysis. Parameters of a best-fit nucleotide model of evolution for Bayesian analysis were determined by hLRT and AIC in MRMODELTEST 2.0 (Nylander 2004), and MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003) was implemented for one million generations, saving every hundredth tree, and with a burn-in of 100,000 trees. Similar analyses were also conducted on a reduced dataset (see Results).

To assess populational relationships, a statistical parsimony network (Templeton et al. 1992) of mtDNA haplotypes was constructed using TCS 1.21 (Clement et al. 2000), with a connection limit of 30 and gaps treated as a 5th state. Differences in allele frequencies among sites were assessed by computing pairwise F_{ST} values with 10,000 permutations and a significance value of 0.05 and by performing Fisher's exact test of population differentiation in ARLEQUIN 3.11 (Excoffier et al. 2005). Isolation-by-distance was tested among individuals with a Mantel test (Mantel 1967) in ALLELES IN SPACE 1.0 (AIS, Miller 2005) with 1,000 permutations.

Results

One hundred twenty-seven *Eurycea* in seven counties were sampled for this study (Table 1 and Appendix A). The 1026-bp cytochrome b alignment of 127 individuals resulted in 26 unique haplotypes (GenBank Accession Nos. HQ713576-HQ713601); with the addition of already published GenBank accessions, the number of individuals was 149 and of unique haplotypes was 44. The model of evolution that best fitted the data was TVM+G (chosen by AIC) and HKY+G (chosen by hLRT) as determined by MODELTEST and GTR+G chosen by AIC and hLRT as determined by MRMODELTEST. The Bayesian phylogram is shown in Fig. 1. NJ, ML, and Bayesian analyses resulted in similar topologies. Similar analyses were performed on a reduced dataset, i.e., only individuals recovered in clade 2 from Fig. 1. HKY+G (chosen by AIC and hLRT) was the model of evolution that best fitted the data as determined by MRMODELTEST; the resultant Bayesian phylogram is shown in Fig. 2.

The statistical parsimony network of 24 unique mtDNA haplotypes in 116 individuals is presented in Fig. 3. Three haplotypes were detected at Jacob's Well; all were unique to that site. In fact, 12 haplotypes were found at only one site: A, B, C, D, E, F, H, I, J, K, L, M, N, and O (Table 2). Three haplotypes were found at multiple sites: G, L, and M (Table 2).

Pairwise F_{ST} values were calculated for groups of individuals recovered in clade 2 from Fig. 1 (Table 3). Most values were not significant, which is likely due to the inclusion of already published GenBank accessions that had very low sample sizes. Very high F_{ST} values were found for *E. nana* ($F_{ST} = 0.927-0.974$) and between a clade containing *E. pterophila* and one containing *E. neotenes* and *E. 'Comal Springs' sp.* ($F_{ST} = 0.849-1.000$). Within the clade containing *E. neotenes* and *E. 'Comal Springs' sp.*, F_{ST} values were 0.356-0.768. Within the clade containing *E. pterophila*, F_{ST} values were 0.571-0.792. Significant genetic differentiation was detected using Fisher's exact test for 22 combinations of sites/species (Table 3). Jacob's Well was different from the other locations within the clade that contains *E. pterophila* sites (Fern Bank Springs and Ott's Spring). All three were different from Comal Springs and Hueco Springs. Again, most tests were not significant, but could be explained by low sample sizes. The Mantel test of only '*neotenes*' and '*pterophila*' individuals (those with haplotypes A, B, C, D, E, F, G,

H, I, J, and K; $n = 100$) revealed significant positive, and large, correlations between genetic distances and geographic distance (i.e., isolation-by-distance) ($r = 0.888$, $P < 0.001$).

Discussion

The unique environmental context of Texas has created tremendous biodiversity both above and below its surface. Texas ranks second behind only California in total diversity, third in total endemism, but also fourth in total extinctions across the USA (Stein 2002). Texas is also fifth in total amphibian diversity, with at least 20% of those species at risk placing it among the top ten for total percentage of amphibian taxa at risk (Stein 2002). The endemic salamander fauna of central Texas has some of the smallest depicted distributions for any amphibian in the United States. These taxa are poorly known, often poorly documented, and provide a confusing array of phenotypes and morphology even among a single spring site. Seemingly the resolution to this would be genetic data, but the variability extends to the genetic results.

One reason for the current levels of uncertainty is simply the novelty of the investigations for these taxa. With the majority of “new” *Eurycea* species having been described in the last few decades, reviews and revisions to the alpha taxonomy of the group have not yet been conducted. The achievement of a stable evolutionary taxonomy supported by evolutionary relationships is the goal, but it cannot be achieved quickly given the diversity, number of sites, and the often contradictory conclusions of systematists examining the data.

We sought a specific answer in our evaluation of the salamanders from Jacob’s Well. Does this location contain a unique lineage of evolutionarily distinct *Eurycea*? The answer to that question pragmatically must include context among other populations, previous studies, and additional data from specimens outside of the study site. In our work we first chose to use an mtDNA marker in order to provide higher resolution than would be possible with similar amounts of nuclear DNA sequence data. We chose to use sequences from cytochrome b (Chippendale et al. 2000), because the database for homologous sequences is larger than that for ND4 (Lucas et al. 2009).

The results demonstrate the underlying instability of the current taxonomy, at least from the perspective of mtDNA marker analyses (Fig. 1). The resulting topology is in close general agreement with previously published phylogenetic relationships (Chippendale et al. 2000; Lucas et al. 2009). The Texas *Eurycea* resolve two major divisions. The northern group contains *E. chisholmensis*, *E. naufragia*, and *E. tonkawae* which form the sister clade to those occurring south and west (*E. troglodytes*, *E. nana*, *E. sosorum*, *E. latitans*, *E. tridentifera*, *E. pterophila*) including the aquifer forms (*E. rathbuni* and *E. waterlooensis*). There are deep genetic divergences between these two sister groups.

Within the northern species group there is a similar deep divergence between *E. naufragia* and the clade containing *E. chisholmensis* and *E. tonkawae*. The southern species

group has much less divergent lineages overall, but discrete units are supported at those lower divergences (Fig. 1). The aquifer-dwelling forms, *E. rathbuni* and *E. waterlooensis*, form a distinct clade that is a sister group to a clade of two groups, *E. pterophila* and *E. troglodytes*. All of the Jacob's Well salamanders fall within the *E. pterophila* clade (Fig. 2).

The samples from Jacob's Well were collected at a variety of depths, including very deep within the cave system (Appendix A). There were no substantial differences among those samples, regardless of depth (Fig. 2). Similarly, there are no substantial differences seen between the samples from Jacob's Well and those from Fern Bank or Ott's Spring (see Lucas et al. 2009 for a map depiction). Furthermore, the data do not resolve any distinction among the Jacob's Well samples and the sequences available for *E. latitans*, *E. tridentifera*, or *E. pterophila* (Fig. 2). In the evaluation by Lucas et al. (2009), the authors note the presence of unique haplotypes for several sites. While we found some haplotypes that were unique to Jacob's Well and to Ott's Spring, Lucas et al. (2009) found shared haplotypes at these sites for another mtDNA gene, ND4 (Table 2). And, while both studies recovered unique haplotypes at Comal Springs and Hueco Springs, we also found a shared haplotype at those two sites (Table 2).

The actual relationships among the haplotypes for salamanders at these localities are more complex than simply having shared haplotypes or not (Fig. 3). As an example, there are many changes between the haplotype from *E. sosorum*, or that from the sample from the Perdenales river site (Fig. 3) and other clades, but even these seemingly obvious divergences may be more anomalous than representative as these are from a single sample from those locations. The results indicate that the Jacob's Well salamanders are part of a broad group of *E. pterophila* populations and that significantly more work will be required before the current taxonomy can be shown to accurately portray the underlying evolutionary relationships among the salamanders from these localities. Our results do not support the current taxonomic structure for species named within the genus *Eurycea* occurring in central Texas, instead our results would support a much reduced species taxonomy reflecting the evolutionary relationships depicted by Fig. 1.

It is not clear from any single study how best to interpret the taxonomic decisions that derive from an understanding of evolutionary relationships. The underlying reason that systematics is cyclical is fundamentally a part of the increasing information available for taxonomic groups over time. For example, we compared average genetic distances (uncorrected p) among multiple taxonomic/phylogenetic levels in the genus *Eurycea* (Fig. 4). We sought to examine the overall genetic divergences among described species within the genus. It was our general expectation that comparisons within species would show less genetic variation than between species and that related species groups would follow a similar trend when compared among such groups. This was not the result. The partitioning of genetic variability into names did not follow a recognizable trend for *Eurycea* in Texas. It may simply be that this is not a good way to characterize the populations, but it may also support our contention that Texas *Eurycea*

need significant, comprehensive examination in order to accurately describe the evolutionary variation for this genus.

Further support for the need of comprehensive revision to the taxonomy can be seen in the evaluation of the accessibility to the populations. We chose to illustrate this approach this by simply comparing the straightline geographic distance from Austin, Texas to the type localities for the species examined here. The *E. troglodytes* complex is the furthest from the Capitol building in Austin and encompasses the largest genetic variation (Fig. 4). The answer to the systematics in *Eurycea* is additional work in the entirety of the group, not just in those taxa proximal to the state capitol. Currently, development pressures are not as high further to the west in the area of *E. troglodytes*, but unfortunately that translates to fewer funding dollars available to examine those populations. Ironically, in completing the work for these salamanders as a whole, the results from the areas under the highest development pressures may be more clearly and effectively understood. It is in the context of generic diversity that species composition and evolutionary relationships can be most clearly ascertained.

Jacob's Well is one of a handful of moderately large to large springs that still flow in central Texas (Brune 1975). The reduction of spring flow is a state-wide phenomenon and one that is unlikely to reverse trend. The community-based efforts at Jacob's Well represent the type of conscientious and involved stewardship required to maintain these unique environments in the face of development pressures and human water needs. The salamanders of Jacob's Well do not represent a distinct evolutionary lineage from others in the area based on our analyses of mtDNA sequence data. However, this spring is unique in its large size compared to adjacent localities, it is already stewarded by an engaged community derived effort, and it is an icon for the nearby community of Wimberley, Texas. The conjunction of all of those benefits increase the overall value of this site as a stable locality of *Eurycea* with all the attendant benefits to future research and conservation goals.

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Table 1. Sampling sites by county, number of samples, haplotypes (number of individuals per haplotype), and GenBank Accession Nos. for *Eurycea* individuals used in this study.

Site	<i>n</i>	Haplotypes	GenBank	Taxon ^a
Bandera Co.				
Lost Maples Natural Area	6	T (5) U (1)	HQ713595 HQ713596 ^b	<i>E. sp.</i>
Sutherland Hollow Spring	1	—	AY014853 ^c	<i>E. troglodytes</i>
Bell Co.				
Salado Springs	1	—	AY014841 ^c	<i>E. chisholmensis</i>
Bexar Co.				
Helotes Creek Spring	2	—	AY014850 ^c , AY528400 ^d	<i>E. neotenes</i>
Comal Co.				
Unknown	1	—	AY260758 ^e	<i>E. latitans</i>
Comal Springs	1	—	AY260759 ^c	<i>E. 'Comal Springs' sp.</i>
Comal Springs Run 1	3	G (1) H (1) I (1)	HQ713582 HQ713583, HQ713584 ^b	<i>E. sp.</i>
Comal Springs Run 3	3	G (1) H (1) I (1)		<i>E. sp.</i>
Honey Creek Cave	1	—	AY014848 ^c	<i>E. tridentifera</i>
Huaco Springs	6	G (4) J (1) K (1)	HQ713585 HQ713586 ^b	<i>E. sp.</i>
Ott's Spring	6	D (6)	HQ713579 ^b	<i>E. sp.</i>
Edwards Co.				
250 m W of RR335, about 8.5 rd km S of jct RR335 & TX Hwy 41	3	V (2) W (1)	HQ713597, HQ713598 ^b	<i>E. troglodytes</i> complex
Gillespie Co.				
1.36 rd mi S jct White Oak Rd & Zenner-Alhorns Rd on Zenner-Alhorns	2	X (1) Z (1)	HQ713599, HQ713601 ^b	<i>E. sp.</i>
Trough Spring	1	—	AY014852 ^c	<i>E. troglodytes</i>
Hays Co.				
Fern Bank Springs	1	—	AY014851 ^c	<i>E. pterophila</i>
Fern Bank Springs	12	E (11) F (1)	HQ713580 HQ713581 ^b	<i>E. sp.</i>
Fern Bank Springs, spring-fed stream near Fern Bank Springs	3	E (3)		<i>E. sp.</i>
Jacob's Well	24	A (22) B (2)	HQ713576 HQ713577 ^b	<i>E. sp.</i>
Jacob's Well 20'	10	A (6) B (4)		<i>E. sp.</i>
Jacob's Well 20'-70'	12	A (7) B (5)		<i>E. sp.</i>
Jacob's Well 70'	7	A (7)		<i>E. sp.</i>
Jacob's Well subsurface	14	A (7) B (5) C (2)		<i>E. sp.</i>
Rattlesnake Cave	2	—	HQ713578 ^b AY014844, AY014845 ^c	<i>E. rathbuni</i>
San Marcos Springs	1	—	AY014846 ^c	<i>E. nana</i>

Spring Lake Below Dam	2	L (1) M (1)	HQ713587 HQ713588 ^b	<i>E. nana</i>
Spring Lake Diversion Springs	2	M (1) N (1)	HQ713589 ^b	<i>E. nana</i>
Spring Lake Hotel Site	2	L (1) O (1)	HQ713590 ^b	<i>E. nana</i>
Kendall Co. Pfeiffer's Water Cave	1	—	AY014849 ^c	<i>E. latitans</i>
Kerr Co. Stockman Spring	5	Y (5)	HQ713600 ^b	<i>E. sp.</i>
Polk Co. (Arkansas) Band's Spring	1	—	AY014854 ^c	<i>E. multiplicata</i>
Smith Co. Tyler	1	—	AY528401 ^d	<i>E. quadridigitata</i>
Travis Co. Balcones Canyonlands, mainstem above Tributary 7	1	Q (1)	HQ713592 ^b	<i>E. tonkawae</i>
Balcones Canyonlands, Tributary 5	2	Q (1) R (1)	HQ713593 ^b	<i>E. tonkawae</i>
Barton Springs	1	—	AY014857 ^c	<i>E. sosorum</i>
Barton Springs Pool	1	—	AY014856 ^c	<i>E. waterlooensis</i>
Hammett's Crossing Spring	1	—	AY014847 ^c	<i>E. 'Pedernales' sp.</i>
SAS canyon	1	S (1)	HQ713594 ^b	<i>E. tonkawae</i>
Stillhouse Springs	2	—	AY014842 ^c , AY691749 ^f	<i>E. tonkawae</i>
Stillhouse Springs	1	P (1)	HQ713591 ^b	<i>E. tonkawae</i>
Sunken Garden Spring	1	—	AY014855 ^c	<i>E. waterlooensis</i>
Williamson Co. Cedar Break Hiking Trail Spring	1	—	AY014843 ^c	<i>E. naufragia</i>

^aSpecies as identified in the field or in GenBank.

^bData from this study.

^cHillis et al. 2001.

^dBonett & Chippindale 2004.

^eWiens et al. 2003.

^fChippindale et al. 2004.

Table 2. Comparison of mtDNA haplotypes (ND4 gene vs. cytochrome b gene) found at nine central Texas sites.

Site	ND4 ^a		cytochrome b ^b	
	Haplotype	Number	Haplotype	Number
Haplotypes unique to one site				
Comal Springs	J, K	34, 9	H, I	2, 2
Devil's Backbone	L, M	2, 7	—	—
Fern Bank Springs	E, G, H	32, 4, 1	E, F	14, 1
Huaco Springs	N	13	J, K	1, 1
Jacob's Well	B	2	A, B, C	49, 16, 2
Ott's Spring	D, F	11, 6	D	6
Spring Lake Diversion Springs	—	—	N	1
Spring Lake Hotel Site	—	—	O	1
Haplotypes shared among sites				
Devil's Backbone, Jacob's Well, Ott's Spring	A	9, 20, 1	—	—
Spring Lake Below Dam, Spring Lake Diversion Springs	—	—	M	2
Spring Lake Below Dam, Spring Lake Hotel Site	—	—	L	2
Spring Lake Below Dam, Spring Lake Diversion Springs, Spring Lake Hotel Site	C	29, 32, 31	—	—
Spring Lake Below Dam, Spring Lake Diversion Springs, Spring Lake Hotel Site	I	4, 5, 2	—	—
Comal Springs, Huaco Springs	—	—	G	6

^aLucas et al. 2009.

^bData from this study.

Table 3. Pairwise F_{ST} values (below diagonal) and P values from Fisher's exact test of population differentiation (above diagonal) for 'species' in clade 2 from Fig. 1. Significant F_{ST} values are shown in bold.

	<i>E. 'Pedernales' sp.</i>	<i>E. nana</i>	<i>E. sosorum</i>	<i>E. latitans</i>		<i>E. tridentifera</i>
	AY014847	Spring Lake ($n = 7$)	AY014857	AY014849	AY260758	AY104848
AY014847	—	0.496	1.000	1.000	1.000	1.000
Spring Lake	0.954	—	0.493	0.497	0.496	0.503
AY014857	1.000	-0.091	—	1.000	1.000	1.000
AY014849	1.000	0.920	1.000	—	1.000	1.000
AY260758	1.000	0.924	1.000	1.000	—	1.000
AY104848	1.000	0.916	1.000	1.000	1.000	—
Comal Springs	0.959	0.933	-0.333	0.817	0.839	0.788
Huaco Springs	0.956	0.927	-0.400	0.795	0.821	0.760
AY014850, AY528400	1.000	0.943	1.000	1.000	1.000	1.000
Fern Bank Springs	0.972	0.948	0.611	0.750	0.869	0.641
Jacob's Well	0.986	0.974	0.786	0.869	0.932	0.811
Ott's Spring	1.000	0.952	1.000	1.000	1.000	1.000

	<i>E. neotenes?</i> and <i>E. 'Comal Springs' sp.</i>			<i>E. pterophila?</i>		
	Comal Springs ($n = 7$)	Huaco Springs ($n = 6$)	AY014850, AY528400	Fern Bank Springs ($n = 16$)	Jacob's Well ($n = 49$)	Ott's Spring ($n = 6$)
AY014847	0.245	0.433	0.336	0.171	<0.050	0.143
Spring Lake	<0.050	<0.050	0.165	<0.050	<0.050	<0.050
AY014857	0.244	0.439	0.334	0.162	<0.050	0.142
AY014849	0.252	0.434	0.335	0.170	<0.050	0.141
AY260758	0.256	0.430	0.331	0.175	<0.050	0.143
AY104848	0.248	0.426	0.331	0.156	<0.050	0.142
Comal Springs	—	0.191	0.106	<0.050	<0.050	<0.050
Huaco Springs	0.356	—	0.072	<0.050	<0.050	<0.050
AY014850, AY528400	0.792	0.768	—	<0.050	<0.050	<0.050
Fern Bank Springs	0.849	0.837	0.916	—	<0.050	<0.050
Jacob's Well	0.913	0.906	0.953	0.792	—	<0.050
Ott's Spring	0.864	0.855	1.000	0.571	0.719	—

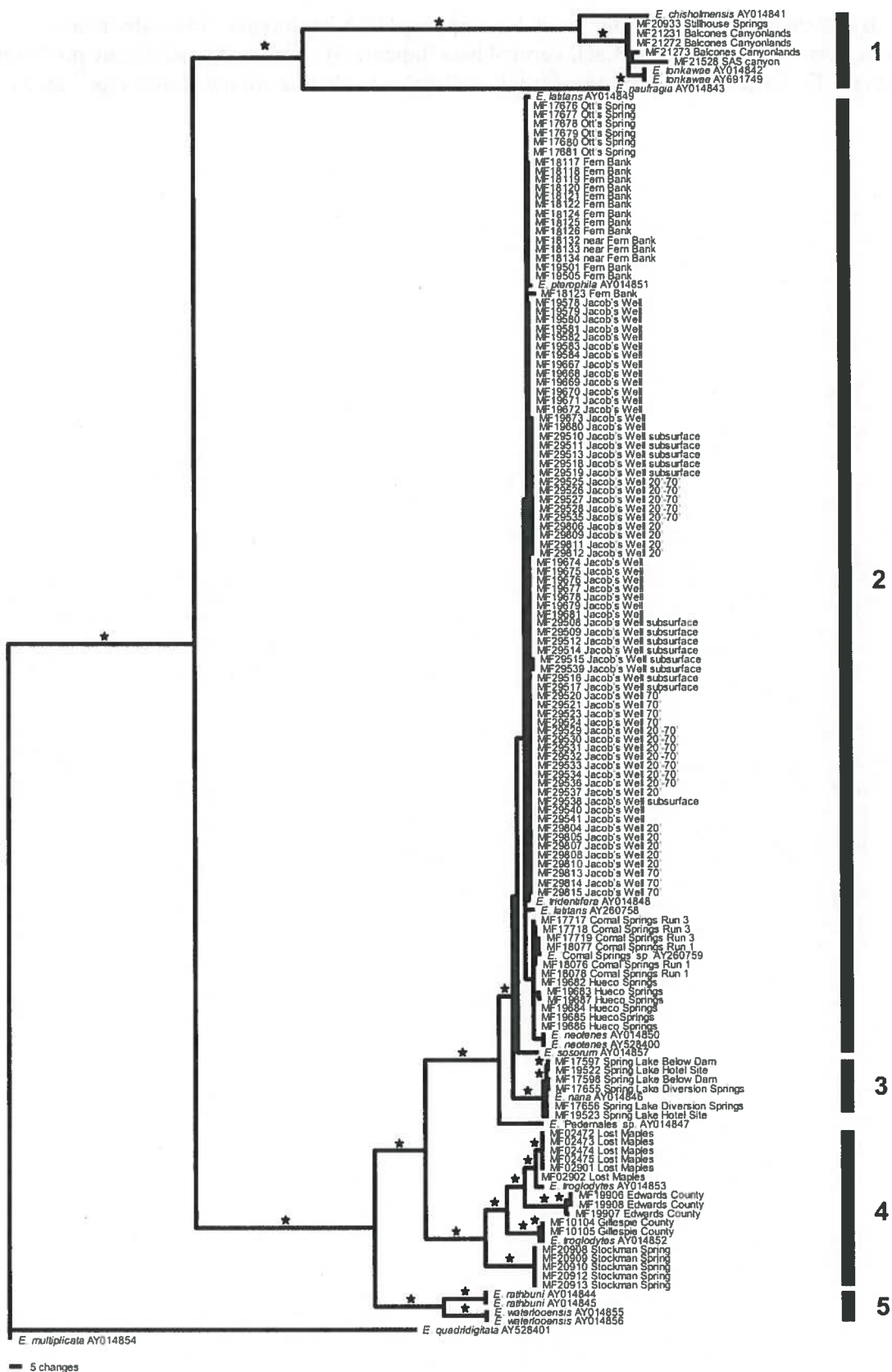
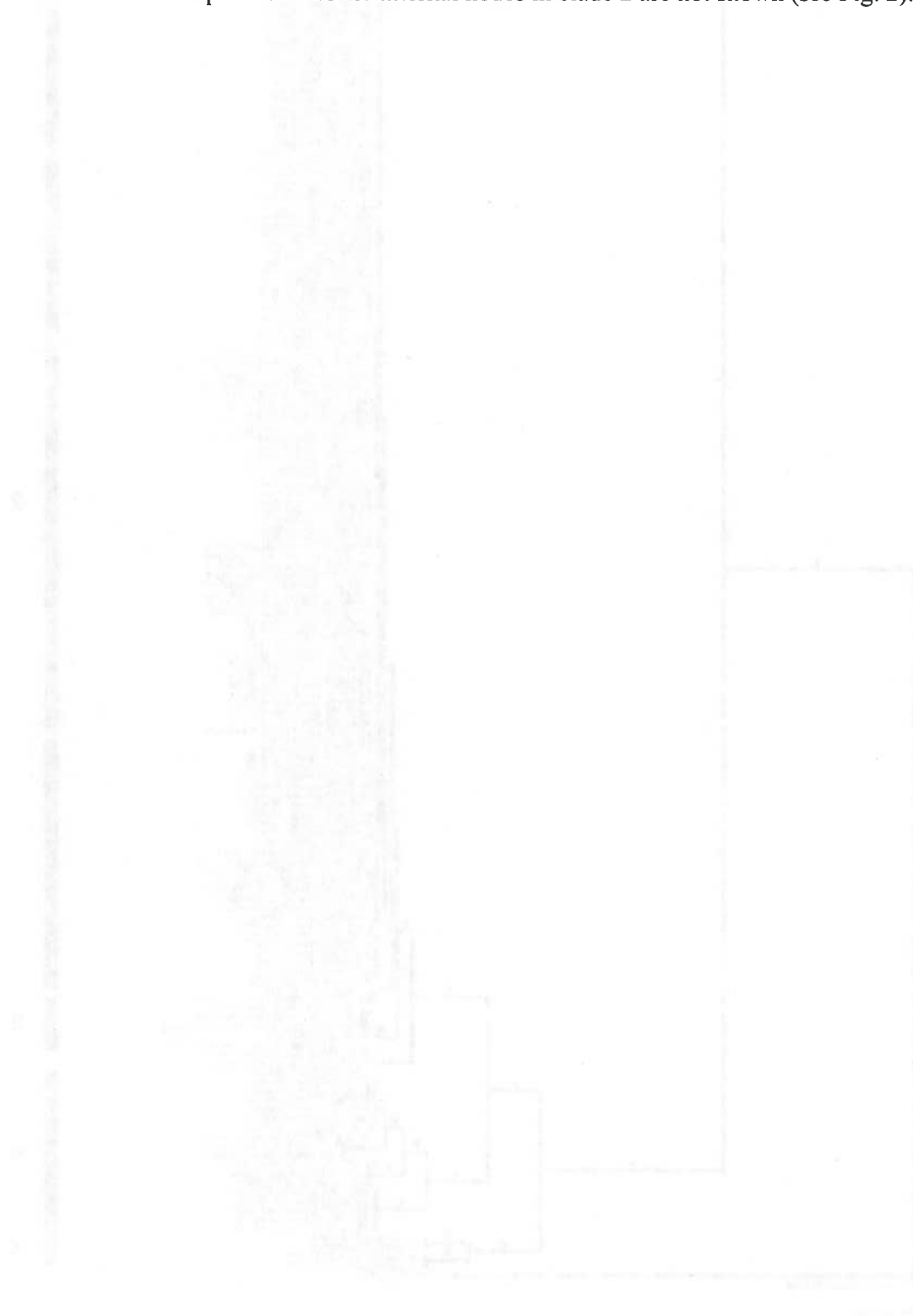


Figure 1. Bayesian consensus phylogram of 43 unique mtDNA haplotypes (149 individuals) rooted with *Eurycea multiplicata*. Black vertical bars indicate five clades. Stars indicate posterior probabilities >87. Posterior probabilities for internal nodes in clade 2 are not shown (see Fig. 2).



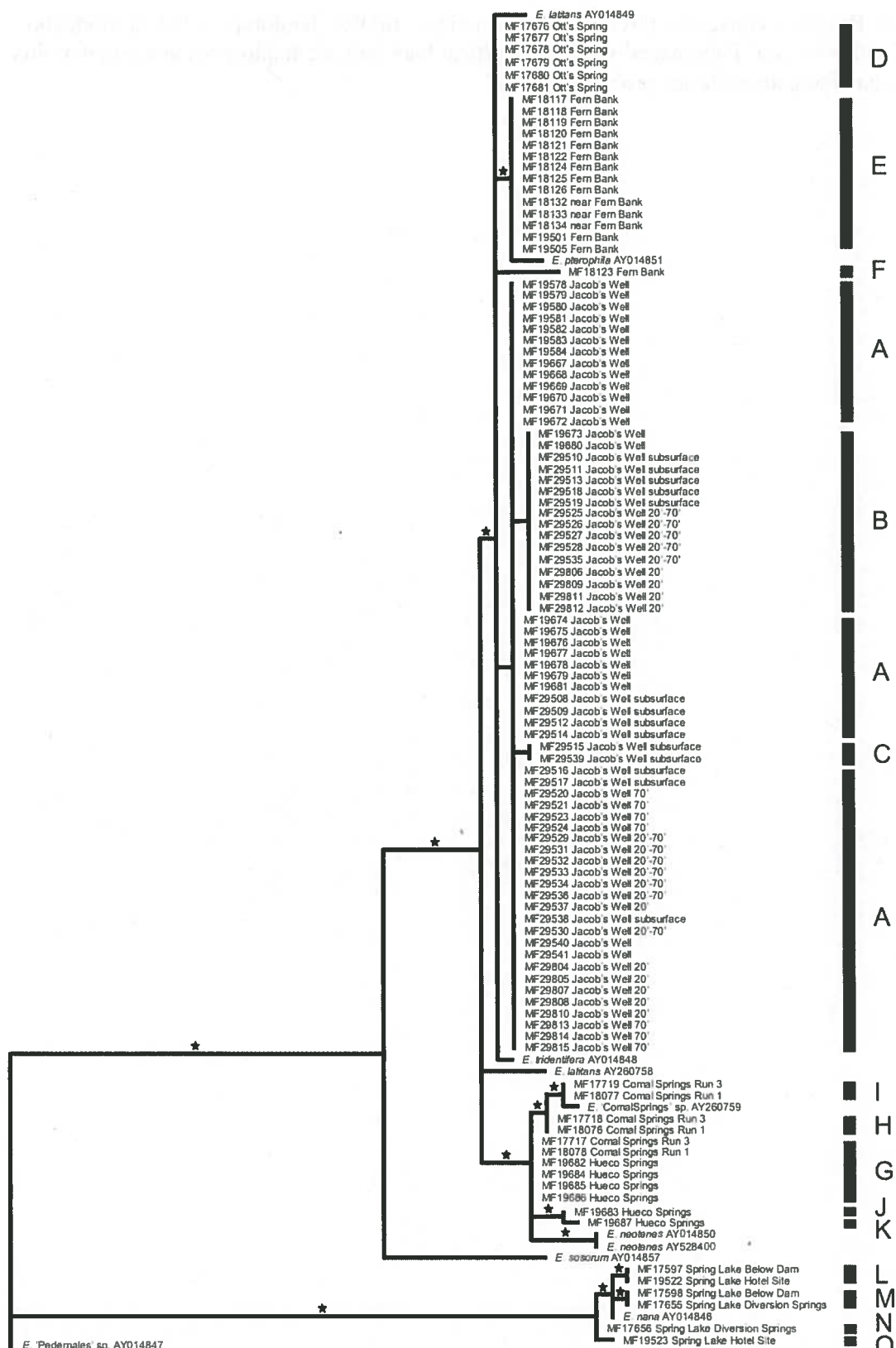
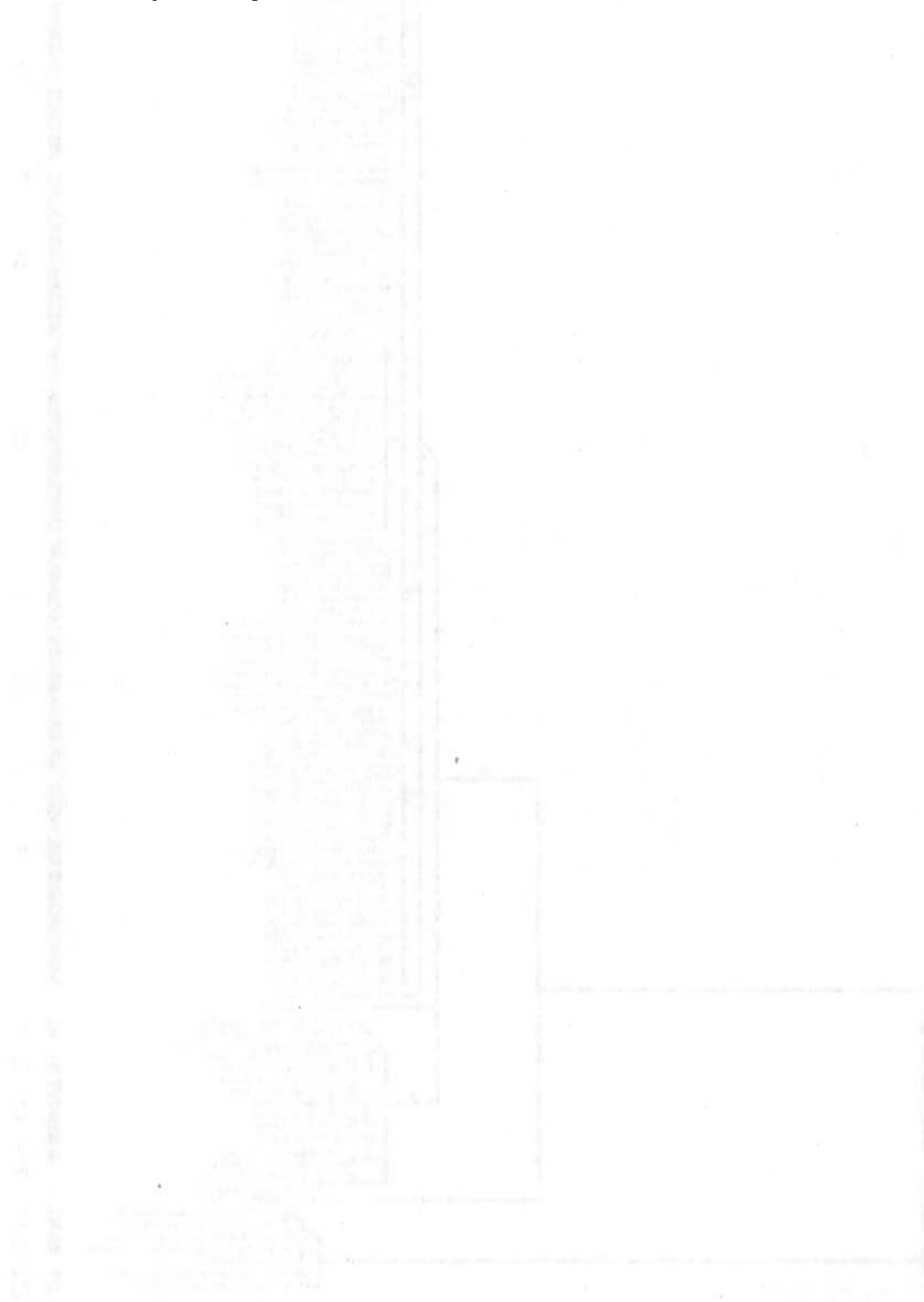


Figure 2. Bayesian consensus phylogram of 24 unique mtDNA haplotypes (116 individuals) rooted with *Eurycea* 'Pedernales' sp. Black vertical bars indicate haplotypes generated in this study. Stars indicate posterior probabilities >92.



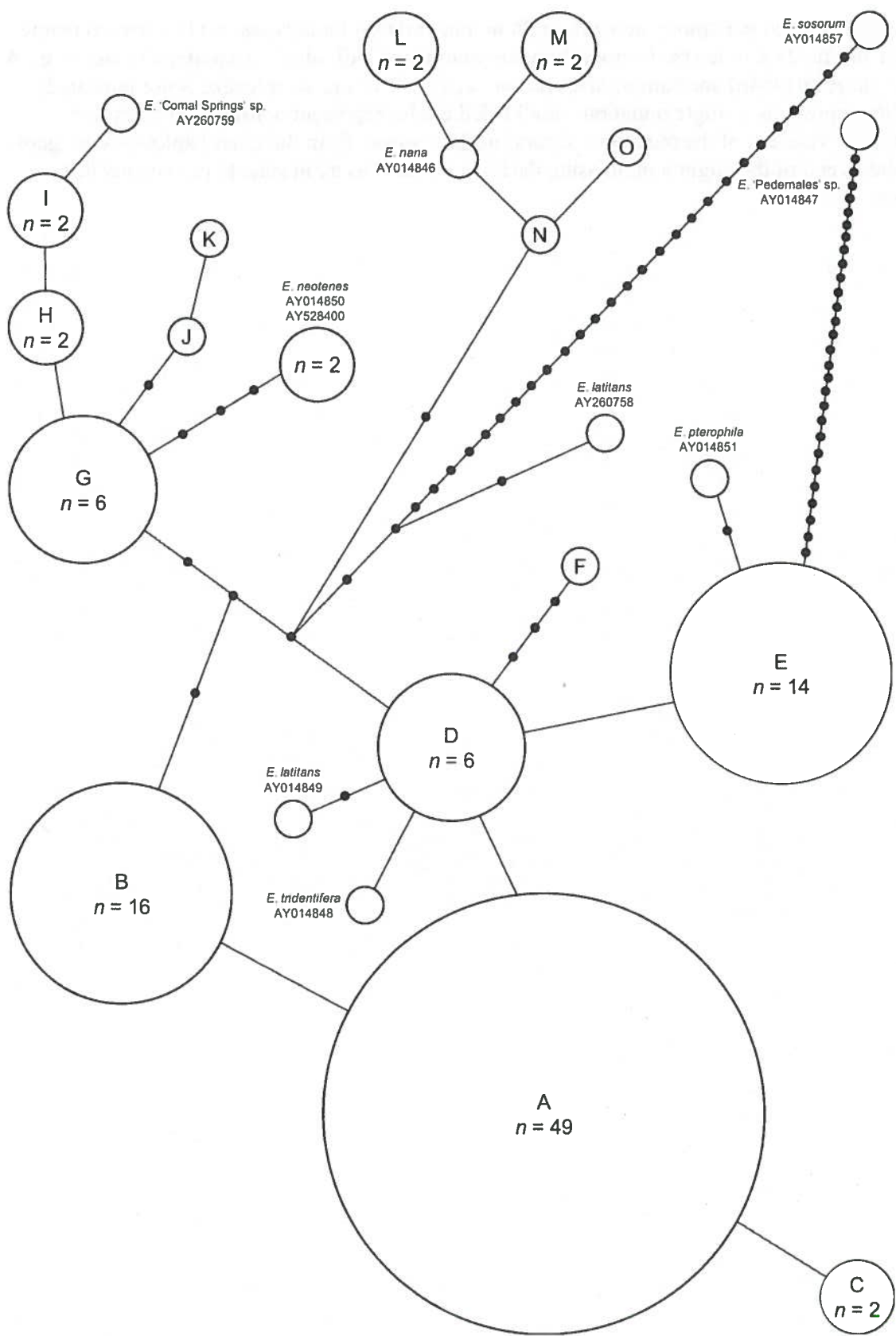


Figure 3. Statistical parsimony network of 24 unique mtDNA haplotypes in 116 *Eurycea* (same as those in Fig. 2). Circle size is proportional to number of individuals. Haplotype names (e.g., A or *E. nana* AY014846) and sample sizes are shown ($n = 1$ where sample size is not indicated). Each line represents a single mutation; small filled circles represent nonsampled or extinct haplotypes. Nineteen of the mutations separating *E. sosorum* from the other haplotypes are gaps from the 5' end of the alignment; missing data were treated as a 5th state to reconstruct this network.



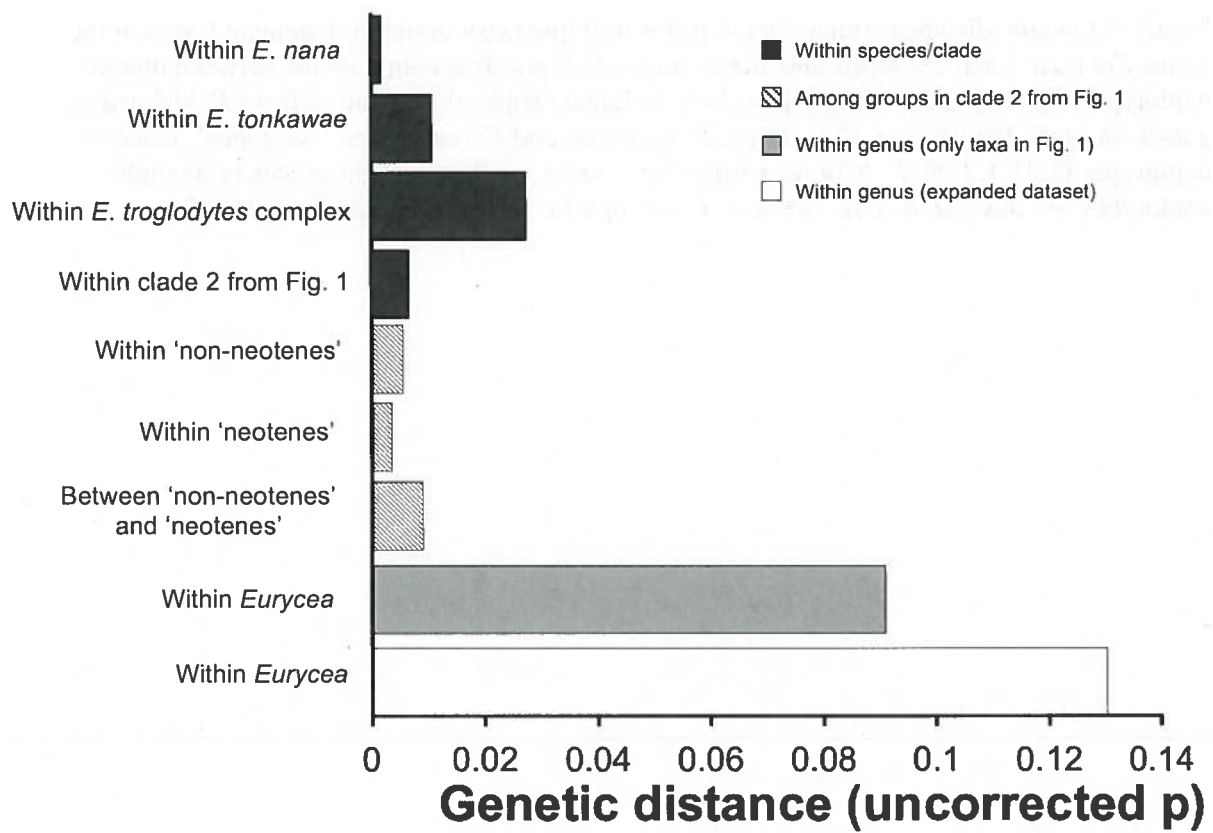
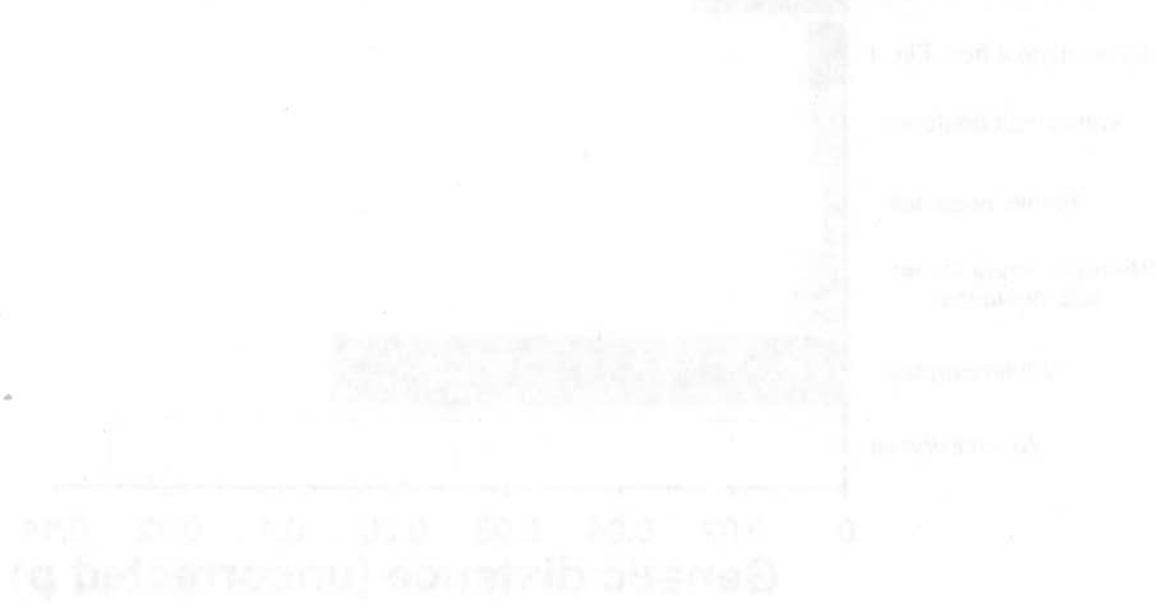


Figure 4. Genetic distances (uncorrected p) for multiple taxonomic/phylogenetic levels in the genus *Eurycea*. Each bar represents the average of all possible comparisons between unique haplotypes. The expanded dataset ($n = 241$) included GenBank sequences from *E. bislineata*, *E. guttolineata*, *E. longicauda*, *E. lucifuga*, *E. spelaesus*, and *E. tynerensis*. 'Neotenes' includes haplotypes G, H, I, J, K, *E. 'Comal Springs' sp.*, and *E. neotenes*. 'Non-neotenes' includes haplotypes, A, B, C, D, E, F, *E. latitans*, *E. pterophila*, *E. sosorum*, and *E. tridentifera*.



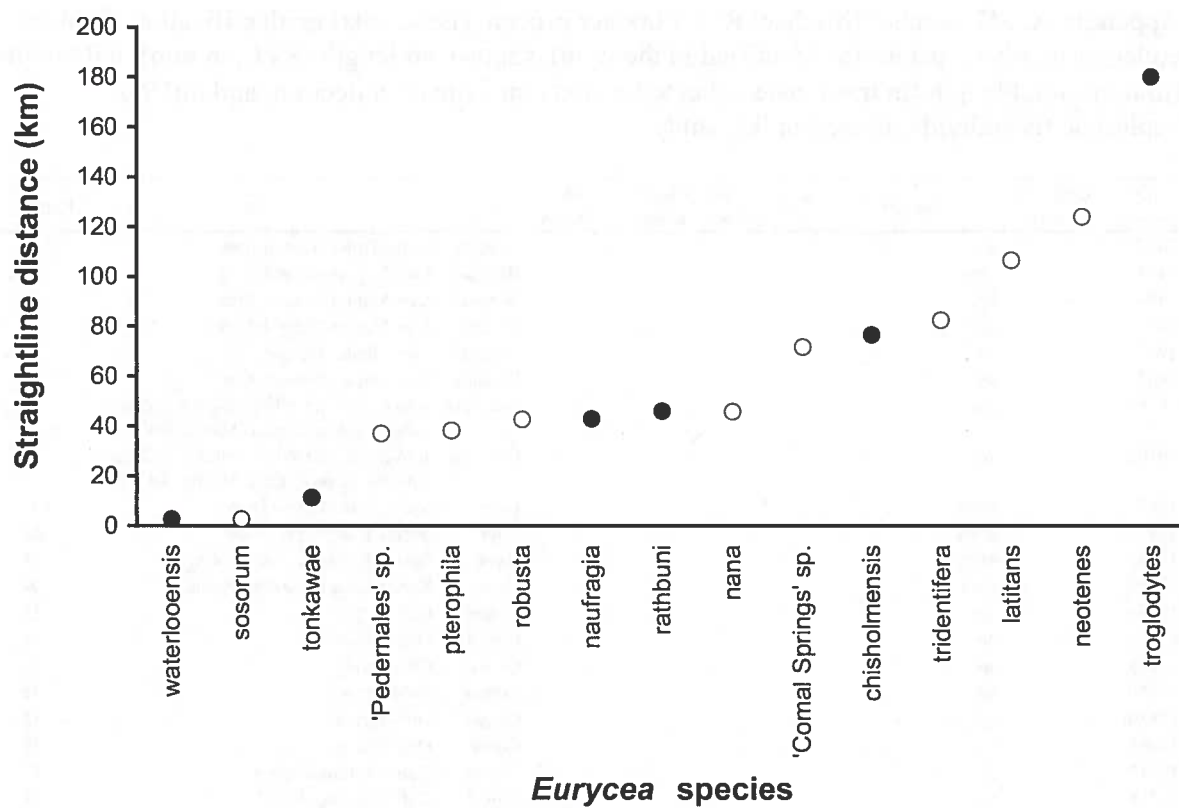


Figure 5. Straightline distance (in km) from the Texas State Capitol building in Austin, Texas, to the type localities of central Texas *Eurycea* species. Open circles represent species which were recovered in clade 2 from Fig. 1.

Appendix A. MF number (Michael R. J. Forstner Frozen Tissue catalog identification number), collector number, species (as identified in the field), snout-vent length (SVL, in mm), tail length (in mm), total length (in mm), date collected, county and site of collection, and mtDNA haplotype for individuals used in this study.

MF Number	Collector Number	species	SVL	Tail Length	Total Length	Date Collected	County	Site	Haplotype
2472		sp.					Bandera	Lost Maples Natural Area	T
2473		sp.					Bandera	Lost Maples Natural Area	T
2474		sp.					Bandera	Lost Maples Natural Area	T
2475		sp.					Bandera	Lost Maples Natural Area	T
2901		sp.					Bandera	Lost Maples Natural Area	T
2902		sp.					Bandera	Lost Maples Natural Area	U
10104		sp.					Gillespie	1.36 rd mi S jct White Oak Rd & Zenner-Alhorns Rd on Zenner-Alhorns Rd	X
10105		sp.					Gillespie	1.36 rd mi S jct White Oak Rd & Zenner-Alhorns Rd on Zenner-Alhorns Rd	Z
17597		nana					Hays	Spring Lake Below Dam	L
17598		nana					Hays	Spring Lake Below Dam	M
17655		nana					Hays	Spring Lake Diversion Springs	M
17656		nana					Hays	Spring Lake Diversion Springs	N
17676		sp.					Comal	Ott's Spring	D
17677		sp.					Comal	Ott's Spring	D
17678		sp.					Comal	Ott's Spring	D
17679		sp.					Comal	Ott's Spring	D
17680		sp.					Comal	Ott's Spring	D
17681		sp.					Comal	Ott's Spring	D
17717		sp.					Comal	Comal Springs Run 3	G
17718		sp.					Comal	Comal Springs Run 3	H
17719		sp.					Comal	Comal Springs Run 3	I
18076		sp.					Comal	Comal Springs Run 1	H
18077		sp.					Comal	Comal Springs Run 1	I
18078		sp.					Comal	Comal Springs Run 1	G
18117		sp.					Hays	Fern Bank Springs	E
18118		sp.					Hays	Fern Bank Springs	E
18119		sp.					Hays	Fern Bank Springs	E
18120		sp.					Hays	Fern Bank Springs	E
18121		sp.					Hays	Fern Bank Springs	E
18122		sp.					Hays	Fern Bank Springs	E
18123		sp.					Hays	Fern Bank Springs	F
18124		sp.					Hays	Fern Bank Springs	E
18125		sp.					Hays	Fern Bank Springs	E
18126		sp.					Hays	Fern Bank Springs	E
18132		sp.					Hays	near Fern Bank Springs	E
18133		sp.					Hays	near Fern Bank Springs	E
18134		sp.					Hays	near Fern Bank Springs	E
19501		sp.					Hays	Fern Bank Springs	E
19505		sp.					Hays	Fern Bank Springs	E
19522		nana					Hays	Spring Lake Hotel site	L
19523		nana					Hays	Spring Lake Hotel site	O
19578		sp.					Hays	Jacob's Well	A
19579		sp.					Hays	Jacob's Well	A
19580		sp.					Hays	Jacob's Well	A
19581		sp.					Hays	Jacob's Well	A
19582		sp.					Hays	Jacob's Well	A
19583		sp.					Hays	Jacob's Well	A
19584		sp.					Hays	Jacob's Well	A
19667		sp.					Hays	Jacob's Well	A
19668		sp.					Hays	Jacob's Well	A
19669		sp.					Hays	Jacob's Well	A
19670		sp.					Hays	Jacob's Well	A

MF Number	Collector Number	species	SVL	Tail Length	Total Length	Date Collected	County	Site	Haplotype
19671		sp.					Hays	Jacob's Well	A
19672		sp.					Hays	Jacob's Well	A
19673		sp.					Hays	Jacob's Well	B
19674		sp.					Hays	Jacob's Well	A
19675		sp.					Hays	Jacob's Well	A
19676		sp.					Hays	Jacob's Well	A
19677		sp.					Hays	Jacob's Well	A
19678		sp.					Hays	Jacob's Well	A
19679		sp.					Hays	Jacob's Well	A
19680		sp.					Hays	Jacob's Well	B
19681		sp.					Hays	Jacob's Well	A
19682		sp.					Comal	Hueco Springs	G
19683		sp.					Comal	Hueco Springs	J
19684		sp.					Comal	Hueco Springs	G
19685		sp.					Comal	Hueco Springs	G
19686		sp.					Comal	Hueco Springs	G
19687		sp.					Comal	Hueco Springs	K
19906		troglydites complex					Edwards	250 m W of RR335, about 8.5 rd km S of jct RR335 & TX Hwy 41	V
19907		troglydites complex					Edwards	250 m W of RR335, about 8.5 rd km S of jct RR335 & TX Hwy 41	W
19908		troglydites complex					Edwards	250 m W of RR335, about 8.5 rd km S of jct RR335 & TX Hwy 41	V
20908		sp.					Kerr	Stockman Spring	Y
20909		sp.					Kerr	Stockman Spring	Y
20910		sp.					Kerr	Stockman Spring	Y
20912		sp.					Kerr	Stockman Spring	Y
20913		sp.					Kerr	Stockman Spring	Y
20933		tonkawae					Travis	Stillhouse Springs	P
21231		tonkawae					Travis	Balcones Canyonlands, mainstem above Tributary 7	Q
21272		tonkawae					Travis	Balcones Canyonlands, Tributary 5	Q
21273		tonkawae					Travis	Balcones Canyonlands, Tributary 5	R
21528		tonkawae					Travis	SAS canyon	S
29508	Zara-5563	sp.			8.2	5/21/10	Hays	Jacob's Well subsurface	A
29509	Zara-5564	sp.	8.5	6.4	14.9	5/21/10	Hays	Jacob's Well subsurface	A
29510	Zara-5564	sp.	13	8.8	21.8	5/21/10	Hays	Jacob's Well subsurface	B
29511	Zara-5564	sp.	10.6	6	16.6	5/21/10	Hays	Jacob's Well subsurface	B
29512	Zara-5564	sp.	9.7	4.6	14.3	5/21/10	Hays	Jacob's Well subsurface	A
29513	Zara-5564	sp.	8.8	4.6	13.4	5/21/10	Hays	Jacob's Well subsurface	B
29514	Zara-5564	sp.	9.2	5.3	14.5	5/21/10	Hays	Jacob's Well subsurface	A
29515	Zara-5564	sp.	12.5	6.6	19.1	5/21/10	Hays	Jacob's Well subsurface	C
29516	Zara-5564	sp.	12.1	7.2	19.3	5/21/10	Hays	Jacob's Well subsurface	A
29517	Zara-5564	sp.	9.2	5.1	14.3	5/21/10	Hays	Jacob's Well subsurface	A
29518	Zara-5564	sp.	11.6	5.8	17.4	5/21/10	Hays	Jacob's Well subsurface	B
29519	Zara-5564	sp.	12.6	6.9	19.5	5/21/10	Hays	Jacob's Well subsurface	B
29520	Zara-5565	sp.	9.1	5	14.1	5/14/10	Hays	Jacob's Well 70'	A
29521	Zara-5565	sp.	8.4	4.7	13.1	5/14/10	Hays	Jacob's Well 70'	A
29522	Zara-5565	sp.	9.4	5	14.4	5/14/10	Hays	Jacob's Well 70'	n/a
29523	Zara-5565	sp.	9.3	6	15.3	5/14/10	Hays	Jacob's Well 70'	A
29524	Zara-5565	sp.	9.4	5.5	14.9	5/14/10	Hays	Jacob's Well 70'	A
29525	Zara-5566	sp.	13	6.6	19.6	6/10/10	Hays	Jacob's Well 20'-70'	B
29526	Zara-5566	sp.	9	4.1	13.1	6/10/10	Hays	Jacob's Well 20'-70'	B
29527	Zara-5566	sp.	9.8	4.7	14.5	6/10/10	Hays	Jacob's Well 20'-70'	B
29528	Zara-5566	sp.	9.2	3.4	12.6	6/10/10	Hays	Jacob's Well 20'-70'	B
29529	Zara-5566	sp.	10.1	5.3	15.4	6/10/10	Hays	Jacob's Well 20'-70'	A
29530	Zara-5566	sp.	9.8	4.9	14.7	6/10/10	Hays	Jacob's Well 20'-70'	A
29531	Zara-5566	sp.	8.8	4.5	13.3	6/10/10	Hays	Jacob's Well 20'-70'	A
29532	Zara-5566	sp.	8.9	4.7	13.6	6/10/10	Hays	Jacob's Well 20'-70'	A
29533	Zara-5566	sp.	9.5	6.6	16.1	6/10/10	Hays	Jacob's Well 20'-70'	A

MF Number	Collector Number	species	SVL	Tail Length	Total Length	Date Collected	County	Site	Haplotype
29534	Zara-5566	sp.	9	5.1	14.1	6/10/10	Hays	Jacob's Well 20'-70'	A
29535	Zara-5566	sp.	8.8	5.2	14	6/10/10	Hays	Jacob's Well 20'-70'	B
29536	Zara-5566	sp.	10.7	6.3	17	6/10/10	Hays	Jacob's Well 20'-70'	A
29537	Zara-5569	sp.				6/5/10	Hays	Jacob's Well 20'	A
29538	Zara-5570	sp.				8/13/09	Hays	Jacob's Well subsurface	A
29539	Zara-5571	sp.				5/28/09	Hays	Jacob's Well subsurface	C
29540	Zara-5576	sp.				5/14/10	Hays	Jacob's Well	A
29541	Zara-5577	sp.				5/14/10	Hays	Jacob's Well	A
29804	Zara-5934	sp.	7.3	3.1	10.4	7/3/10	Hays	Jacob's Well 20'	A
29805	Zara-5934	sp.	8.1	6.2	14.3	7/3/10	Hays	Jacob's Well 20'	A
29806	Zara-5934	sp.	10.9	5.8	16.7	7/3/10	Hays	Jacob's Well 20'	B
29807	Zara-5934	sp.	9.7	4.7	14.4	7/3/10	Hays	Jacob's Well 20'	A
29808	Zara-5934	sp.	9.7	5.4	15.1	7/3/10	Hays	Jacob's Well 20'	A
29809	Zara-5934	sp.	13.8	9.5	23.4	7/3/10	Hays	Jacob's Well 20'	B
29810	Zara-5934	sp.	18.6	6.9	25.5	7/3/10	Hays	Jacob's Well 20'	A
29811	Zara-5934	sp.	17.6	12.5	30.1	7/3/10	Hays	Jacob's Well 20'	B
29812	Zara-5935	sp.	31.3	24.4	55.7	7/3/10	Hays	Jacob's Well 20'	B
29813	Zara-5936	sp.	8.6	4.1	12.7	8/4/10	Hays	Jacob's Well 70'	A
29814	Zara-5936	sp.	10	4.8	14.8	8/4/10	Hays	Jacob's Well 70'	A
29815	Zara-5936	sp.	9.8	5.3	15.1	8/4/10	Hays	Jacob's Well 70'	A

Primary Research Paper

Ecology of the Jollyville Plateau salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization^{*}

Beth Davis Bowles^{1,*}, Mark S. Sanders² & Robert S. Hansen³

¹Plant Sciences Graduate Program, Integrative Biology, School of Biological Sciences, University of Texas, Austin, TX 78712, USA

²City of Austin Water Utility, Balcones Canyonlands Preserve, 3635 Hwy. 620 South, Austin, TX 78738, USA

³Alan Plummer Associates, Inc., 6300 La Calma, Suite 400, Austin, TX 78752-3825, USA

(* Author for correspondence: Tel.: (512) 471-4997; Fax: (512) 232-3402; E-mail: beth.davis@mail.utexas.edu)

Received 17 May 2004; in revised form 17 March 2005; accepted 29 March 2005

Key words: *Eurycea tonkawae*, perennibranchiate salamander, Edwards Plateau, Texas, springs, impervious cover

Abstract

The Jollyville Plateau salamander, *Eurycea tonkawae* Chippindale, Price, Wiens, and Hillis, inhabits springs and wet caves of the Jollyville segment of the Edwards Plateau, Texas. The known range of this species is limited to six stream drainages, and most known localities are at risk of impairment from urban development. Our purpose was to gather needed autecological information on *E. tonkawae* and evaluate factors that may affect the distribution and abundance of the species. We conducted visual salamander surveys at nine stream sites across the Jollyville Plateau between December 1996 and December 1998. The survey sites were classified as undeveloped or developed based on watershed impervious cover estimates. We characterized the habitat for each site, including substrate type, discharge, and water quality. Salamander counts varied seasonally, but generally were higher during spring and summer. Salamander densities across sites were positively correlated with rubble and cobble substrate density as preferred cover, and negatively correlated with the standard deviation of water temperature, as expected for a spring-adapted species. In addition, we found that mean salamander densities at sites occurring in undeveloped watersheds were significantly higher than at developed sites, where specific conductance of the water was higher. The results of this study suggest that while habitat and seasonal factors influence surface salamander densities, *E. tonkawae* populations may be most vulnerable to effects associated with urbanization.

Introduction

Urban sprawl has impaired over 50,000 km of streams and rivers in the United States (USEPA, 2000). Impervious cover in watersheds elevates the frequency and intensity of storm flows and reduces baseflow in receiving streams (reviewed in Leopold, 1968; Schueler, 1994; Novotny, 2003) increases erosion and downcutting of the stream channel

(Arnold et al., 1982; Booth & Jackson, 1997), and contributes nutrient and toxic pollutant loads (Pitt et al., 1995; Novotny, 2003). The diversity and abundance of benthic invertebrates and fishes are consistently and dramatically lower in urban relative to non-urban catchment streams (reviewed in Paul & Meyer, 2001). The threshold of measurable degradation of stream habitat and loss of biotic integrity consistently occurs with 6–15% impervious cover in contributing watersheds (e.g., Klein, 1979; Schueler, 1994; Booth & Jackson, 1997; Wang et al., 2001; Morse et al., 2003; Roy et al., 2003).

^{*} This work was conducted while B.D. Bowles and R.S. Hansen were employed by the City of Austin Watershed Protection Department Austin Texas.

The karst aquifers of the Edwards Plateau, in central Texas, contribute to thousands of springs (Brune, 1981) that are home to approximately 90 endemic animal species, including subterranean and surface-dwelling invertebrates and salamanders, and several species of fish (Bowles & Arsuffi, 1993). The Jollyville Plateau salamander, *Eurycea tonkawae* Chippindale, Price, Wiens, and Hillis, a perennibranchiate member of the family Plethodontidae, is endemic to springs and caves of the Jollyville segment of the Edwards Plateau. Similar to other populations of *Eurycea*, *E. tonkawae* is restricted to the vicinity of wet caves, springs, and spring-dominated surface flows. Several characteristics of these aquatic habitats have been used to explain the highly localized distribution of perennibranchiate *Eurycea*, including temporal and thermal flow reliability, minimal substrate siltation and calcium carbonate deposition (Tupa & Davis, 1976; Sweet, 1982), and the availability of subsurface refugia and corridors (Dowling, 1956; Rudolph, 1978; Sweet, 1982; Chippindale et al., 1993; Tumblison & Cline, 1997). *Eurycea* salamanders are commonly observed occupying areas under or near rocks, aquatic plants and algae, silt, sand, and organic debris (Tupa & Davis, 1976; Tumblison et al., 1990; Chippindale et al., 1993).

Available information specific to *E. tonkawae* is limited to the systematic description of the species (Chippindale et al., 2000) and anecdotal observa-

tions. The known range of this species is limited to six stream drainages, and most known localities are at risk of impairment from urban development due to their small, localized recharge areas (Chippindale et al., 2000). For example, recharge to springs in Bull Creek, which possesses the largest populations of *E. tonkawae*, primarily is from infiltration of rainwater on the plateau and runoff captured by local sinkholes (Johns, 1994). The paucity of ecological and life history information is a hindrance to the development of a watershed management policy that would promote effective protection of the species and its habitat in a region subject to urban expansion. The purpose of this paper is to document the relative abundance of surface-dwelling populations of *E. tonkawae*, identify the existing range of habitat conditions in which the salamanders occur, and provide a preliminary assessment of factors that may regulate the abundance and distribution of the species.

Materials and methods

Study sites

We selected six stream sites for salamander surveys on a monthly or bimonthly basis between December 1996 and December 1998, and two additional sites were surveyed quarterly (Table 1). The selection of sites was based on the consistent

Table 1. *Eurycea tonkawae* survey sites in Travis County, Texas with estimates of watershed impervious cover

Study Site	Survey frequency	Location	Watershed impervious cover estimate (%)
Spicewood Spring and Tributary (SP)	Monthly or bimonthly	30°21'46" N, 97°44'51" W	45
Stillhouse Hollow Spring and Tributary (ST)	Monthly or bimonthly	30°22'18" N, 97°45'49" W	22
Barrow Hollow Tributary (BA)	Monthly or bimonthly ¹	30°22'16" N, 97°46'02" W	27
Long Hog Hollow Tributary (T3)	Monthly or bimonthly	30°23'49" N, 97°46'10" W	16
Tanglewood Spring ² and Tributary (TA)	Quarterly	30°25'50" N, 97°46'54" W	30
Bull Creek Tributary 6 @ Hank's Tract (T6)	Monthly or bimonthly	30°25'30" N, 97°48'51" W	15
Bull Creek Tributary 5 @ Hank's Tract ³ (T5)	Monthly or bimonthly	30°25'37" N, 97°49'04" W	5
Bull Creek @ Franklin Tract ⁴ (FR)	Monthly or bimonthly	30°25'08" N, 97°48'40" W	3
Whelless Spring and Tributary (WH)	Quarterly	30°27'53" N, 97°52'25" W	0

¹Surveys began July 1998.

²Also known as Canyon Vista Spring.

³Also known as Bull Creek Spring.

⁴Also known as New Bull Creek Spring.

occurrence of *E. tonkawae* and available access. No random or systematic site selection protocol was attempted due to the limited number of sites available for study. The Barrow Hollow survey site (BA) was added late in the project and therefore was not included in most analyses. All data collection was conducted under approximate baseflow conditions.

Study sites were classified into two groups based on watershed impervious cover estimates (Table 1) grouped here as "developed" (>10%) and "undeveloped" (<10%) following literature threshold values cited above. Impervious cover was estimated from GIS maps of roads and buildings developed using 1997 aerial photos, and an additional 46.45 m² was applied to the impervious cover estimates for each building unit to account for driveways and sidewalks (City of Austin, unpubl.). Agricultural activity in these watersheds is minimal, if present at all, and was not detected in the GIS analysis of the aerial photos.

Salamander surveys

We defined the boundaries of the salamander survey areas by the extent of salamander occurrence in the stream reach at the first survey, the practicality of search effort, and representativeness of the habitat type. We divided survey sites into sections based on habitat type: riffle/run (flowing with gravel/cobble substrate), pool (deep or shallow with no flow), bedrock glide (shallow flow with bedrock substrate), or combinations of these types. A minimum of three sections was surveyed at each site and the maximum depth among sections surveyed was approximately 0.3 m. Individuals were assigned to one of two relative size classes based on a visual estimate of total length (tip of snout to tip of tail): large (>2.5 cm) or small juvenile (≤2.5 cm). No consistent attempts were made to determine sex or verify sexual maturity of individual salamanders.

Salamander surveys were conducted at approximately the same day of the month, when possible, and between 9 am and 3 pm. Each survey involved searching the wetted surface of the entire section, including in and under available cover and in the top layer of sediment or detritus. We created

stream maps to estimate wetted surface area for each section. The field and survey procedures employed in this project were selected to minimize disturbance to the habitat and avoid direct handling of salamanders. We made no attempt to search for salamanders in subsurface habitats. Numbers of sunfish (*Lepomis* spp.), black bass (*Micropterus* spp.) and crayfish (*Procambarus* sp.) longer than approximately 5 cm encountered during salamander surveys were recorded as potential predators.

Habitat

We recorded habitat observations on the same dates as the salamander surveys, including a visual estimate of the percent of the substrate covered by rocks, algae and plants, leaves, and woody debris. Rock substrates were classified by size based on a modified Wentworth scale (Wentworth, 1922). Percent embeddedness of cobble substrate was a visual estimate of the percent of the rock surface surrounded by carbonate deposits, sediment, sand, or organic detritus. The estimate for each section represents the average embeddedness value for 5–10 rocks. Substrate items were classified as bedrock when they were highly embedded in the substrate and could not be moved with reasonable effort. We estimated flow, or discharge (m³ s⁻¹), using a Marsh McBirney Model 2000® portable velocity meter following the methodology of Gordon et al. (1992).

On the final sampling date at all sites, we employed a grid design to select 50–100 points in each section to record substrate type and size. Size and type of cover items used by each salamander we encountered also were recorded. We then calculated standardized selection ratios following Manly et al. (1993). Standardized selection ratios represent the probability of use of each cover type by the species based on the number of cover items used and the number available, assuming equal availability of all cover items (Manly et al., 1993).

Water chemistry

We collected surface and spring water samples monthly from all sites on the same date, with the exception of WH where water samples were

collected on the same day as each salamander survey. Water temperature ($^{\circ}\text{C}$), pH, conductivity ($\mu\text{S cm}^{-1}$) and dissolved oxygen concentration (mg l^{-1}) were taken at each site with a calibrated Hydrolab[®] (Hydrolab, Austin, TX, USA). Samples from springs were collected from flowing water as close to the rock orifice as possible. Preservation and chemical analysis methods followed protocols in United States Environmental Protection Agency (1983). Water temperature ($^{\circ}\text{C}$) also was measured in each section during each survey.

Statistical analysis

We conducted statistical analyses using SYSTAT 10 statistical software (vers. 1.0.0.1) or according to Zar (1984). Analyses were evaluated at 95% confidence and conducted using the section means or site means for each parameter as independent replicates. The section mean for each parameter was calculated by averaging all data collected for that parameter in the section over the 2 year study. Similarly, the site mean for a single parameter was calculated by averaging the site data for that parameter over the 2 year period.

To investigate potential habitat preferences by salamanders, we compared the section mean salamander densities among habitat groups: riffle/run ($n = 11$), pool ($n = 11$), and bedrock glide ($n = 6$) using Kruskal-Wallis non-parametric analysis of variance. Spring pools and sections that contained a combination of habitat types were not included in this analysis.

We used Spearman rank correlation analysis to test the significance of relationships of salamander counts or densities (per m^2 wetted area) versus crayfish counts, substrate cover estimates, substrate embeddedness estimates, water temperature, and flow rates. The sequential Bonferroni procedure described by Rice (1989) was used to assess the significance of the p -values at a table-wide significance level of 0.05.

We conducted t -test two-sample mean comparisons on the site means of salamander densities and selected habitat and water chemistry parameters to determine significant differences between the impervious cover groups. Correlation

analysis was rejected for these comparisons because the relationships were nonlinear (Allan, 2004).

Results

Salamander counts

Numbers of *E. tonkawae* we observed at the surface were highly variable among the study sites during the 2 year study (Table 2), primarily due to seasonal fluctuations observed in counts (Fig. 1), and were highest during the spring and summer months. In particular, the number of small juvenile salamanders relative to the total number of salamanders was distinctly higher from March to August in both years (Fig. 1). This pattern was apparent at all sites when viewed individually and in the two sites monitored quarterly (not shown), except where low flows reduced wetted surface area during the summer months. We never observed salamander eggs during the course of this study, but occasionally encountered gravid females (based on observation of eggs through the abdominal wall). Gravid females generally were observed from November through February, however no consistent effort was made to inspect individuals for eggs.

Habitat

Mean salamander densities were significantly higher in riffles/runs and pools than in bedrock glides ($H = 9.9$, $p < 0.01$), and mean salamander numbers were positively correlated to the estimated mean area of rubble and cobble by section (Table 3). The standardized selection ratios from the fall/winter 1998 surveys indicate a preference by large *E. tonkawae* for larger rock substrates as cover (Fig. 2). We observed few salamanders under leaves or vegetation relative to the amount of those items available. On the contrary, we found that the probabilities of use of rubble, cobble, and boulder substrates were higher and progressively increased with rock size. The use of leaves as cover may have been minimally underestimated due to the difficulty of locating salamanders in large leaf packs. Additionally, we could not calculate standardized selection ratios

Table 2. Mean (\pm 1 SD) and range of salamander counts and habitat and water chemistry variables measured at nine *Eurycea tomkowi* sites across all sampling dates. Water chemistry data include spring and surface measurements (n.d. = below detection limit)

Parameter	SP	ST	BA ¹	T3	TA	T6	T5	FR	WH
No. of large salamanders (>2.5 cm)	15 \pm 9 (2-38)	14 \pm 12 (1-41)	7 \pm 2 (5-9)	28 \pm 24 (0-92)	11 \pm 6 (3-19)	20 \pm 10 (5-41)	40 \pm 21 (3-76)	84 \pm 69 (1-280)	37 \pm 19 (8-66)
No. of sm. juvenile salamanders (\leq 2.5 cm)	2 \pm 4 (0-12)	2 \pm 2 (0-8)	0	6 \pm 12 (0-45)	1 \pm 2 (0-8)	2 \pm 3 (0-13)	4 \pm 5 (0-17)	11 \pm 4 (0-63)	16 \pm 26 (0-81)
Wetted area (m ²)	50.0 \pm 8.8 (35.2-58.9)	43.6 \pm 10.6 (30.4-57.3)	57.1 \pm 28.2 (15.0-74.0)	195.6 \pm 62.0 (31.8-246.8)	44.0 \pm 2.7 (38.5-47.4)	123.9 \pm 39.1 (43.2-165.0)	117.8 \pm 53.3 (32.3-246.0)	103.2 \pm 7.4 (13.3-134.0)	53.2 \pm 31.8 (2.3-87.9)
Rubble + cobble (m ²)	14.1 \pm 3.6 (8.2-20.0)	9.3 \pm 5.3 (2.7-18.5)	8.1 \pm 5.4 (2.1-15.2)	8.4 \pm 3.6 (3.2-13.9)	4.3 \pm 0.9 (3.1-6.9)	10.1 \pm 3.5 (6.1-18.0)	19.1 \pm 7.5 (3.1-28.6)	20.3 \pm 7.6 (3.3-36.8)	7.2 \pm 5.5 (0.5-16.7)
Flow (m ³ s ⁻¹)	0.003 \pm 0.003 (0-0.010)	0.002 \pm 0.002 (0-0.007)	0.004 \pm 0.004 (0-0.009)	0.015 \pm 0.018 (0-0.070)	0.007 \pm 0.006 (0.001-0.023)	0.039 \pm 0.042 (0-0.134)	0.018 \pm 0.015 (0.002-0.057)	0.062 \pm 0.013 (0-0.238)	0.007 \pm 0.014 (0-0.046)
Temperature (°C)	21.9 \pm 1.8 (19.0-32.0)	18.8 \pm 2.8 (12.2-24.2)	18.6 \pm 3.2 (16.0-25.6)	20.2 \pm 5.0 (10.6-30.0)	21.2 \pm 3.5 (15.0-26.8)	19.5 \pm 3.9 (10.9-27.8)	19.6 \pm 3.1 (14.0-24.9)	18.5 \pm 3.0 (13.0-24.8)	19.4 \pm 2.1 (13.5-22.2)
% Embeddedness	12 \pm 15 (0-50)	19 \pm 17 (0-50)	3 \pm 10 (0-30)	13 \pm 13 (0-50)	9 \pm 12 (0-60)	12 \pm 13 (0-50)	16 \pm 13 (0-50)	18 \pm 15 (0-60)	16 \pm 11 (0-40)
Specific conductance (μ S cm ⁻¹)	1010 \pm 135 (644-1124)	991 \pm 30 (863-1120)	935 \pm 25 (914-967)	819 \pm 136 (727-1310)	846 \pm 83 (712-1101)	920 \pm 40 (850-984)	611 \pm 20 (575-645)	556 \pm 22 (523-600)	612 \pm 32 (567-671)
Nitrate-N (mg l ⁻¹)	3.9 \pm 0.9 (0.6-5.4)	5.5 \pm 1.3 (3.0-8.0)	5.1 \pm 1.2 (2.9-7.9)	1.1 \pm 0.5 (0.3-2.1)	1.9 \pm 0.5 (0.3-2.9)	0.5 \pm 0.2 (0.1-1.0)	0.4 \pm 0.1 (0.1-0.6)	0.1 \pm 0.1 (n.d.-0.4)	0.1 \pm 0.02 (0.03-0.1)
Dissolved oxygen (mg l ⁻¹)	7.1 \pm 1.0 (5.3-9.5)	7.9 \pm 0.7 (7.0-10.0)	8.2 \pm 1.5 (7.1-9.9)	10.4 \pm 1.6 (8.5-13.6)	7.7 \pm 1.1 (5.7-10.4)	9.5 \pm 2.0 (5.7-12.4)	7.0 \pm 1.4 (4.4-9.0)	7.1 \pm 1.4 (4.1-8.9)	5.6 \pm 1.4 (3.1-7.3)
pH	6.9 \pm 0.4 (6.1-8.1)	7.2 \pm 0.4 (6.4-7.9)	7.8 \pm 0.2 (7.4-8.2)	7.7 \pm 0.4 (7.0-8.4)	7.4 \pm 0.5 (6.4-9.3)	7.5 \pm 0.4 (6.6-8.3)	7.2 \pm 0.4 (6.6-8.2)	7.4 \pm 0.4 (6.7-8.3)	7.3 \pm 0.4 (6.4-7.9)

¹Burrow was not included in most study analyses (water quality data collected from March 1997–December 1998).

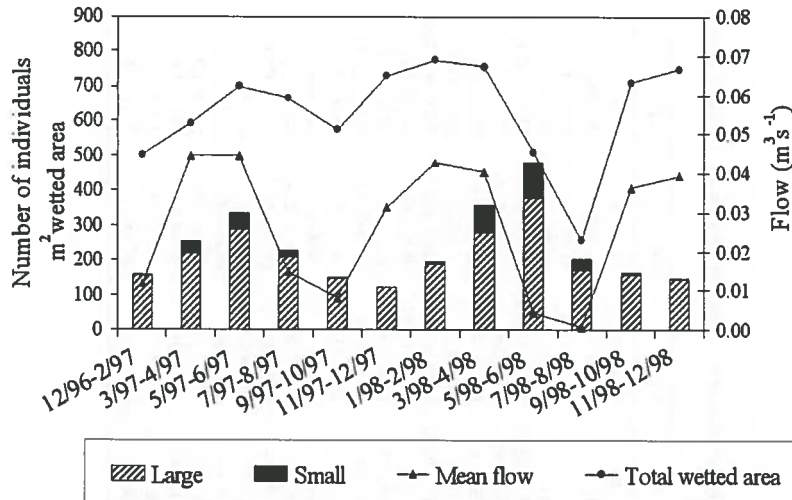


Figure 1. Total *Eurycea tonkawae* counts by size class, mean flow and total wetted area at six sites surveyed between December 1996 and December 1998. The data include sites surveyed at least bimonthly and a mean was used if a site was surveyed both months.

Table 3. Results of the Spearman rank correlation analysis of *Eurycea tonkawae* counts or densities versus selected habitat and water quality variables. All variables are the computed means over the period of the study

Dependent variable	Independent variable	Analysis scale	N	r_s	p	Significant?*
No. of salamanders	Rubble + cobble (m ²)	Section	37	0.74	< 0.001	Y
No. of salamanders	no. of crayfish	Section	37	-0.008	> 0.1	N
No. of salamanders/m ²	% embeddedness	Section	37	0.17	> 0.1	N
No. of salamanders/m ²	Mean temperature (°C)	Section	37	-0.35	< 0.05	N
	SD of temperature			-0.45	< 0.01	Y
No. of salamanders/m ²	Flow (m ³ s ⁻¹)	Site	8	-0.071	> 0.1	N

* Significance of p was determined following Rice (1989).

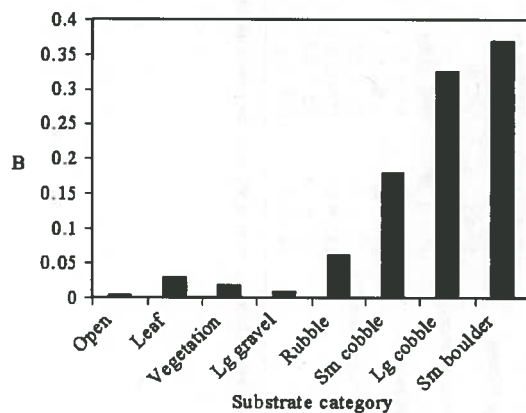


Figure 2. Standardized selection ratios (b) (Manly et al., 1993) representing probability of use by large *Eurycea tonkawae* for available substrate types.

for small juvenile salamanders because of the low number of individuals observed in the fall/winter 1998 survey ($n = 2$). However, we commonly observed small juvenile salamanders in shallow areas (≤ 5 cm) near the bank under rubble, small cobble, vegetation, and woody debris over the course of the study.

We did not detect any relationships between salamander densities and embeddedness estimates (Table 3). Rocks were primarily embedded in loose organic detritus or sand and this did not adversely affect salamander presence. Additionally, small substrate particles were rare in glide habitats, which also possessed relatively lower salamander densities.

The maximum and minimum temperatures recorded during this study were 32.0 °C and

10.6 °C (Table 2). Mean salamander densities were negatively associated with standard deviation in water temperature across sections (Table 3), indicating that salamander densities were lowest in sections that were least influenced by springflow.

Baseflow (discharge) rates at the study sites ranged from 0 to 0.238 m³ s⁻¹ (Table 2). Our data suggest that mean salamander densities were not linearly related to mean discharge across sites (Table 3). Rather, we observed that baseflow at these sites affected salamander numbers to the extent that low flows reduced wetted surface area (Fig. 1) or high flows created inhospitable currents in riffles and bedrock glides. For example, when flow rates were high at FR, salamanders were conspicuously absent from riffles with high water velocity. The high water velocity may have scoured available cover and exceeded the capacity of individual salamanders to maintain position in the channel. We were unable to determine if individual salamanders were flushed downstream or retreated to subsurface refugia (e.g., Rudolph, 1978).

Potential predators

We found no significant relationships between salamander abundance and crayfish abundance within or among sites (Table 3). Moreover, we noted few negative interactions between salamanders and crayfish and no increased incidence of missing tails in the presence of crayfish (e.g., Tumlison et al., 1990). In a single instance, we observed a crayfish actively feeding on a salamander held in its cheliped. However, this occurred at the Spicewood site on the same date that other salamanders were observed dead or moribund (cause unknown), suggesting that the crayfish was likely a scavenger and not a predator.

We rarely observed fish in the study areas over the course of the study; consequently no comparison to salamander numbers across sections was conducted. Although direct predation of centrarchid fish on *Eurycea* salamanders has been observed (Tupa & Davis, 1976; Nelson, 1993; R. Hansen, personal observation), *E. tynerensis* apparently reduces fish predation rates relative to other species by retreating into gravel substrate

(Rudolph, 1978). We noted that Jollyville Plateau salamanders frequently retreated into the substrate after cover was removed by the surveyors, suggesting they also possess this anti-predation behavior.

Developed vs. undeveloped tributaries

Mean salamander densities were significantly lower in the developed tributaries relative to the undeveloped tributaries (Fig. 3). Our estimates of rubble and cobble substrate, baseflow (discharge) rates and % embeddedness were slightly higher in undeveloped sites, while mean temperature and SD of temperature were lower. However, none of the parameters were significantly different between the impervious cover groupings (Table 4). Three of the developed sites had a relatively higher proportion of bedrock substrate compared to the other sites. The results of the habitat comparisons suggest that this may have contributed to lower densities at those sites.

Mean water specific conductance ($\mu\text{S cm}^{-1}$) was higher in developed tributaries (Fig. 3) due to increased concentrations of chloride, magnesium, nitrate-nitrogen, potassium, sodium, and sulfate that were present at these sites. Most notably, SH averaged 5.5 mg l⁻¹ nitrate-nitrogen (with a max-

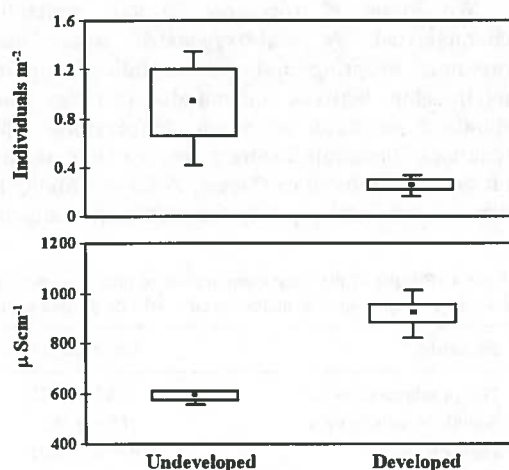


Figure 3. Box and whisker plots of *Eurycea tonkawae* densities and water specific conductance at the undeveloped ($n = 3$) and developed ($n = 5$) sites. The boxes represent the mean \pm SE. The data point and the whiskers represent the mean and the range, respectively.

imum measurement of 8.0 mg l^{-1}) (Table 2). Because we recorded dissolved oxygen levels at different times of day across sites these values are not directly comparable. Mean pH values were similar between developed and undeveloped tributaries (Table 2).

Discussion

Basic natural history information is necessary to effectively gauge anthropogenic impacts on populations. Unfortunately, such information is frequently lacking or woefully inadequate, particularly for rare species. Our goals in this study were to fill gaps in knowledge of the autecology of *Eurycea tonkawae* and begin to evaluate the impacts of urban development on populations of the species.

Surface abundances of *E. tonkawae* at these sites were higher in the spring and summer months and were not dependent on stream flow rates. While the increase of small juveniles observed during the same time period superficially indicates a seasonal reproduction pattern, the ability of *E. tonkawae* to enter subterranean habitats presently precludes drawing conclusions about seasonal reproduction and survival in these study populations, as well as population size.

We found *E. tonkawae* habitats generally characterized by well-oxygenated water and proximity to springs and seeps, as indicated by the relationship between salamander densities and standard deviation of water temperature. The reliance of perennibranchiate *Eurycea* salamanders on springhead habitats (Sweet, 1982) potentially is due to a minimal capacity for metabolic compen-

sation below the ambient springflow temperature range (McAllister & Fitzpatrick, 1989).

We found a strong relationship between available rock cover and densities of *E. tonkawae*. This is consistent with similar studies on other aquatic salamanders (Davic & Orr, 1987; Parker, 1991; Welsh & Ollivier, 1998; Smith & Grossman, 2003) and explains the relatively low salamander densities in bedrock glides. Additionally, we found that embeddedness of rock substrates did not affect salamander density, likely due to the loose nature of the interstitial particles. Tumilson et al. (1990) noted that *Eurycea taylori* densities were highest at sites where embeddedness was near 50% of rock bottom surface, and he hypothesized that the small particles provided spaces for foraging and cover.

Predation risk to populations of *E. tonkawae* at the surface appears to be minimal. We found no sound evidence to suspect crayfish are predators to the salamanders. While anecdotal evidence shows sunfish and black bass are predators, these fish occurred rarely in the salamander habitats.

The impervious cover site groups identified in this study were well-differentiated into those having high salamander densities and low specific conductance (undeveloped), or relatively lower salamander densities and high specific conductance measurements (developed). This likely indicates a mutual response to impacts associated with urbanization. Increased levels of ions in surface water associated with urbanization in Bull Creek and nearby Barton Creek were attributed to wastewater line leaks, roadway runoff, and land use practices such as fertilizer application and irrigation (Johns, 1994; Johns & Pope, 1998).

Table 4. Results of the *t*-test comparisons of mean salamander densities and selected habitat and water quality variables (± 1 SE) at *Eurycea tonkawae* sites in undeveloped versus developed watersheds

Parameter	Undeveloped ($n = 3$)	Developed ($n = 5$)	<i>t</i>	<i>p</i>
No. of salamanders m^{-2}	0.93 ± 0.27	0.26 ± 0.04	3.34	0.016
Rubble + cobble (m^2)	15.5 ± 4.2	9.3 ± 1.6	1.69	0.141
Flow ($\text{m}^3 \text{s}^{-1}$)	0.029 ± 0.017	0.013 ± 0.007	1.03	0.341
Specific conductance ($\mu\text{S cm}^{-1}$)	593 ± 19	917 ± 38	-6.18	0.001
Mean water temperature ($^{\circ}\text{C}$)	19.2 ± 0.3	20.3 ± 0.5	-1.48	0.190
SD of water temperature	2.7 ± 0.3	3.4 ± 0.5	-0.88	0.412
% Embeddedness	17 ± 1	13 ± 2	1.64	0.151

Pollutants expected with these sources include toxic hydrocarbons and heavy metals (Novotny, 2003). In addition, the high current velocities associated with elevated discharge in urban watersheds during storm events results in increased instability of substrates (Booth & Jackson, 1997) thereby dislodging and removing cover for salamanders (Orser & Shure, 1972) and potentially exceeding their ability to maintain position in the channel. Other impacts to the populations of *E. tonkawae* we observed at the developed sites in the course of this study further highlight their vulnerability to human activities. Among these are dead salamanders evidently crushed under rocks, and discharges of chlorinated pool water into salamander habitats. Several salamanders were found dead or moribund during the October 1998 survey at SP, but the cause is unknown. In addition, we observed salamanders with spinal scoliosis at ST over the course of the study. We do not know the cause of this deformity, and it merits further investigation to determine if it is a naturally-occurring phenomenon or a product of anthropogenic disturbance (Ryan, 1998).

Amphibians are sensitive indicators of environmental degradation (Barinaga, 1990) and prior research has shown a reduction of salamander densities associated with urban impacts to streams (Orser & Shure, 1972; Willson & Dorcas, 2003). Reduction in habitat quality, due to changes in the natural flow regime and degradation of ground and surface water quality, may be the largest threats from urbanization facing these populations and must be considered in future conservation efforts.

Acknowledgements

This work was funded by the City of Austin, Texas Watershed Protection Department and is the product of hard work and diligence of many City of Austin staff members too numerous to cite in entirety. However, we would like to specifically recognize the extensive contributions of DeeAnn Chamberlain, Ed Peacock, David Johns, Martha Turner, and Chris Herrington. The Aquatic Biological Assessment Team members (R. Cole, V. Hutchison, L. Roesner, M. Schramm and J. Yelderman, Jr.) were instrumental in pro-

viding the framework for the study design. We would also like to thank the Lower Colorado River Authority, A. and D. Spencer and the Great Hills Golf Course for access to salamander sites on their properties. Andrew Price, David Bowles and two anonymous reviewers provided valuable guidance and reviewed earlier versions of this paper.

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