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PHYLOGENETIC RELATIONSHIPS AND SYSTEMATIC REVISION OF CENTRAL TEXAS HEMIDACTYLIINE PLETHODONTID SALAMANDERS

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ABSTRACT: Genetic variation and phylogenetic relationships of central Texas nontransforming spring and cave salamanders, genera *Eurycea* and *Typhlomolge* (Plethodontidae: Plethodontinae: Hemidactyliini), were examined using 25 allozyme loci and DNA sequence data for a maximum of 356 bp of the mitochondrial cytochrome *b* gene. Monophyly of the central Texas hemidactyliines is well supported. High levels of divergence occur among many populations and groups of populations, and there clearly are many more species in the group than previously recognized. Many have extremely restricted distributions in isolated islands of aquatic habitat. Several major monophyletic groups were identified that correspond to geographically circumscribed areas of the Edwards Plateau region. The deepest phylogenetic split in the group occurs between populations northeast versus southwest of the Colorado River. Species that have been assigned to the genus *Typhlomolge* are phylogenetically nested within the central Texas *Eurycea*; therefore, the genus *Typhlomolge* is placed in the synonymy of *Eurycea*. Continued recognition of the species *E. latitans, E. nana, E. neotenes, E. pterophila, E. sosorum, E. tridentifera,* and *E. troglodytes* is recommended, but *E. neotenes* appears to be restricted in range to a small geographic area, and is not widespread in the region as previously thought. The *E. latitans* and *E. troglodytes* species complexes are recognized; each consists of spring and cave populations that include those at the type localities of the latter two species, plus other populations to which they appear most closely related. Three new species from northeast of the Colorado River are described.

Key words: Caudata; Plethodontidae; *Eurycea*; *Typhlomolge*; *Eurycea chisholmensis* new species; *Eurycea naufragia* new species; *Eurycea tonkawae* new species; Phylogeny; Speciation; Central Texas; Allozymes; Cytochrome *b*

THE EDWARDS PLATEAU region of central Texas is characterized by Cretaceous limestones uplifted since at least mid-Tertiary times, dissected and eroded to form numerous springs and caves (Sweet, 1978*a*; Potter and Sweet, 1981; Abbot and Woodruff, 1986; Woodruff and Abbott, 1986; and Veni, 1994 review the geologic history of the area). These habitat islands are inhabited by a variety of endemic aquatic organisms, many with extremely restricted distributions. Predominant among the aquatic vertebrate fauna of the region are plethodontid salamanders of the genera *Eurycea* and *Typhlomolge* (tribe Hemidactyliini), almost all of which are perennibranchiate (i.e., retain gills and other larval morphological features throughout their lives). Members of this group exhibit a wide range of morphologies that vary primarily according to whether they occupy surface or subterranean habitats. *Typhlomolge rathbuni,* the first member of the group to be described, was discovered after the drilling of an artesian well at San Marcos, Hays Co. in 1895 (Stejneger, 1896). This large cave salamander immediately captured the attention of the scientific community due to its bizarre morphology, including tiny nonfunctional vestiges of eyes, loss of pigmentation, long slender legs, and broad flattened head; this species and its presumed sister species *T. robusta* exhibit some of the most extreme cave-associated morphologies known among vertebrates (Potter and Sweet, 1981; Sweet, 1986). Rec-

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ognized as a plethodontid by Emerson (1905) and later placed in the tribe Hemidactyliini by Wake (1966), the relationships of *Typhlomolge* to other hemidactyliines have remained controversial (Mitchell and Reddell, 1965; Mitchell and Smith, 1972; Potter and Sweet, 1981; Lombard and Wake, 1986). For the remainder of this paper, we will use the genus name *Eurycea* for these two species, a taxonomic change proposed by Mitchell and Reddell (1965) and Mitchell and Smith (1972). Molecular phylogenetic evidence presented here and by Chippindale (1995) supports this taxonomic shift.

Additional central Texas hemidactyliines were not recognized until 1937, when Bishop and Wright described *Eurycea neotenes* from a spring near Helotes in Bexar Co. In subsequent decades, several more spring and cave species were described: *E. nana* Bishop 1941 from San Marcos Springs in Hays Co., *E. latitans* Smith and Potter 1946 from Cascade Caverns in Kendall Co., *E. pterophila* Burger, Smith and Potter 1950 from Fern Bank Springs in Hays Co., *E. troglodytes* Baker 1957 from Valdina Farms Sinkhole in Medina Co., *E. tridentifera* Mitchell and Reddell 1965 from Honey Creek Cave in Comal Co., and *E. sosorum* Chippindale, Price and Hillis 1993 from Barton Springs in Travis Co. The status and relationships of these taxa have been problematic; most recently Sweet (1978*a,b,* 1984) questioned the status of *E. pterophila* (which he synonymized under *E. neotenes*) as well as *E. latitans* and *E. troglodytes,* which he considered hybrids between other species.

With the exception of Bogart's (1967) chromosomal studies, all inferences of relationships among the central Texas hemidactyliines have been based on morphology, and no truly phylogenetic analysis of the group has been attempted. Morphological variation in the group has proven confusing; many surface dwellers from throughout the region appear similar to one another based on external morphology (e.g., Mitchell and Smith, 1972; Hamilton, 1973; Sweet, 1978*a,* 1982; Chippindale et al., 1993), while subterranean dwellers display a spectrum of degrees of cave-associated morphology (Mitchell and Reddell, 1965; Mitchell and Smith, 1972; Sweet, 1978*a,* 1984; Potter and Sweet, 1981). Based on biogeographic and geologic considerations, multiple invasions of subterranean habitat are likely to have occurred (Mitchell and Smith, 1972; Sweet, 1978*a,* 1982, 1984; Potter and Sweet, 1981), but in at least some cases (especially in the areas inhabited by *E. tridentifera, E. latitans,* and perhaps *E. troglodytes*) there is the potential for subterranean gene flow among cave populations (Sweet, 1978*a,* 1984).

A key issue is whether the central Texas hemidactyliines are monophyletic. Wake (1966) and Potter and Sweet (1981) suggested that members of what they considered the genus *Typhlomolge* may be derived from an early Tertiary invasion of the area whereas *Eurycea* arrived later (perhaps in the late Miocene or Plio-Pleistocene). According to this scenario, the extreme cave-associated morphologies of *E. rathbuni* and *E. robusta* (the two species formerly placed in *Typhlomolge*) may reflect the long period of time spent underground. The range of (less extreme) cave-associated morphologies seen in other central Texas subterranean *Eurycea* could then represent convergences with these species, varying in degree according to the length of time spent underground and constrained by the putative common ancestry of members of the Edwards Plateau *Eurycea* group exclusive of *E. rathbuni* and *E. robusta.* Potter and Sweet (1981) found that the taxa they considered *Typhlomolge* and some central Texas cave *Eurycea* exhibit the same general evolutionary trends in head form (broadening and flattening of the skull). However, Potter and Sweet (1981) demonstrated that the osteological basis of these changes differs in the two groups. Given this result, Potter and Sweet (1981) recommended that the genus *Typhlomolge* continue to be recognized, a reasonable move given the information then available.

Extreme cave-associated morphologies are seen in some other non-Texan hemidactyliine plethodontids, including *Typhlotriton spelaeus* from the Ozark region and

Haideotriton wallacei from northern Florida and Georgia. We included these taxa in the present study; both are thought to have close affinities to *Eurycea* and/or what has been considered *Typhlomolge* (Wake, 1966; Lombard and Wake, 1986). We also included representatives of all species groups currently recognized within *Eurycea.* Use of multiple outgroups is particularly important because hemidactyliine relationships are poorly understood, and the closest relatives of the central Texas group are uncertain (but see Sweet, 1977*b* for additional discussion).

Given the nature of morphological variation in the group and the potential for parallel or convergent morphological evolution, we used mainly molecular markers to investigate the evolutionary history and relationships of the central Texas hemidactyliines. Here we present the results of allozyme and mitochondrial DNA (mtDNA) sequence studies that we have used to characterize genetic variation and diversity, identify species boundaries, and reconstruct the phylogenetic history of the group. We also offer a taxonomic revision of the group and descriptions of three new species.

Materials and Methods

Sampling

Salamanders were collected from springs and caves throughout the Edwards Plateau region and returned to the laboratory alive, where they were dissected for appropriate tissues (see below) after anaesthesia with MS-222 (Sigma). Specimens were deposited into the Texas Natural History Collection (Texas Memorial Museum, University of Texas at Austin). Sampling localities are listed in Appendix I and mapped in Fig. 1A. The generalized range of members of the group with reference to counties and major cities is shown in Fig. 1B.

Outgroup Taxa

For all parsimony analyses (see below), eight outgroup taxa were used to root the trees. We chose taxa that span the range of morphological and genetic divergence in the genus *Eurycea,* plus other hemidactyliine genera that are suspected to be closely related to or nested within *Eurycea.* We based the choice of outgroup members on published morphological work (Wake, 1966; Sweet, 1977*b*; Lombard and Wake, 1986) and molecular data from a study in progress of higher-level hemidactyliine relationships (Chippindale, unpublished). The outgroup consisted of *E. bislineata* (Renfrew Co. Ont.), *E. wilderae* (Watauga Co. NC), *E. quadridigitata* (yellow-bellied form, Tyler Co. TX), *E. quadridigitata* (silver-bellied form, Charleston Co. SC), *E. l. longicauda* (Baltimore Co. MD), *E. m. multiplicata* (Polk Co. AR), *Haideotriton wallacei* (Jackson Co. FL), and *Typhlotriton spelaeus* (Stone Co. MO).

Allozyme Electrophoresis

We examined a total of 357 individuals of central Texas *Eurycea* for allozyme variation, representing 64 populations or taxa. Early in the study, we homogenized many salamanders whole in a solution of 0.001 M EDTA and 0.010 M Tris, pH 7.5, using approximately 1:1 w/v proportions of tissue to grinding solution. We subsequently found that destruction of entire specimens was unnecessary, and for later allozyme work we used a combination of skeletal muscle, heart, liver, and gut homogenized approximately 1:1 w/v in the above solution using an electric tissue grinder. Homogenates were spun for 3–5 min at about 12,000 rpm in a microcentrifuge, and 8–10 µl of the resulting supernatant was used to soak filter paper wicks for electrophoresis. Electrophoretic methods and staining procedures generally followed Murphy et al. (1996); electrophoretic conditions used for resolution of different enzyme-encoding loci are listed in Table 1. Enzyme system names and Enzyme Commission numbers follow the recommendations of the Expert Protein Analysis System, Swiss Institute of Bioinformatics (website: www.expasy.ch/enzyme/). We screened 25 loci for which banding patterns were readily interpretable and activity was strong, and rejected many others for which activity levels were highly variable among individuals, resolu-

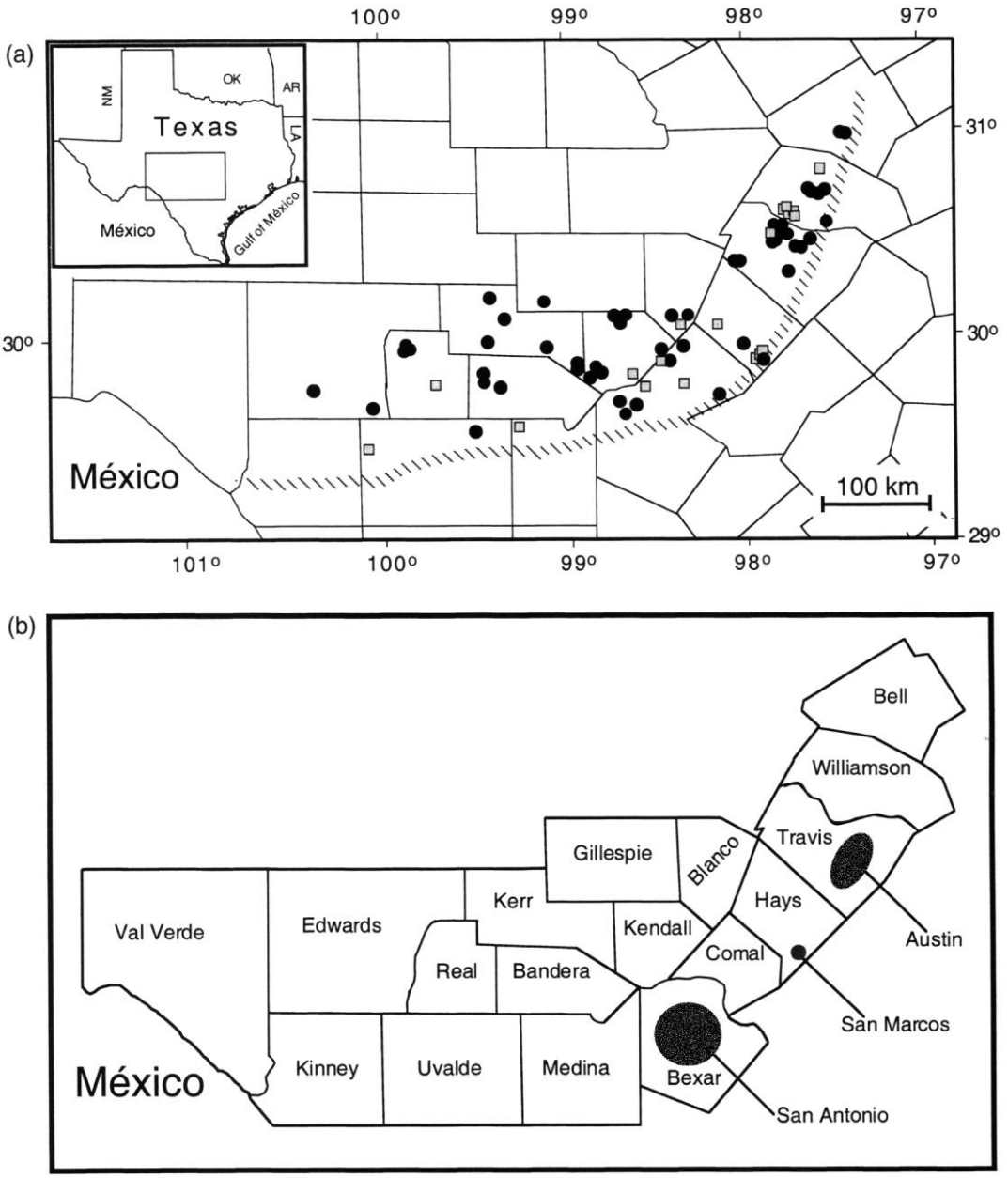

FIG. 1.—A. The Edwards Plateau region of central Texas, with collection localities for salamanders sampled in this study. Filled circles represent surface spring localities and stippled squares represent cave localities. The hatched line represents the southern and eastern margin of the Edwards Plateau (the Balcones Escarpment). Allozyme data were obtained for all localities shown, and cytochrome *b* sequence data were obtained for a subset of these localities described in the text. B. Counties in which cave and spring hemidactyliine plethodontid salamanders are known or suspected to occur; collecting localities are shown in Fig. 1A. Major cities are shown for reference and their outlines are greatly simplified.

TABLE 1.—Electrophoretic conditions used to resolve products of 25 enzyme-encoding loci in central Texas hemidactyliine plethodontid salamanders, plus outgroup taxa. Refer to Murphy et al. (1996) for composition of buffers 1, 3, 4, and 5, and Chippindale (1989) for composition of buffer 2. 1 = Tris-citrate II; 2 = "Poulik" pH 9.5; 3 = Tris-borate-EDTA pH 8.6; 4 = Tris-citrate-EDTA pH 7.0; and 5 = Tris-borate.

Enzyme system	Locus	E.C. number	Electrophoretic conditions
Aconitate hydratase	Acon-1	4.2.1.3	1
Adenylate kinase	Ak	2.7.4.3	1, 4
Aspartate aminotransferase (cytosolic)	sAat	2.6.1.1	5
Creatine kinase	Ck-1	2.7.3.2	1
Creatine kinase	Ck-2	2.7.3.2	1
Leucyl (cytosol) aminopeptidase	Cap	3.4.11.1	1
Glucose-6-phosphate isomerase	Gpi	5.3.1.9	2
Glutathione reductase	Gr	1.6.4.2	1
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	1.2.1.12	3
Glycerol-3-phosphate dehydrogenase	G3pdh	1.1.1.8	3
Isocitrate dehydrogenase (NADP+)	Idh-1	1.1.1.42	1
Isocitrate dehydrogenase (NADP+)	Idh-2	1.1.1.42	1
L-Lactate dehydrogenase	Ldh-A	1.1.1.27	5
L-Lactate dehydrogenase	Ldh-B	1.1.1.27	5
Malate dehydrogenase (NAD+)	Mdh-1	1.1.1.37	1
Malate dehydrogenase (NAD+)	Mdh-2	1.1.1.37	1
Malate dehydrogenase (oxaloacetate decarboxylating) (NADP+)	Mdhp-1	1.1.1.40	1
Mannose-6-phosphate isomerase	Mpi	5.3.1.8	2
Peptidase (cytosol nonspecific) (glycyl-L-leucine)	Pep-A	3.4.13.8	3
Tripeptide aminopeptidase (L-leucylglycylglycine)	Pep-B	3.4.11.4	1
XAA-Pro dipeptidase (L-phenylalanyl-L-proline)	Pep-D	3.4.13.9	1
Phosphoglucomutase	Pgm	5.4.2.2	3
6-Phosphogluconate dehydrogenase	Pgdh	1.1.1.44	4
Pyruvate kinase	Pk	2.7.1.40	4
Superoxide dismutase (cytosolic)	sSod	1.15.1.1	2

tion was poor, or mobility patterns were not consistently reproducible. Changes to the stain recipes described by Murphy et al. (1996) are as follows: (1) Half volumes were used for all liquid stains except Gr, G3pdh, Idh-1, Idh-2, and Pgdh, and third or quarter volumes were used for agar overlays; (2) For IDH stains, we substituted 0.08 g dry isocitric acid for the liquid form, and used pH 7.0 Tris/HCl buffer, because Idh-1 often showed little activity at pH 8.0; (3) In stains for Ak, Ck, and Pk, 200 U of hexokinase were used (rather than the recommended 20 U); (4) Glucose-1-phosphate (Sigma G-1259) was used as the Pgm substrate; (5) To stain for Gr, we used 13.0 ml of Tris/HCl pH 8.0, 0.5 ml 0.5 mg/ml DCIP, 0.002 g FAD, 0.01 g NADH, 0.02 g oxidized glutathione, and 0.5 ml 5 mg/ml MTT; and (6) To stain for sAat, we used 12.5 ml of the following stock (to which we added 12.5 ml Tris/HCl pH 8.0 and 0.05 g Fast Blue BB): 500 ml water, 0.37 g alpha-ketoglutaric acid, 1.33 g L-aspartic acid, 2.50 g polyvinyl pyrrolidone, 0.5 g Na_2EDTA, and 14.20 g Na_2HPO_4.

DNA Amplification and Sequencing

Sequence data were gathered for single individuals from 34 populations of central Texas *Eurycea,* including representatives from throughout the geographic range sampled for allozyme variation, populations that proved substantially divergent based on the allozyme data, and all described species (whether currently recognized or not) except *T. robusta,* for which the subterranean habitat is now inaccessible. We amplified a fragment of roughly 400 bp from the 5′ end of the mitochondrial cytochrome *b* (cyt *b*) gene using the

polymerase chain reaction (PCR), with the following primers (slightly modified from Moritz et al., 1992).

light strand: primer MVZ 15: GAACTA ATGGCCCACAC(AT)(AT)TACG(ACGT) AA
heavy strand: primer CB2H: CCCCTC AGAATGATATTTGTCCTCA

A map of the cyt *b* gene and the locations of these primers is provided by Moritz et al. (1992); CB2H is a truncated version of their cyt-b2 primer.

For most specimens, DNA was extracted from tail or liver tissue using the STE method described by Hillis et al. (1996); 1–2 µl of the resulting solution was then diluted in 50 µl of 1X TE for PCR. For *Eurycea troglodytes* (for which little allozyme data could be obtained, and which may now be extinct) we used supernatant from an allozyme sample prepared in the mid-1970's by S. Sweet and provided by D. Wake, and applied a modification of the Chelex extraction method (Walsh et al., 1991). This method also was used for specimens from Bat Well, Greenwood Valley Ranch Springs, and Cloud Hollow Spring, and *E. tridentifera* (Ebert Cave) and *E. latitans*. The method is as follows: Fifty µl of allozyme supernatant (*E. troglodytes*) or a tiny fragment of liver or muscle (other specimens) was added to 500 µl of an autoclaved 5% solution of Chelex-100 (Biorad) in distilled water. Samples were placed in a 55 C water bath for about 3 h, shaking occasionally. The samples were then vortexed briefly, heated to 95 C for 15 min, vortexed again, and centrifuged briefly to precipitate the Chelex.

PCR was performed using an MJ Research PTC-100 or Ericomp thermal cycler. PCR conditions that yielded the most consistent amplifications were as follows. Reactions consisted of 3–6 µl dilute DNA (for Chelex extractions, 2 µl of DNA solution plus 2 µl of a 1 in 50 dilution of Chelex solution), 0.1 µM each primer, 40 µM dNTPs, standard *Taq* polymerase buffer (1.5 mM $MgCl_2$), and 1–2 U *Taq* polymerase in a total volume of 50 µl. Temperature cycling usually used was: Step 1: 94 C 1.5 min (X 1)/ Step 2: 94 C 30 s, 50 C 30 or 45 s, 72 C 1 min (X 34)/ Step 3: 72 C 5 min (X 1).

Amplified DNA was purified using the method of Zhen and Swank (1993): 20–25 µl of the PCR sample was electrophoresed through a nonsubmerged 1.5% agarose gel and the band of interest was removed from a well cut in the gel containing 15% PEG 800 and 2X TAE. Cycle sequencing (e.g., Hillis et al., 1996) was usually used, with 3′ ^{32}P end-labelled primers. Reactions were electrophoresed through standard 6% acrylamide DNA sequencing gels. Gels were dried into Whatman 3M filter paper without fixation and exposed to Kodak X-Omat AR or Biomax MR film for 1–4 days at room temperature without intensifying screens. Details of sequencing procedures are given by Chippindale (1995).

We sequenced each sample using both MVZ 15 and CB2H as sequencing primers, with substantial overlap (typically 100–200 bp) in the middle region of the fragment for most samples. Some samples proved difficult to sequence using MVZ 15, so we also designed an internal sequencing primer: 5′TC(ACT)TTTATTGA (CT)CTCCCAGC 3′. Nucleotide sequences were unambiguously alignable by eye, and no insertions or deletions were observed.

To confirm that we were working with the mitochondrial cyt *b* gene, we purified mtDNA from a specimen from the Sutherland Hollow population and compared its cyt *b* sequence to that of another individual from the same population for which total cellular DNA was used. The sequences were identical for all readable base pairs.

Cyt *b* sequences were deposited in GenBank (accession numbers AF252340–252380).

Assessment of Levels of Variation and Cluster Analyses

An IBM PC version of Swofford and Selander's (1981) Biosys-1 program was used to calculate measures of allozyme variation and genetic distances, and to assess overall divergence based on allozymes. To assess deviations from Hardy-Weinberg equilibrium, we used chi-square tests and applied

Levene's (1949) correction for small sample size. We treated almost all localities as separate OTUs for similarity clustering. However, we combined several localities that were geographically proximal and identical or near-identical in allelic composition and frequency as single units to yield a total of 59 "populations". These were: Barrow Hollow + Stillhouse Hollow Springs; Knight + Cedar Breaks Springs; Pedernales Spring 1 + Spring 2; Murphy's Spring + Sabinal Canyon Spring; Greenwood Valley Ranch Springs 1 + 2 + 3; Cherry Creek Spring + Cloud Hollow Spring; and *E. rathbuni* from Ezell's Cave + Rattlesnake Cave + Diversion Spring.

No activity was observed for the Gr locus in any of the five *E. rathbuni* screened for allozyme variation, nor could we detect activity in any of the six Greenwood Valley Ranch Springs individuals for Mdh-2. For all analyses, we treated these individuals as homozygous for unique "null" alleles at these loci. In doing so we assumed that individuals in these populations possess a unique form of each enzyme, and that the differences have a genetic basis.

Overall divergence based on allozyme data was assessed using UPGMA (e.g., Sneath and Sokal, 1973) with Manhattan (Prevosti) distances (e.g., Wright, 1978); we also used Nei's (1978) unbiased distance and Rogers' (1972) distance for comparison. To depict relative levels of sequence divergence among central Texas *Eurycea,* we calculated uncorrected sequence divergences among populations and taxa and performed neighbor-joining cluster analysis using PAUP* v. 4.0b2 (Swofford, 1999).

Parsimony Analyses

For parsimony analyses of allozyme data, we employed a method of frequency-based coding that involved treatment of each different observed array of allele frequencies for a given locus (character) as a unique state. Manhattan distances (*D*) among states were calculated using Biosys-1 (Swofford and Selander, 1981). These distances were then converted to whole numbers (we rounded to two digits) and used as the numbers of steps among states in a step matrix, implemented using PAUP* (version 4.0.d055 and 4.0b3; Swofford, 1998, 1999). Berlocher and Swofford (1997) and Wiens (1995, 1999) provided a detailed description of this method.

TABLE 2.—Occurrence of alternative nucleotide combinations in partial mitochondrial cytochrome *b* sequence, summarized by codon position. Values in cells represent total number of observations of combinations of alternative nucleotides (or invariant nucleotides) summed across the indicated positions.

Nucleotide combination	Codon position		
	1	2	3
A	24	21	15
C	16	25	4
G	25	18	0
T	31	41	4
AC	3	0	0
AG	4	2	11
AT	1	1	1
CG	0	0	0
CT	8	6	48
GT	0	0	0
ACG	1	1	4
ACT	1	0	15
AGT	1	0	7
ACGT	0	0	7

For parsimony analysis of sequence data, we partitioned characters into first, second, and third codon positions, followed by combinatorial weighting of changes among bases using Wheeler's (1990) method with Rodrigo's (1992) correction for invariant positions. Values in the resulting three transformation matrices were used as the numbers of steps among alternative bases in three PAUP* step matrices (corresponding to first, second, and third codon positions). Frequencies of alternative nucleotide combinations are shown in Table 2 and transformation matrices in Table 3. This approach allowed incorporation of information on the frequency and direction of different kinds of changes, based on observed patterns in the data set. Each step matrix was rendered symmetric by averaging across the diagonal to minimize effects of sampling error with respect to estimates of change frequency (see Swofford et al., 1996). For parsimony analyses using combined allozyme and sequence data, we used the allozyme characters, coded as described

TABLE 3.—Transformation matrices calculated from the observed nucleotide changes, scaled to a maximum of 100 steps for the rarest changes (see text for details).

	Codon position 1				Codon position 2					Codon position 3				
	A	C	G	T		A	C	G	T		A	C	G	T
A	—	50	46	70	A	—	100	58	100	A	—	64	29	65
C	78	—	99	39	C	100	—	100	54	C	100	—	100	17
G	70	100	—	100	G	62	100	—	i	G	80	100	—	100
T	100	32	100	—	T	89	44	i	—	T	89	13	84	—

above, plus the sequence data partitioned by codon position with the application of combinatorial weights. For these analyses, we scaled the DNA step matrix values to 100 for equivalence with changes at each allozyme locus (because use of the Manhattan distance approach for allozymes allowed up to 100 "steps", or distance units, between states, i.e., alternative allele frequency arrays).

We conducted heuristic parsimony searches using three treatments of the data: 1) allozyme data only; 2) sequence data only; and 3) allozyme plus sequence data. Each involved 50 random-taxon-addition replicates to reduce the chances of recovering only trees within a particular "tree island" (Maddison, 1991). We assessed phylogenetic confidence in particular nodes through use of nonparametric bootstrapping (Felsenstein, 1985), implemented using PAUP*. One hundred heuristic bootstrap pseudoreplicates were conducted for each data treatment; bootstrapping for data treatment 3 involved 10 random-taxon-addition sequence replicates per bootstrap pseudoreplicate, but this was not feasible for data treatments 1 and 2 so only a single taxon-addition-sequence was used within each pseudoreplicate for these analyses.

Population Groups

The large number of populations included in the allozyme portion of this study made it impractical to include each as a separate unit in parsimony analyses, and sequencing of representatives from all populations was impractical. To reduce the number of working units, we constructed 24 population groups based on consideration of geographic location and proximity plus similarity in allelic composition and frequency. Membership of these groups is shown in Figs. 2 and 3, and localities are listed in Appendix I. Although most of these groups likely represent real evolutionary units (species or monophyletic groups of species), several are somewhat arbitrary. Especially problematic assemblages of populations are the Carson Cave group and the *E. latitans* complex. These issues, and the potential impact of the grouping approach on the analyses, are addressed below (see Discussion).

Osteological Examination, Measurements, and Morphometric Analyses

Osteological variation among the three new species of *Eurycea* that we describe here was examined following clearing-and-staining of specimens using the method of Dingerkus and Uhler (1977). Specimens examined are listed below. For morphometric analyses, we used the following measurements of external morphology: AG (axilla-groin length), ALL (anterior limb length, from anterior insertion to tip of third toe), ED (eye diameter, measured as anterior-posterior diameter of the externally visible dark disc of the eye), HLA (head length A, distance from tip of snout to center of gular fold), HLB (head length B, distance from posterior margin of eye to posteriormost gill insertion), HLC (head length C, distance from tip of snout to posteriormost gill insertion), HLL (hind limb length, from groin to tip of third toe), HW (head width immediately posterior to jaw articulation), IOD (interocular distance), SL (standard length, distance from tip of snout to posterior margin of vent), and TL (tail length, from posterior margin of vent to tip). ED was measured at ×64 magnification with backlighting using an ocular micrometer, AG, SL, and TL were

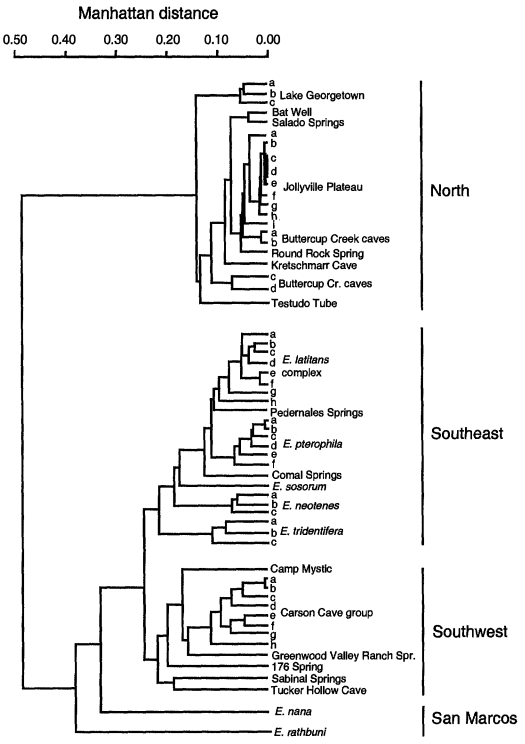

FIG. 2.—Similarity (UPGMA) clustering of central Texas hemidactyliine plethodontid salamanders based on allozyme Manhattan distances. Major geographic regions are indicated. Within groups, populations are as follows. Lake Georgetown group: a = Avant's Spring, b = Knight and Cedar Breaks Springs, c = Buford Hollow Spring; Jollyville Plateau group: a = Balcones Park Spring, b = Barrow Hollow and Stillhouse Hollow Springs, c = Horsethief Hollow Spring, d = New Bull Creek Spring, e = Schlumberger Spring, f = Wheless Spring, g = Hanks' Spring, h = Canyon Creek Spring, i = Canyon Vista Spring; Buttercup Creek Caves group: a = Ilex Cave, b = Buttercup Creek Cave, c = T.W.A.S.A. Cave, d = Treehouse Cave; *E. latitans* complex: a = Bear Creek Spring, b = Cibolo Creek Spring, c = Honey Creek Cave Spring, d = Kneedeep Cave Spring, e = Cherry Creek and Cloud Hollow Springs, f = Less Ranch Spring, g = Rebecca Creek Spring, h = Pfeiffer's Water Cave; *E. pterophila*: a = Boardhouse Springs, b = Peavey's Springs, c = Zercher Spring, d = Fern Bank Spring, e = T Cave, f = Grapevine Cave; *E. neotenes*: a = Helotes Creek Spring, b = Leon Springs, c = Mueller's Spring; *E. tridentifera*: a = Honey Creek Cave, b = Ebert Cave, c = Badweather Pit; Carson Cave group: a = Carson Cave, b = West Nueces Spring, c = Sutherland Hollow Spring, d = Smith's Spring, e = Robinson Creek Spring, f = WB Spring, g = Trough Spring, h = Fessenden Springs.

measured with digital calipers, and the remaining measures were taken at ×7.5 magnification with an ocular micrometer. Specimens measured were the following (all are from the Texas Natural History Collection, University of Texas at Austin). *Eurycea tonkawae,* Jollyville Plateau/Brushy Creek drainage: Balcones Park Spring (TNHC 55132–134); Barrow Hollow Spring (TNHC 50933, 50938); Brushy Creek Spring (TNHC 50988, 54225–226, Canyon Creek Spring (TNHC 55141–144); Krienke Spring (TNHC 53466, 53469, 53472, 53475–476); Schlumberger Spring (TNHC 50985–986); Stillhouse Hollow Spring (TNHC 50947, 50950, 50952, 50956–957); Wheless Tract Spring (TNHC 55150, 55152, 55155–157. *Eurycea naufragia,* Georgetown area: Avant's Spring (TNHC 51027–029); Buford Hollow Spring (TNHC 51008, 51013–014, 58860–861; Cedar Breaks Hiking Trail Spring (TNHC 51000–002). *Eurycea chisholmensis,* Salado Springs: TNHC 51139–142, 51143 (TL not measured), 51144 (HLA not measured), 51145–146, 58859. Summaries of measurements are given in Table 4.

We assessed the contributions of different variables to total morphometric variance among individuals using principal components analysis (PCA) implemented via STATISTICA v. 4.5 (1993, Statsoft, Tulsa, OK); each variable was subjected to a $\log_{10}$ transformation prior to analysis. We then used a one-way analysis of variance of individual scores on factor 2 to assess differences among the three new species we describe here, and performed a post hoc Scheffe test (Sokal and Rohlf, 1981) for significance of differences between species.

RESULTS

Intrapopulation Allozyme Variation

Twenty-two of the 25 allozyme loci examined displayed polymorphism among and/or within populations of central Texas hemidactyliines (Appendix II). However, levels of intrapopulation genetic variation generally were low. Direct-count heterozygosity (H) ranged from 0% in several

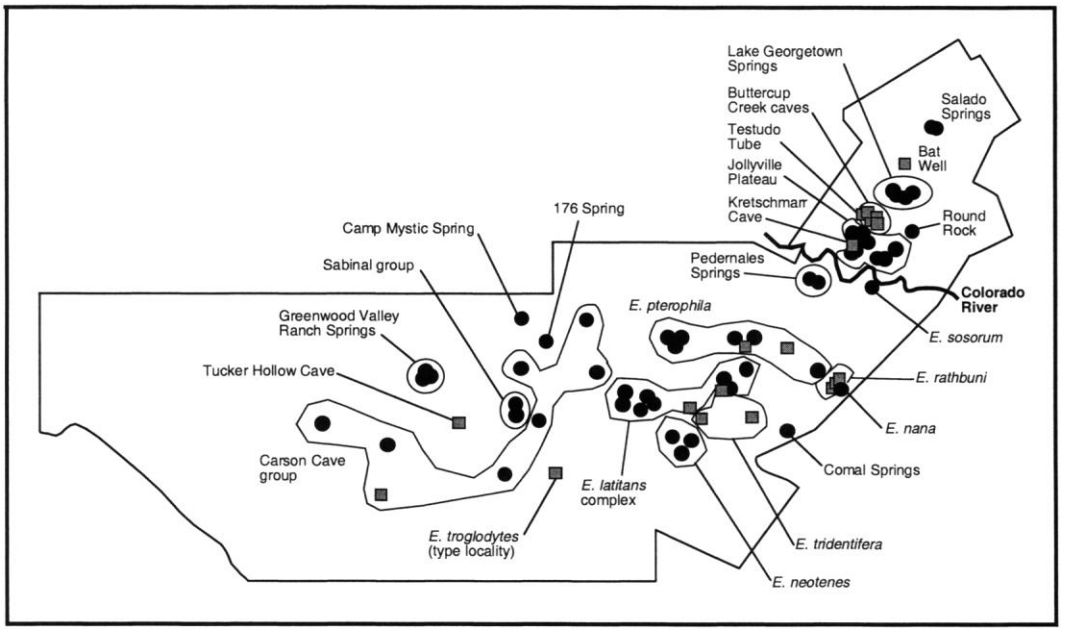

FIG. 3.—Population groups to which central Texas *Eurycea* were assigned for DNA sampling and parsimony analysis; these assignments were based largely on initial allozyme studies, the results of which are summarized in Fig. 2. The region outlined corresponds to the counties in which these salamanders are known to occur, detailed in Fig. 1. Filled circles represent surface spring localities and stippled squares represent cave localities.

populations to 12.0% in the T.W.A.S.A. Cave population (represented by a single specimen); the next highest observed *H* was 10.8% in *Eurycea tridentifera* from Badweather Pit (five specimens examined). Mean *H* across all populations was 2.92% (S.E. = 0.042). The percentage of polymorphic loci (*P*) ranged from 0% in several populations to 32% in *E. sosorum* with a mean of 9.69% (SE = 0.128), and the average number of alleles per locus (*A*) ranged from 1.0 to 1.2 with a mean of 1.09 (SE = 0.001). Sixteen significant deviations from Hardy-Weinberg equilibrium were detected (chi-square test, $P < 0.05$), of a total of 1600 populations × loci examined. There were 145 cases in which a locus exhibited intrapopulation variation. Using this value to conduct sequential Bonferroni tests (Rice, 1989), at most four deviations were significant.

TABLE 4.—Summary statistics for measurements of north central Texas species of *Eurycea* used in morphometric analyses. Sample sizes are in parentheses following species names; values given are mean (in mm) ± 1 standard deviation.

Variable	Species		
	E. tonkawae (N = 29)	*E. naufragia* (N = 13)	*E. chisholmensis* (N = 7)
SL	30.49 (3.29)	28.97 (4.49)	32.93 (2.53)
AG	16.44 (2.09)	15.77 (2.87)	18.17 (1.84)
TL	23.67 (3.94)	21.04 (4.84)	24.58 (3.54)
HLA	6.26 (0.76)	5.90 (0.71)	6.48 (0.67)
HLB	5.13 (0.52)	5.17 (0.54)	5.79 (0.68)
HLC	8.08 (0.78)	8.16 (0.84)	8.90 (0.99)
HW	4.67 (0.49)	4.50 (0.52)	5.01 (0.76)
IOD	1.47 (0.26)	1.47 (0.19)	1.73 (0.27)
HLL	6.30 (0.76)	6.39 (0.59)	7.20 (0.67)
ALL	5.60 (0.71)	5.33 (0.47)	6.25 (0.55)
ED	1.31 (0.10)	1.23 (0.16)	0.98 (0.14)

Interpopulation Allozyme Variation

The UPGMA tree constructed from Manhattan distances (Fig. 2) reveals a high degree of genetic differentiation among some populations and groups of populations. In particular, members of the "northern" group (populations from northeast of the Colorado River in Travis, Williamson, and Bell counties) are extremely

divergent from all other central Texas *Eurycea*. Average Manhattan *D* between the northern and other populations exceed 0.45, which correspond here to average Nei's (1978) *D* over 0.65 and Rogers's (1972) *D* over 0.45. These distances reflect numerous differences in allelic composition from all other central Texas *Eurycea* examined; most of these differences are fully or nearly fixed, or mutually exclusive. South of the Colorado River, *E. rathbuni* from the San Marcos region appears as the next most divergent member of the group with a Manhattan *D* exceeding 0.40 from all other taxa examined, and *E. nana* (also from the San Marcos area) is next most divergent (Manhattan *D* over 0.30). Of the remaining populations, there is a division between a "southeastern group" (all populations east of extreme eastern Kerr Co., corresponding primarily to the southeastern drainages of the Edwards Plateau) and a "southwestern group" (corresponding primarily to southwestern drainages of the plateau). While *E. nana* and *E. rathbuni* each possess unique alleles at several loci (Appendix II), differentiation among members of the southeastern and southwestern groups primarily is based on allele frequency variation. UPGMA trees constructed using Nei's (1978) unbiased distance and Rogers's (1972) distance had topologies nearly identical to that of the Manhattan *D* tree except at the very lowest clustering levels, and are not shown here. Major groups based on similarity analyses of allozyme data are shown in Fig. 3.

Levels and Patterns of Sequence Variation

Across the maximum 356 bp of cyt *b* sequenced, 133 sites (37.5%) were variable including outgroup taxa; within the ingroup 101 sites (28.5%) varied (aligned sequences are shown in Appendix III). Of the 118 codons examined, 15 (12.7%) exhibited amino acid variation considering all taxa; excluding the outgroup, 13 (11.0%) were variable. Base composition of the light strand (G = 0.16, A = 0.26, T = 0.36, C = 0.23) was very similar to that reported for plethodontid salamanders of the genus *Ensatina* by Moritz et al. (1992). The neighbor-joining tree based on uncorrected percent sequence divergence is shown in Fig. 4. In most respects, the major patterns of divergence seen in the allozyme-based cluster analysis also occur in the DNA-based cluster analysis. The northern populations are strongly differentiated from all others, exhibiting over 14% uncorrected sequence divergence on average (Fig. 4). As in the allozyme-based cluster analysis, *Eurycea rathbuni* appears as next most distinct, with an average sequence divergence of approximately 9% from other non-northern populations. The same division between southeastern and southwestern populations (exclusive of *E. rathbuni*) occurs as in the allozyme-based cluster analysis, with average sequence divergences over 7%. A key difference between the allozyme tree and that based on sequence data is that in the DNA-based tree, *E. nana* appears with the southeastern group, whereas based on allozymes it is strongly differentiated and appears outside all other non-northern populations except *E. rathbuni*. Most allozyme differences represent autapomorphies for *E. nana* and thus are not informative in a parsimony context (see below). The other major difference between the allozyme cluster analysis tree and that based on DNA is that the DNA tree reflects the extremely low levels of sequence variation among most taxa in the southeastern region. In contrast, substantial allozyme variation is present in the southeastern group (Fig. 2 and Appendix II).

Geographic Patterns of Genetic Differentiation

Cluster analyses of both the allozyme and sequence data identify several major groups of populations, most of which correspond to geographically circumscribed regions of the Edwards Plateau area: a northern group, a southeastern group, a southwestern group, *Eurycea rathbuni* from San Marcos, and *E. nana* from San Marcos (*E. nana* is strongly differentiated based on allozymes only). The latter four, all from southwest of the Colorado River, will be referred to collectively here as the "southern group". The named taxa *E. neo-*

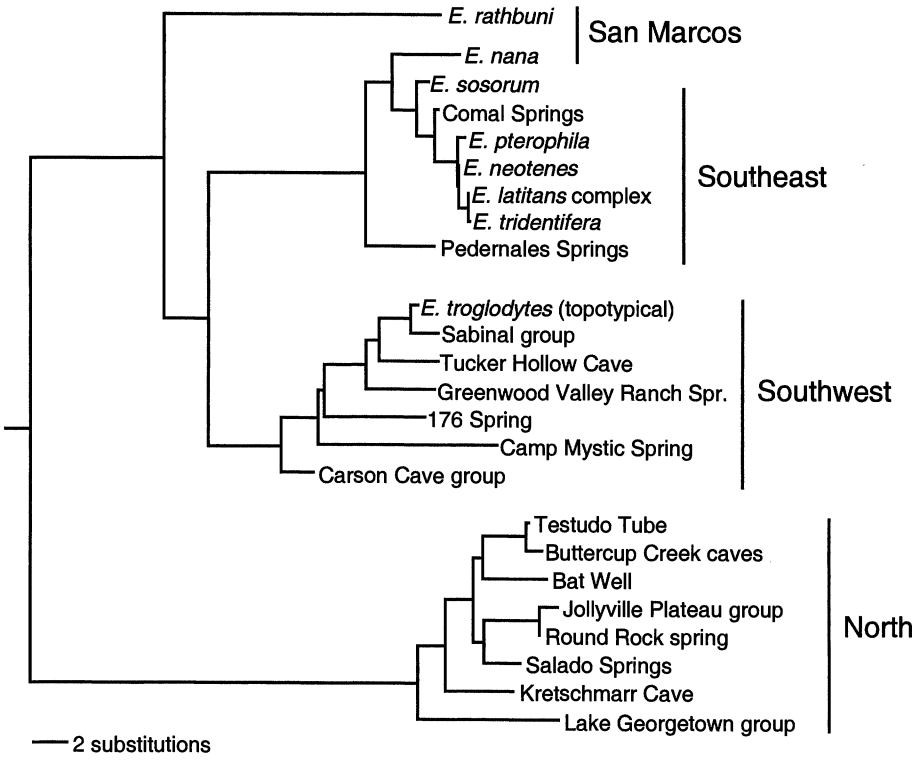

FIG. 4.—Neighbor-joining tree for central Texas hemidactyliine plethodontid salamanders based on uncorrected cytochrome *b* sequence divergence. Rooting is based on positions of outgroup taxa (included in analysis but not shown). Species and informal groups correspond to those listed in text and shown in Figs. 2 and 3. Major geographic regions are indicated.

tenes, E. latitans, E. pterophila, E. sosorum, and *E. tridentifera* all cluster within the southeastern region based on allozymes and DNA. The only remaining named taxon (exclusive of *E. rathbuni* and *E. nana*) is *E. troglodytes,* which clusters with members of the southwestern group based on sequence data (allozyme data were not available for this population). Cave populations from the northern, southeastern, and southwestern regions all cluster with spring populations in the same regions based on both allozymes and DNA, regardless of their degree of morphological divergence. The only exception is *E. rathbuni,* which is strongly differentiated both morphologically and based on allozymes and DNA from all other members of the southern assemblage.

We found no evidence that the cave-dwelling taxa *E. latitans* and *E. troglodytes* are of hybrid origin. None of the five *E. latitans* examined for allozyme variation displayed a unique Mdhp-1 allele that appears fixed or at very high frequency in the three populations of *E. tridentifera* that we examined (Appendix II). As described above, analyses of sequence data place topotypical *E. troglodytes* with populations in the southwestern region and do not support a close relationship between this population and the southeastern *E. tridentifera* (but see Discussion for caveats regarding use of mitochondrial sequence data).

Parsimony Analyses

Results of parsimony analyses are shown in Figs. 5–7. Analysis of allozyme data alone yielded three equally parsimonious trees of length 138.52 (we have divided the number of steps in each tree by 100, so that the tree lengths reflect the number of character changes, i.e., a fixed differ-

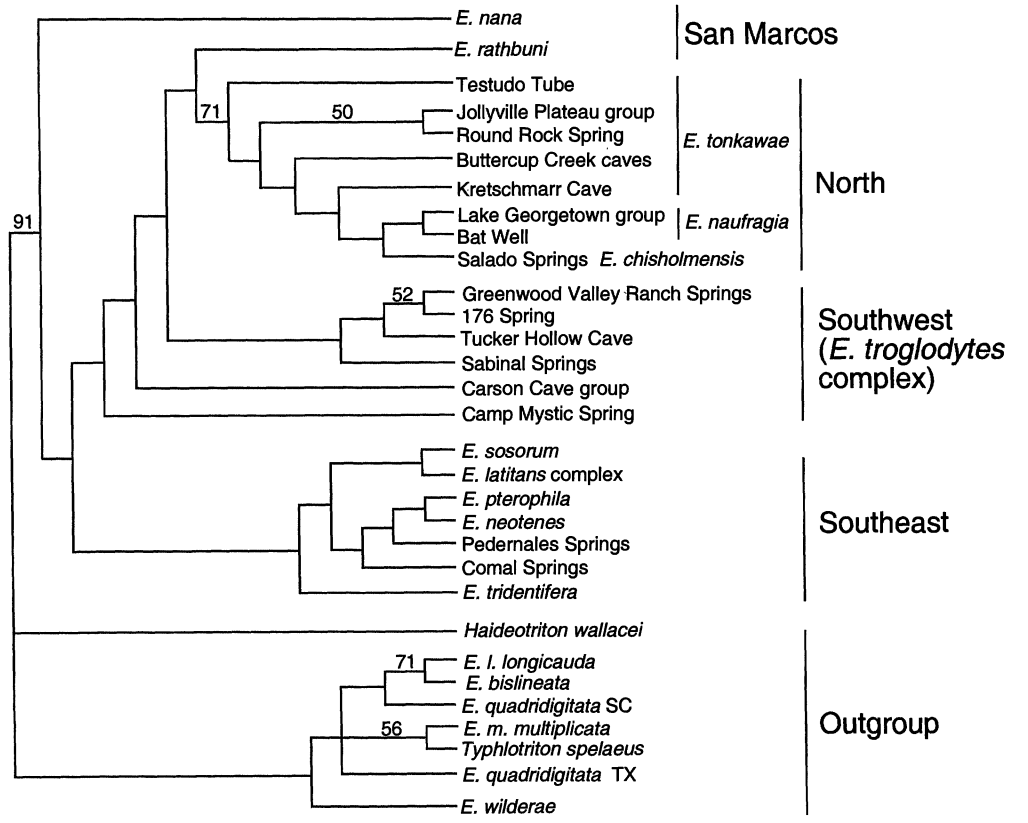

FIG. 5.—Strict consensus of the three equally parsimonious trees for central Texas hemidactyliine plethodontid salamanders that resulted from a heuristic search (with 50 random taxon addition sequence replicates), using allozyme data alone with Manhattan distance step matrices. Bootstrap support (100 pseudoreplicates) is shown for nodes with 50% or greater support based on a separate nonparametric bootstrap analysis.

ence at one locus would result in one step between two taxa). The strict consensus of these trees is shown in Fig. 5. Analysis of cyt *b* sequence data alone (with combinatorial weighting and partitioning by codon position) yielded 90 equally parsimonious trees of length 137.38; the strict consensus is shown in Fig. 6. The single most-parsimonious tree (length 285.24) that resulted from combined analysis of the allozyme and sequence data is shown in Fig. 7.

Results of all three parsimony analyses shown here are consistent with monophyly of the central Texas hemidactyliines. The lowest bootstrap value for the node uniting the Texas hemidactyliines is 59% using sequence data alone; using allozymes alone the bootstrap value for this node is 91%, and the combined analysis yields a value of 98%.

All parsimony analyses support monophyly of the northern group of populations. The weakest support occurs in the allozymes-only trees, with a bootstrap value of 71% (Fig. 5). However, support for all other nodes except ingroup monophyly is weak using the allozyme data alone. Members of the northern group not only are characterized by diagnostic alleles at several allozyme loci and unique sequence substitutions, but also by unique amino acid substitutions at four codons. Unambiguous and potential molecular synapomorphies for this and the other groups recognized here are listed in detail by Chippindale (1995) and summarized in the species accounts given in the Discussion.

The southern group appears as monophyletic in both analyses that include the

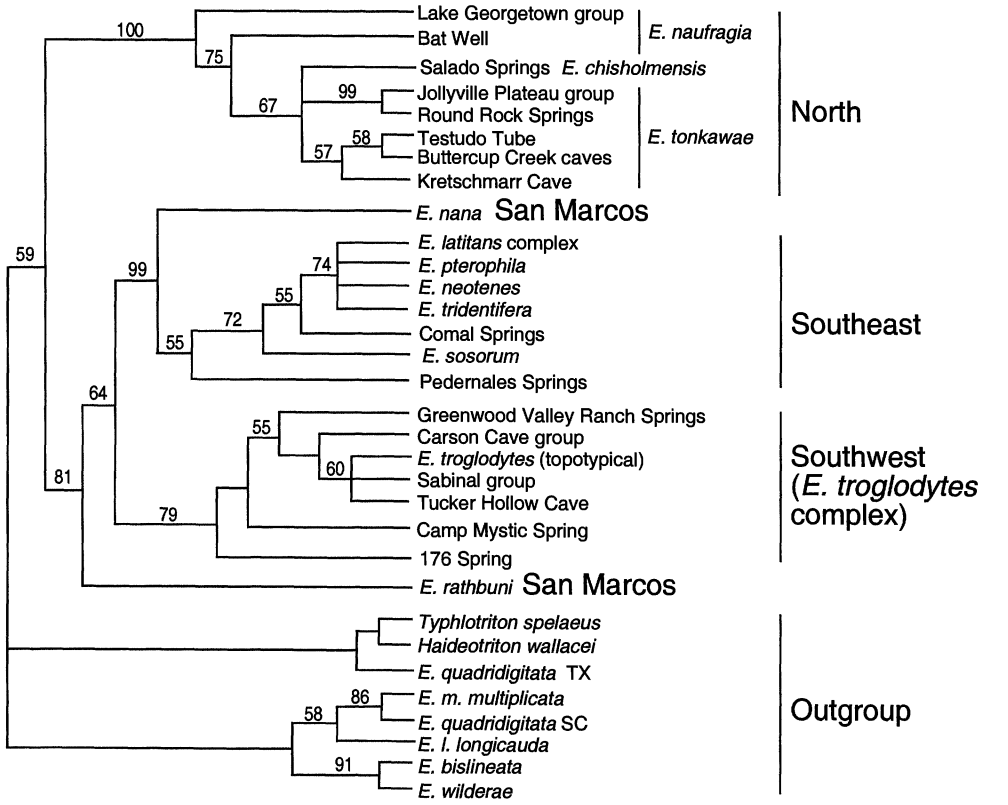

FIG. 6.—Strict consensus of the 90 equally parsimonious trees for central Texas hemidactyliine plethodontid salamanders that resulted from a heuristic search (with 50 random taxon addition sequence replicates), using cytochrome *b* data alone with partitioning by codon position and combinatorial weighting. Bootstrap support (100 pseudoreplicates) is shown for nodes with 50% or greater support based on a separate nonparametric bootstrap analysis.

sequence data, but not in the tree based on allozyme data alone (Fig. 5). This analysis places *Eurycea rathbuni* as the sister lineage to the northern group, with members of the southwestern group appearing successively sister to this grouping (rendering the southwestern group nonmonophyletic). A monophyletic southeastern group is sister to this grouping, and *E. nana* appears as sister to all other central Texas hemidactyliines. Support for these relationships is weak (bootstrap values below 50% for almost all relevant nodes), indicating little allozyme character support for any relationships except monophyly of all central Texas hemidactyliines and monophyly of the northern group. Use of the sequence data alone (Fig. 6) provides relatively strong support for monophyly of the southern group inclusive of *E. rathbuni* (bootstrap value 81%), and combination of the allozyme and sequence data provides even stronger support (90%). *Eurycea rathbuni* appears as the sister lineage to all other southern taxa in both analyses that include the sequence data.

The southeastern populations plus *Eurycea nana* appear as a well-supported monophyletic group in the analyses that include the sequence data (bootstrap values for this node are 99% based on sequence alone and 97% based on the combined data). Using allozymes alone, the southeastern clade is present but the position of *E. nana* is problematic, as explained above. Similarly, relationships of the southwestern populations are poorly supported (and the group is not monophyletic) based on allozymes alone, but they appear as a well-supported monophyletic

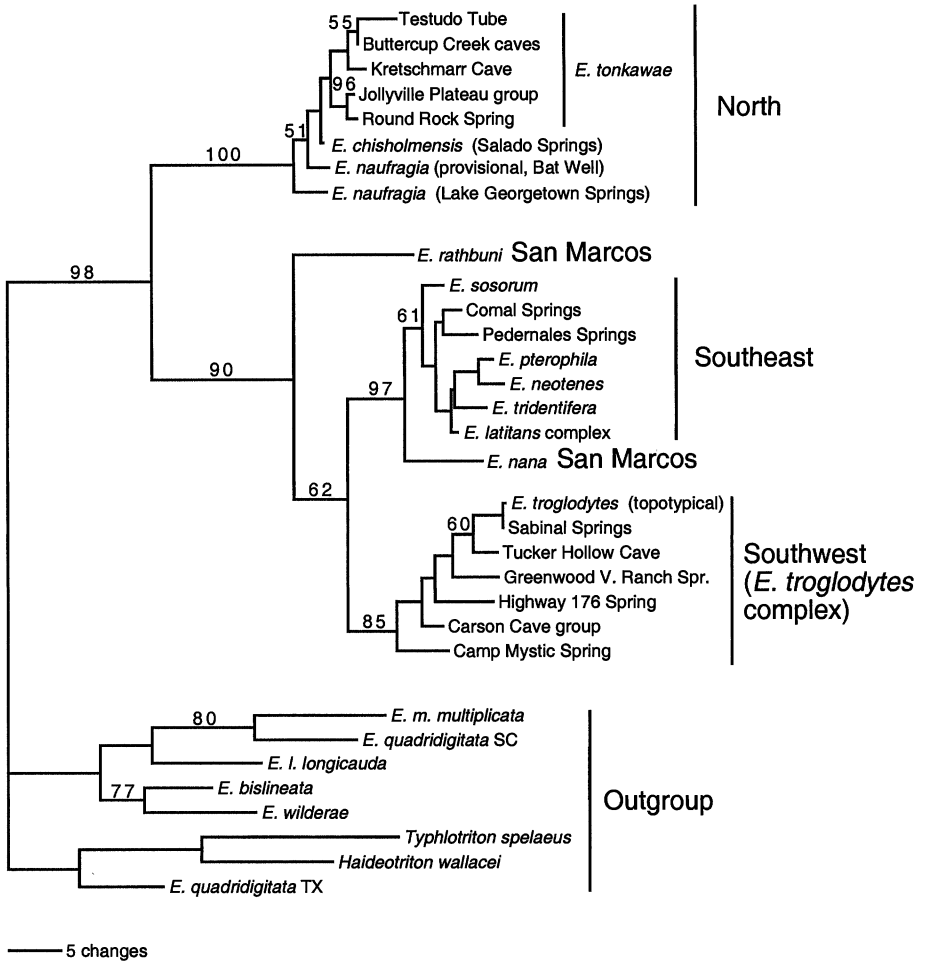

FIG. 7.—The single most parsimonious tree that resulted from a heuristic search (with 50 random taxon addition sequence replicates) of combined allozyme and cytochrome *b* sequence data for central Texas hemidactyliine plethodontid salamanders (allozymes were coded using Manhattan distance step matrices and cytochrome *b* sequence data with partitioning by codon position and combinatorial weighting). Relative branch lengths are shown. Bootstrap support (100 pseudoreplicates) is shown above nodes with 50% or greater support based on a separate nonparametric bootstrap analysis.

group in the DNA-only (bootstrap 79%) and combined (85%) analyses. Both analyses that include the sequence data support a sister-group relationship between the southeastern and southwestern groups (bootstrap 64% for DNA only and 62% for combined data).

Biogeographic and Taxonomic Patterns Based on Parsimony Analyses

Our preferred phylogenetic hypothesis for the central Texas hemidactyliines (that based on the combined allozyme and cyt *b* sequence data; Fig. 7) divides them into a monophyletic "northern" clade from northeast of the Colorado River, and a "southern" clade from southwest of the Colorado River. The southern clade is composed of *Eurycea rathbuni* from San Marcos, a "southeastern" subgroup corresponding primarily to the eastern drainages on the southern portion of the Edwards Plateau exclusive of the San Marcos region (*E. nana* from San Marcos appears to be the sister lineage to this group), and a "southwestern" subgroup generally cor-

TABLE 5.—Factor loadings and coefficients resulting from principal components analysis of external measurement variables for northern central Texas *Eurycea*. Two factors were extracted; the first explains 65.3% of the total variance, and the second explains 12.0%. Values shown are for unrotated axes, and have been rounded to three decimal places.

	Factor 1		Factor 2	
Variable	Loading	Coefficient	Loading	Coefficient
SL	−0.920	−0.128	−0.209	−0.158
AG	−0.862	−0.120	−0.298	−0.226
TL	−0.741	−0.103	−0.371	−0.280
HLA	−0.885	−0.123	−0.026	−0.020
HLB	−0.855	−0.119	0.275	0.208
HLC	−0.887	−0.124	0.184	0.139
HW	−0.857	−0.119	0.076	0.057
IOD	−0.774	−0.108	0.351	0.265
HLL	−0.808	−0.113	0.107	0.081
ALL	−0.857	−0.119	0.037	0.028
ED	−0.118	−0.016	−0.895	−0.677

responding to the western drainages in the southern portion of the Edwards Plateau region. This pattern of phylogenetic relationships is consistent in most ways with the pattern of similarities revealed by the cluster analyses of both allozyme and sequence data (except for the placement of *E. nana* based on the allozyme data).

Relationships of Outgroup Taxa

Among the outgroup taxa used, there was relatively little consistency in inferred relationships among analyses. With the exception of the allozymes-only analyses, there generally was strong support for a sister-group relationship between *Eurycea bislineata* and *E. wilderae,* consistent with previous inferences that these taxa are part of a distinct group within *Eurycea* (e.g., Jacobs, 1987). *Eurycea quadridigitata* from Texas and *E. quadridigitata* from South Carolina never clustered together, suggesting that this nominal taxon may be composed of at least two species. Depending on the analysis, the monotypic cave-dwelling genera *Haideotriton* and *Typhlotriton* occur in various places in the tree, but were nearly always imbedded within the genus *Eurycea.*

Morphometric Analyses

PCA extracted two factors. Loadings and coefficients for each variable on each factor are listed in Table 5. The first accounts for 65.3% of the total variance among individuals, and appears to be based primarily on body size. The second factor explains another 12.0% of the total variance, and appears to be based primarily on eye diameter. A bivariate plot of individual factor scores (not shown) demonstrated wide overlap between two of the three new species that we describe here (those from the Jollyville Plateau and Georgetown areas), whereas most individuals from the Salado population occupied a distinct region of factor space. For simplicity, we show a bivariate plot of ED vs. AG in the species description for *Eurycea chisholmensis* (below). This includes two additional animals (TNHC 51143 and 51144) that were not included in the factor analysis because some measurements could not be taken.

ANOVA revealed significant differences among the three new species that we describe here, based on individual scores on factor 2 (F-test, df = 46, $P < 0.0001$). Pairwise comparisons between species, using a post-hoc Scheffe test, revealed that *Eurycea chisholmensis* from Salado Springs differed significantly from *E. tonkawae* Jollyville Plateau/Brushy Creek drainage ($P < 0.0001$) and *E. naufragia* Georgetown ($P < 0.05$), whereas the latter two species were not significantly differentiated from each other based on morphometric proportions alone.

DISCUSSION

Intrapopulation Allozyme Variation

Shaffer and Breden (1989) found that nontransforming salamanders usually ex-

hibit lower levels of genetic variation (as measured by allozyme markers) than do transforming species. Our results for most populations of central Texas hemidactyliines are consistent with this observation, given the mean heterozygosity (H) across all populations of 2.9% (however, there are several notable exceptions, particularly among cave-dwellers; see Appendix II and below). Shaffer and Breden (1989) suggested that this pattern may be due in part to the ephemeral nature of the habitats of many nontransforming species: perennibranchiate salamanders often inhabit bodies of water in areas that are relatively hot and arid, and periodic drying of these aquatic habitats may result in genetic bottlenecks. Our field observations (see Chippindale, 1995) and those of Hamilton (1973) and Sweet (1977*b*) support this hypothesis for at least some populations of spring-dwelling central Texas hemidactyliines.

Sweet (1978*a*) suggested that many populations of cave hemidactyliines in central Texas may have originated from spring-dwellers that followed the water column underground when it dropped because of drought, or when erosion led to stream capture. Some of the highest levels of heterozygosity observed were among cave dwellers from subterranean aquatic systems that likely are relatively extensive (especially *Eurycea tridentifera* from caves of the Cibolo Sinkhole Plain in Comal, Kendall, and Bexar counties); perhaps these systems support large numbers of individuals and/or are buffered against the drastic changes in water availability to which surface populations are subject. However, the relationship between heterozygosity and habitat type is far from clear-cut: a few spring populations exhibit relatively high levels of heterozygosity, and even within a given cave system, estimates of heterozyosity can vary widely from site to site (e.g., for *E. tridentifera* from Honey Creek Cave vs. Badweather Pit, and for the various populations of *E. tonkawae* from the Buttercup Creek Cave system in Williamson Co.).

Interpopulation Sequence Differentiation and Variability of Allozymes Versus Mitochondrial DNA

The levels of nucleotide variation seen for cyt *b* here are very similar to those reported by Moritz et al. (1992) for plethodontine plethodontids of the genus *Ensatina* plus the outgroups *Aneides* and *Plethodon*. Including outgroups, we found 37.5% of the 356 positions surveyed variable (28.5% for ingroup members only), while Moritz et al. (1992) reported 37.0% of positions variable for the 681 bp they sequenced across all *Ensatina, Plethodon,* and *Aneides* they examined. Moritz et al. (1992) found almost twice the amino acid sequence variation in their study as did we (22.0% of amino acids variable including ingroup and outgroups, compared to 12.7% here with outgroups and 11.0% without). The reason for the apparently greater level of conservation in hemidactyliine cyt *b* amino acid sequences is unknown.

On a broad scale, the patterns of geographic variation exhibited by both allozymes and cyt *b* sequences are similar. However, much more variation was revealed by allozymes than cyt *b* sequences in the southeastern region, where most populations and taxa exhibited identical or near-identical sequences. This observation demonstrates that even a mitochondrial gene (cyt *b*) generally regarded as fairly fast-evolving (e.g., Graybeal, 1993; Meyer, 1994; Johns and Avise, 1998) may fail at some level to exhibit useful variation, while nuclear allozyme markers continue to be informative. Although some studies of genetic variation in salamanders have found the opposite (i.e., substantial mitochondrial variation but low levels of allozyme variation; see Routman, 1993*a* regarding *Cryptobranchus* and Routman, 1993*b* regarding *Ambystoma*), McKnight et al. (1991) described a situation similar to that in the Texas *Eurycea* for the plethodontid *Phaeognathus hubrichti*. Based on a finding of substantial allozyme variation and low mitochondrial restriction site variation, McKnight et al. (1991) suggested that a bottleneck may have been extreme

enough to reduce effective mitochondrial population sizes to the point where diversity was drastically diminished and particular haplotypes became fixed, whereas effective population sizes for nuclear genes remained large enough to maintain allozyme diversity (see also Birky et al., 1983, 1989; Wade et al., 1994). Perhaps the ancestor of the southeastern Edwards Plateau *Eurycea* experienced a similar bottleneck.

Geographic Patterns of Genetic Differentiation

Cluster analyses of both the allozyme and sequence data (Figs. 2, 4) reflect the high degree of genetic subdivision present in the group, particularly with respect to the groups of populations and taxa from north versus south of the Colorado River. The large number of sequence substitutions between the northern and southern groups, coupled with cyt *b* amino acid substitutions and numerous fixed or near-fixed allozyme differences, indicates that these groups are completely isolated from each other and probably have been for millions of years. This conclusion is reinforced by the results of flow cytometric studies of nuclear genome size: C-values (nuclear DNA mass) are 12–13% higher on average for members of the northern group than the southern group, and there is no overlap in C-value distributions for the two groups [Chippindale and Lowcock, unpublished; Licht and Lowcock, 1991 (note that Licht and Lowcock erroneously listed the mean C-value for members of the northern group as 25.8; the correct value is 28.5)].

Although Sweet (1978*a*) recognized the potential of the Colorado River as a barrier to gene flow, he (1978*a*, 1982) identified the few northern populations known then as *Eurycea neotenes* due to the high degree of morphological similarity between most surface-dwelling populations from the northern and southern areas. The type locality for *E. neotenes* is in Bexar Co., in the southern region. Based on the molecular data, however, the northern group consists of strongly differentiated, long-isolated species. The Colorado River is thought by many to be one of the oldest features of the Edwards Plateau (Abbott, 1975; Sweet, 1978*a*; Veni and Associates, 1992), and probably has cut down through the elevated limestones of the plateau throughout its existence, dividing the Edwards (Balcones Fault Zone) Aquifer into two major sections with little or no hydrologic connection (Slade et al., 1986). Thus it is not surprising that salamanders from either side of the river are strongly differentiated; this pattern of vicariant isolation by waterway also has been observed in other groups of salamanders (e.g., Good and Wake, 1992).

The similarity-based trees also illustrate the relatively high levels of genetic divergence of the taxa from the San Marcos Pool of the Edwards Aquifer in Hays Co. (southern region). *Eurycea rathbuni* and *E. nana* clearly are distinct species long isolated from gene flow with the other populations examined. This result is consistent with the occurrence of numerous other endemic species of aquatic vertebrates, invertebrates, and plants at San Marcos (e.g., Holsinger and Longley, 1980), and likely is related to the high degree of isolation of the San Marcos Pool of the Edwards Aquifer from the remaining southern portions of the aquifer (e.g., Potter and Sweet, 1981).

The subdivision of members of the southern group exclusive of the San Marcos taxa (*Eurycea rathbuni,* plus *E. nana* for allozymes) into southeastern and southwestern components corresponds roughly to the eastern versus western drainages of the southern plateau region. These drainages are of more recent origin and less deeply incised than the Colorado River (e.g., see Sweet, 1978*a*, 1982; Veni, 1994). The southeastern and southwestern groups are not as strongly differentiated from one another as are the northern and southern groups, or the San Marcos taxa compared to all others: most of the allozyme-based differentiation constitutes allele frequency variation rather than fixed differences. However, lack of current gene flow between the regions is especially apparent based on the mitochondrial sequence differences that separate all south-

eastern populations from all southwestern ones.

Of the non-San Marcos southeastern populations, only peripheral ones from the northeastern edge of the area display substantial sequence differentiation, especially the recently-discovered populations from springs along the Pedernales River in Travis Co. These populations are located in an isolated outcrop of Cow Creek limestone and there is little potential for direct connection of this aquatic system with other drainages known to be inhabited by *Eurycea.*

Phylogenetic Relationships and Species Boundaries

We believe that the best estimates of phylogeny are derived from treatments of the data that incorporate as much information as possible about evolutionary processes. Simulation and congruence studies suggest that, in general, incorporation of realistic evolutionary parameters into character coding and weighting increases the likelihood that the correct tree will be recovered, for both DNA sequence (e.g., Hillis et al., 1994; Huelsenbeck, 1995) and allele frequency data (e.g., Wiens, 1998, 2000; Wiens and Servedio, 1997, 1998). Although application of frequency information in parsimony analysis has been controversial (e.g., see Buth, 1984; Crother, 1990; Murphy, 1993; Murphy and Doyle, 1998), compelling arguments for use of frequency-based methods have been made (Swofford and Berlocher, 1987; Wiens, 1995, 2000). Simulation and congruence studies indicate that methods that incorporate frequency data tend to be more accurate than those that do not use this information (Cunningham, 1997; Wiens, 1998, 2000; Wiens and Servedio, 1997, 1998). Given that results of simulation studies (Wiens and Servedio, 1997, 1998) were upheld by congruence analyses of both morphological and allozyme data (Wiens, 1998, 2000), claims that the simulations are questionable because of unrealistic model assumptions (Kornet and Turner, 1999) appear to be unwarranted. The Manhattan distance/step matrix approach allows use of allele frequency information on a locus by locus (i.e., individual character) basis while avoiding the peculiar sampling properties of some other commonly used genetic distances, such as Nei's (1972) *D* (Hillis, 1984; Frost and Hillis, 1990). Wiens (1995) and Berlocher and Swofford (1997) pointed out that this method is equivalent to use of Swofford and Berlocher's (1987) MANOB criterion, previously deemed computationally impractical. Our choice of differential weighting schemes for changes among sequence-data character-states [Rodrigo's (1992) correction of Wheeler's (1990) combinatorial weighting method] allows incorporation of more detailed information on patterns of nucleotide change than would a simple transition versus transversion treatment, and Hillis et al. (1994) demonstrated in simulation studies that symmetrically weighted parsimony performs substantially better in recovering the true phylogeny than does uniform weighting of change probabilities.

The issue of whether or not to combine data sets in phylogenetic analysis has been the subject of considerable controversy (reviewed by Chippindale and Wiens, 1994; Miyamoto and Fitch, 1995; Hillis, 1995; de Queiroz et al., 1995; Huelsenbeck et al., 1996). Most workers agree that it is desirable to examine trees reconstructed based on both separate and combined analyses, and we consider the results of both kinds of analyses here. Our preferred hypothesis of phylogeny is that derived from combination of the allozyme and DNA data, the former treated with frequency-based coding and the latter with combinatorial weighting and codon position partitioning. Despite the apparently poor performance of the allozyme data alone in resolving most relationships, their use in combination with the sequence data substantially increased support for several relationships indicated by the sequence data alone (and in most cases in which node support decreased as a result of data combination, the changes were relatively minor).

The major regional similarity groupings identified by cluster analyses of the allozyme and DNA data appear in the phylo-

genetic trees as monophyletic assemblages (with the exception of the southwestern group using allozymes alone), and all analyses support monophyly of the central Texas hemidactyliines. There is support for monophyly of the northern group in all analyses, and it is clear (as discussed above) that these populations do not represent the species *Eurycea neotenes*. Consideration of all data supports recognition of at least three separate species in the northern group; formal descriptions are presented below. The basal split between populations of *Eurycea* from north versus south of the Colorado is consistent with the view of the Colorado as an ancient, strong barrier to gene flow that has divided the group into two major clades. *Eurycea rathbuni* is phylogenetically embedded within the Texas *Eurycea* in all analyses; thus we concur with Mitchell and Reddell (1965) and Mitchell and Smith (1972) that the genus *Typhlomolge* should be subsumed within the genus *Eurycea*. Analyses based on either DNA alone or combined allozyme and sequence data indicate a sister-taxon relationship between *E. rathbuni* (plus, presumably, *E. robusta*, which was unobtainable) and the remaining members of the southern group. This result suggests an early divergence for the ancestor of *E. rathbuni* and *E. robusta*. The other San Marcos taxon, *E. nana*, is more problematic, and its placement varies depending on which subset of the data is used. The sequence data place it with the southeastern group, not surprising given its minimal sequence divergence from them, and the combined data treatment provides very strong support for this species as the sister taxon to the southeastern group (San Marcos is located in the southeastern Edwards Plateau region but in many respects exhibits a unique aquatic biota with many endemic taxa; e.g., see Holsinger and Longley, 1980). However, the high level of divergence of *E. nana* based on allozymes leads to a weakly supported result in which it is placed outside all other central Texas hemidactyliines when the allozyme data alone are considered (Fig. 5).

Phylogenetic analyses based on the sequence and combined data support the existence of monophyletic southeastern and southwestern groups. Beyond this, there is little support based on the phylogenetic analyses for any particular pattern of relationships within the major groups that we have identified. For all treatments of the data, within-group bootstrap values are nearly all below 70%, the level found in simulation studies by Hillis and Bull (1993) to correspond to a 95% probability that a given clade is real.

With respect to taxonomy, none of our analyses support the previous view (e.g., Baker, 1961; Brown, 1950, 1967*c*; Sweet, 1978*a*, 1982, 1984) of *Eurycea neotenes* as widespread throughout the region. We restrict *E. neotenes* to springs in the area of the type locality at Helotes Creek, Bexar Co., resurrect the name *E. pterophila* Burger, Smith and Potter 1950 for Blanco River drainage populations, continue to recognize the species *E. tridentifera*, *E. sosorum*, and *E. nana*, and suggest that many more, as yet unnamed, species exist. All named species in the group occur in the southeastern region except *E. troglodytes* from Valdina Farms Sinkhole in Medina Co. This taxon [which may now be extinct due to habitat modification (Veni and Associates, 1987; G. Veni, personal communication to PTC)] was synonymized by Sweet (1978*a*, 1984) under both *E. neotenes* and *E. tridentifera* because he considered this population a hybrid swarm. Although allozyme data are not available, cyt *b* sequence for a specimen from the type locality places this population within the southwestern group, where it occurs geographically. Given the maternal inheritance of the mitochondrial genome, there conceivably could be male-based flow of genes (in the form of *E. tridentifera* from the southeast, one of the putative parent species), and thus the population could still consist of hybrids. However, we doubt this, because there is no other evidence of widespread gene flow in this group. The other putative hybrid taxon whose name was synonymized by Sweet, *E. latitans*, does not appear to be a hybrid between *E. tridentifera* and surface *Eurycea* based on the allozyme data. The geographic location of populations of *E. latitans* is much closer

to the known range of *E. tridentifera* than is that of *E. troglodytes*, and gene flow in the hydrologic system of this area seems much more plausible than between this region and the portion of the southwestern region in which *E. troglodytes* occurs, 75 km distant from the known range of *E. tridentifera*. For these reasons, we recognize *E. troglodytes* and *E. latitans*. At a minimum the name *E. troglodytes* should apply to the Valdina Farms locality; we recommend that it be extended to include all members of the southwestern group in the "*E. troglodytes* complex" pending formal description of other species in the region. *Eurycea latitans* applies at least to populations of the Cascade Caverns system of Kendall Co., and here we include several other populations in the area as members of a species complex containing this taxon.

Species Boundaries in the Central Texas Eurycea

Prior to the molecular work detailed here, nearly all assessments of species diversity and boundaries in the central Texas hemidactyliines were based on morphological criteria. This approach resulted in the recognition of a relatively small number of taxa in the group, at most six species or subspecies of *Eurycea* (e.g., see Baker, 1961; Brown, 1967*a–c*) plus two species of *Typhlomolge*. Several authors suggested, however, that additional species remained to be discovered in the group (e.g., Baker, 1961; Bogart, 1967; Brown, 1950, 1967*c*; Mitchell and Smith, 1972). Sweet (1978*a,b*, 1982, 1984) assigned most spring and cave populations from throughout the Edwards Plateau region to the species *E. neotenes*. This left three recognized species of *Eurycea* in the group (*E. neotenes, E. tridentifera,* and *E. nana*), plus a fourth taxon from Barton Springs in Travis Co. that Sweet (1978*a*, 1984) considered a distinct species based on morphology, later described by Chippindale et al. (1993) as *E. sosorum*. Potter and Sweet (1981) also recognized two species in the genus *Typhlomolge, T. rathbuni* and *T. robusta*, which we consider here to represent members of the genus *Eurycea*.

We follow the evolutionary species concept sensu Wiley (1978) and Frost and Hillis (1990). According to this view of species, one seeks evidence that a population or interbreeding group of populations constitutes a distinct evolutionary lineage that will continue to maintain a separate identity from other such lineages until it becomes extinct or undergoes additional speciation. This evidence can be based on many kinds of characters (e.g., morphological, molecular, behavioral, etc.) and geographic considerations (i.e., the potential for isolation from gene flow) also are relevant. By accepting the Evolutionary Species Concept, we emphasize what species are (namely, distinct, independently evolving historical lineages) rather than emphasizing any particular attributes of historical lineages (cf. Mayr, 1982; Paterson, 1985). We also accept all evidence for the independence of historical lineages, rather than restricting ourselves to a limited or arbitrary set of criteria (cf. Cracraft, 1989; Highton et al., 1989; Highton, 1990, 1995).

Below, we present our interpretation of species boundaries in the central Texas hemidactyliines and provide a brief account of each of the groups that we used for phylogenetic analyses. Species to which we assigned each of the populations sampled, and their geographic distributions, are shown in Fig. 8. Considerable ambiguity remains with respect to fine-scale relationships in the Texas *Eurycea*, and this is reflected by our tentative recognition of possibly heterogeneous groups such as the "Carson Cave group" in the southwestern Edwards Plateau region. We identify two groups of populations as species complexes: the *E. latitans* complex in the southeastern plateau region and the *E. troglodytes* complex in the southwestern plateau region. We provide synonymies for each of the species that we recognize, detail the evidence that led us to separate them, recommend a formal name for members of the group if one currently is in use or is available for resurrection, indicate what taxonomic problems remain, and suggest ways to solve these problems. We also describe three new species from the northern region. Our primary aims are to doc-

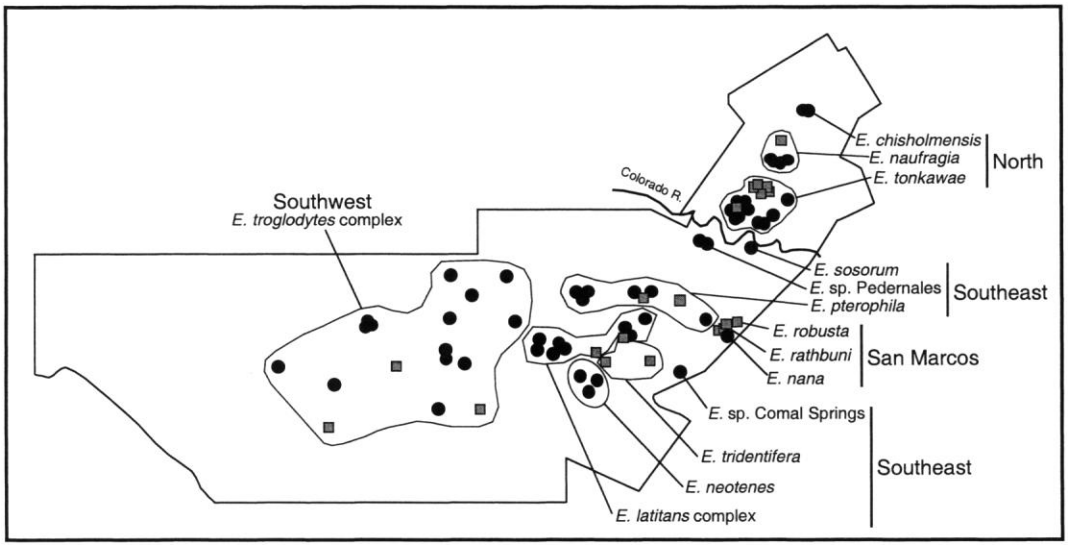

FIG. 8.—Geographic distribution of species of central Texas *Eurycea* recognized in this study. The region outlined corresponds to the counties in which these salamanders are known to occur, detailed in Fig. 1. Filled circles represent surface spring localities and stippled squares represent cave localities.

ument the remarkable diversity in the group, identify populations or groups of populations that appear to represent distinct species, and provide a framework within which future studies of this diversity can proceed.

In the following accounts, we have organized the species and informal groups that we recognize by geographic region. Appendix I provides precise locality information for all populations examined. The designation of a San Marcos region (inhabited by *Eurycea nana, E. rathbuni,* and *E. robusta*) is simply one of convenience, because the taxa from this area are highly divergent and the placement of *E. nana* is uncertain; no close relationship between *E. nana* and the other two species at San Marcos is implied. Museum acronyms follow Leviton et al. (1985). Distinguishing molecular character states are listed; for sequence characters, these refer to the position within cyt *b* relative to the first nucleotide in the alignment of the sequence we obtained (Appendix III).

SYSTEMATIC ACCOUNTS

I. Southern Region

This assemblage includes all populations of *Eurycea* (including the two species formerly assigned to the genus *Typhlomolge*) from southwest of the Colorado River, ranging from western Travis County to eastern Val Verde County. We have examined specimens from 12 of the 14 counties in the southern region in which salamanders are known or thought to occur. The exceptions are Kinney Co., in which salamanders are likely to occur but no populations are known, and Val Verde Co. For the latter, repeated visits to the two known salamander localities at Del Rio (Fourmile Cave and San Felipe Springs; see Sweet, 1978*a*) failed to yield specimens. We expect that these salamanders would fall within the southwestern group that we recognize (see below) based on their location. Very late in the study, *Eurycea* were discovered by Riley Nelson and co-workers north of Del Rio in springs in the Devil's River drainage. Superficially, these appear similar to other surface populations from the southwest, but we have not yet examined them for molecular markers. The southern group is strongly supported as monophyletic in phylogenetic analyses of the molecular data that include mitochondrial sequence.

IA. San Marcos Region

This area is located within Hays Co. in the southeastern region, but displays a unique aquatic fauna in many respects, and the salamanders found here (*Eurycea rathbuni* and *E. robusta* underground and *E. nana* on the surface) are highly divergent from others in the Edwards Plateau region based both on morphology and molecular markers. Synonymies and basic information are as follows:

Eurycea nana

Eurycea nana Bishop 1941:6.
Eurycea neotenes nana: Schmidt 1953:55.

Holotype.—UMMZ 89759.

Type locality.—"Lake at the head of the San Marcos River, Hays County, Texas".

Comments.—Chippindale et al. (1998) discussed the confusion in the literature regarding the status and distribution of this taxon and provided molecular and morphological evidence that it is restricted to the outflows of San Marcos Springs, Hays Co. All recent authors have recognized this species, a position which we support.

Distinguishing molecular character states are as follows. Allozymes: Unique *a* allele fixed at Ak; *d* allele fixed at Acon-1 (an allele with this mobility is also seen in the outgroup taxa *Haideotriton* and *Typhlotriton*); unique *e* allele fixed at sAat; *c* allele fixed at Gr (also seen in the outgroup taxon *Typhlotriton*); *d* allele fixed at Pep-B (also present at low frequency in *E. rathbuni* and in the outgroup taxon *E. wilderae*); unique *h* allele at Pep-D. Sequence: unique T at 24; unique A at 217.

Eurycea rathbuni

Typhlomolge rathbuni Stejneger 1896:620.
Eurycea rathbuni: Mitchell and Reddell 1965:23.

Holotype.—USNM 22686.

Type locality.—"subterranean waters near San Marcos, Texas" (the specimens examined by Stejneger were from the 188-foot-deep Artesian Well at what is now Southwest Texas State University in San Marcos).

Comments.—Three species have at various times been regarded as members of the genus *Typhlomolge*. *Typhlomolge rathbuni* Stejneger 1896 was the first to be described, and is known from several caves and wells that intersect the San Marcos Pool of the Edwards Fault Zone Aquifer (Russell, 1976; Potter and Sweet, 1981; Longley, 1986). In 1981, Potter and Sweet redescribed another species of *Typhlomolge, T. robusta,* based on a single specimen collected in 1951 from a hole drilled in the bed of the Blanco River northeast of San Marcos. Longley (1978) unintentionally provided the original description of this taxon (e.g., see Potter and Sweet, 1981) because in a government report he used the name *T. robusta,* originally applied in a master's thesis by Potter (1963), and offered an extremely brief description of the type specimen while acknowledging Potter as the author of the name. Dixon (1987) rejected Longley's description and cited several ways in which it deviated from taxonomic practices specified by the 1985 revised International Code of Zoological Nomenclature; authorship of the name remains controversial. Wake (1966) initially recognized a third species in the genus, *T. tridentifera,* originally described by Mitchell and Reddell (1965) as *Eurycea tridentifera.* Sweet (1977*a*) listed additional applications of this combination. However, Wake's assessment was based on a very limited number of specimens, and subsequent morphological work using additional material led Mitchell and Smith (1972) to place members of the genus *Typhlomolge* in the synonymy of the genus *Eurycea.* This conclusion is strongly supported by our molecular work.

Since the discovery of *Eurycea rathbuni,* relationships of the taxon have remained controversial. Emerson (1905) first recognized that *E. rathbuni* was a plethodontid, and Wake (1966) assigned the genus to the tribe Hemidactyliini. Based on pre-phylogenetic analyses, Wake (1966) regarded what was then considered *Typhlomolge* as part of a "pre-*Eurycea*" Miocene radiation of hemidactyliine plethodontids in southeastern and south-central North America. Mitchell and Reddell

(1965) and Mitchell and Smith (1972) viewed members of what had been considered *Typhlomolge* simply as extremes in a continuum of cave-associated morphological changes in the Texas *Eurycea*. Potter and Sweet (1981) concurred with Wake's (1966) interpretation of the time scale for invasion of the Edwards Plateau region by ancestors of what they considered *Typhlomolge* and recommended (based on morphological analyses) that the genus *Typhlomolge* should continue to be recognized. Using parsimony methods, Lombard and Wake (1986; and D.B. Wake, personal communication) investigated relationships among genera of plethodontids based primarily on characters of the feeding apparatus (especially the tongue), but were unable to reliably reconstruct the position of *Typhlomolge* except to verify that it belonged within the Hemidactyliini.

Allozyme and mitochondrial sequence data support the monophyly of the central Texas hemidactyliines inclusive of *Eurycea rathbuni*, which is deeply nested in the group (*E. robusta* was unobtainable for molecular studies, but we assume based on morphology that it is the sister species to *E. rathbuni*; see Potter and Sweet, 1981, and Russell, 1976, for further details). *Typhlomolge* cannot be retained as a genus under current rules of nomenclature if it is a subset of *Eurycea*. In order to minimize the number of necessary taxonomic changes, we therefore synonymize the genus *Typhlomolge* under *Eurycea*. Such a move is consistent with the recommendations of Mitchell and Reddell (1965) and Mitchell and Smith (1972), and thus simply represents a reapplication of this combination for *E. rathbuni*. Following the recommendations of Chippindale (1995 and personal communication), Petranka (1998) also used the name *Eurycea rathbuni*, and synonymized *T. robusta* under *Eurycea*.

Frost (1985) gave the range of *Eurycea rathbuni* as "Underground waters in Hays, Kendall, and Comal counties, central Texas, USA", but there is no reliable evidence that this species occurs anywhere but the San Marcos Pool of the Edwards Aquifer, Hays Co. (see Potter and Sweet, 1981 for further discussion).

Distinguishing molecular character states are as follows. Allozymes: *e* allele fixed at Acon-1 (also seen in *Eurycea* sp. from Comal Springs and the outgroup taxon *Typhlotriton*); unique, presumed null allele fixed at Gr; unique *b* allele fixed at Mdh-2; unique *g* allele at Pep-D at 75% frequency. Sequence: T at 9 (shared with *Typhlotriton*); unique A at 24; unique A at 28; unique C at 36; T at 48 (shared with *Typhlotriton*); A at 69 (shared with *Typhlotriton*); T at 78 (shared with some outgroup members); C at 174 (shared with Greenwood Valley Ranch Springs, southwestern group); T at 201 (shared with the outgroup taxon *E. bislineata*).

Eurycea robusta

Typhlomolge robusta Longley, *in* Potter and Sweet 1981:70.
Eurycea robusta: Petranka 1998:275.

Holotype.—TNHC 20255.

Type locality.—"Beneath the Blanco River, 178 m elevation, 5 airline km NE of the Hays County Courthouse, San Marcos, Hays County, Texas".

Comments.—Above we recommend synonymy of the genus *Typhlomolge* under *Eurycea*, which renders this taxon *Eurycea robusta*. Petranka (1998) was the first to formally apply the combination *Eurycea robusta*, also used by Chippindale (1995) in a Ph.D. dissertation.

IB. Southeastern Region

This region encompasses western Travis, Hays (except San Marcos, which we treat separately; see above), Blanco, Comal, Kendall, Bexar, and extreme eastern Kerr counties. It contains the type localities for most of the central Texas *Eurycea* that have been recognized: *Eurycea neotenes*, *E. latitans*, *E. pterophila*, *E. tridentifera*, and *E. sosorum*. It is not clear whether the problematic taxon *E. nana* (from San Marcos Springs) should be considered part of a monophyletic southeastern clade: this species is highly divergent based on allozymes but falls within or sister to the southeastern populations based on cyto-

chrome *b* sequence. Our preferred hypothesis of relationships (based on combined allozyme and sequence data) suggests that *E. nana* is the sister species of all southeastern taxa.

We divide the southeastern clade into seven taxa: *Eurycea neotenes, E. pterophila, E. sosorum, E. tridentifera,* Comal Springs, Pedernales Springs, and the *E. latitans* complex. It is likely that all except the last represent single, distinct species. The evidence of species status is particulary strong for *E. tridentifera, E. sosorum,* and the Pedernales populations, each of which exhibit diagnostic molecular markers and (for the former two) unique morphological features (the Pedernales populations have not yet been examined in detail morphologically). *Eurycea neotenes,* the *E. latitans* complex, *E. pterophila,* and the Comal Springs population are distinguished primarily based on substantial allele frequency differences at allozyme loci.

Eurycea neotenes

Eurycea neotenes Bishop and Wright 1937:142.

Eurycea neotenes neotenes: Schmidt 1953: 55.

Holotype.—USNM 103161 (Cochran, 1961).

Type locality.—"Culebra Creek, 5 miles north of Helotes, Bexar County, Texas"; corrected by Brown (1942) to the headspring of Helotes Creek in Bexar Co.

Comments.—Schmidt (1953) first suggested that *Eurycea neotenes* from the type locality, plus most other populations of *Eurycea* in the Edwards Plateau region, be considered the subspecies *E. n. neotenes,* and he also recognized the subspecies *E. n. nana* and *E. n. pterophila* (see above and below, respectively, for detailed accounts of the latter two taxa). The subspecific designation was ignored by some authors (e.g., Baker, 1961). Brown (1967*b,c*) argued for recognition of *E. nana* as a full species but continued to recognize the subspecies *E. n. neotenes* and *E. n. pterophila.* Sweet (1978*b*) formally synonymized *E. pterophila* (or *E. n. pterophila*) under *E. neotenes,* and subspecies within *E. neotenes* have not generally been recognized since. Many authors (e.g., Baker, 1961; Behler and King, 1979; Brown, 1950; Conant, 1958, 1975; Conant and Collins, 1991; Mitchell and Smith, 1972; Schmidt, 1953; Sweet, 1977*b,* 1978*a,b,* 1982, 1984) have regarded *E. neotenes* (or *E. n. neotenes*) as widespread in springs and caves of the Edwards Plateau region. Based on our molecular evidence, we disagree, and recommend restriction of the name *E. neotenes* to spring populations from the vicinity of the type locality. The three populations that we examined which we place in *E. neotenes* are those from the type locality at Helotes Creek Spring, Bexar Co., plus those at Leon Springs, Bexar Co., and Mueller's Spring, Kendall Co.

Distinguishing molecular character states are as follows. Allozymes: These populations exhibit the *b* allele at high frequency at sAat, otherwise absent or at low frequency in other southeastern populations and taxa (except *E. tridentifera* and the Comal Springs and Pedernales populations); possess the *b* allele at Pgm at high frequency, otherwise seen only in the Comal Springs and Camp Mystic (southwestern group) populations and the outgroup member *E. quadridigitata* from Texas; and exhibit the Mpi *b* allele at high frequency, otherwise seen only in the Pedernales populations, some members of the *E. pterophila* group, and the Fessenden and 176 Springs populations from the southwest. Sequence: No diagnostic sequence substitutions were found.

Eurycea pterophila

Eurycea pterophila Burger, Smith, and Potter 1950:51.

Eurycea neotenes pterophila: Schmidt 1953:56.

Eurycea neotenes: Sweet 1978*b*:106 (in part).

Holotype.—Floyd Potter Coll. No. A993 (private collection, now presumed lost).

Type locality.—"shallow stream flowing from Fern Bank Spring, 6.3 miles northeast of Wimberley on the Blanco River Road, Hays County, Texas".

Comments.—Sweet (1978*b*) demon-

strated that the morphological character states used by Burger et al. (1950) to distinguish *Eurycea pterophila* from other Edwards Plateau *Eurycea* were either more widespread than previously thought or were erroneous (e.g., supposedly short digits were actually due to loss of tips through bacterial infection). Sweet concluded that there was no reason to recognize the Fern Bank Spring population as a separate taxon, and relegated this taxon to synonymy with *E. neotenes.* Based on the information then available this was a logical assessment. Hamilton (1973) also was unable to distinguish Fern Bank Spring *Eurycea* from other populations in the Blanco River drainage and elsewhere in the southeastern Edwards Plateau region based on morphometric analyses. However, the allozyme evidence here shows that the Fern Bank Spring population plus all others in the Blanco River drainage share a high degree of similarity in allele frequencies (although no alleles are diagnostic), suggesting recent or ongoing gene flow. This, coupled with the restriction of this group to a single drainage, leads us to recognize *E. pterophila,* especially because the allozyme and geographic evidence indicates that these populations almost certainly are isolated from gene flow with true (topotypical) *E. neotenes.* Further study will be necessary to clarify the status of this taxon. Populations that we have examined and assign to this taxon are: Fern Bank Spring, Zercher Spring, Boardhouse Springs, Peavey's Springs, Grapevine Cave, and T Cave.

Eurycea sosorum

Eurycea neotenes: Brown 1950:29 (in part).

Eurycea neotenes neotenes: Brown 1967*c*: 36.1 (in part; mapped locality only).

Eurycea sp.: Sweet 1984:429.

Eurycea sosorum Chippindale, Price, and Hillis 1993:249.

Holotype.—TNHC 51184.

Type locality.—"outflow of Parthenia (Main) Springs in Barton Springs Pool, Zilker Park, Travis Co., Texas (30° 15′ 49″ N, 97° 46′ 14″ W)."

Comments.—A detailed account of the morphological and molecular features that distinguish this species from other central Texas *Eurycea* was provided in the original description (Chippindale et al., 1993). Subsequent molecular work has reinforced the conclusion that this population represents a distinct species isolated from gene flow from all others. Our recognition of this taxon as a distinct species is consistent with Sweet's (1978*a,* 1984) conclusions based solely on morphology.

Distinguishing molecular character states based on the data presented here are as follows. Allozymes: For Pep-A the unique *e* allele is present at 83% frequency, and the other (*d*) allele present is otherwise seen only in members of the northern group, *Eurycea nana,* and the Pedernales populations; and at Pep-D the *a* allele is fixed, otherwise seen only in geographically distant and otherwise very divergent members of the northern and southwestern groups. Sequence: C at position 195 is shared only with Pedernales, Smith's Spring (southwest) and Carson Cave (southwest) populations and *E. nana.*

Eurycea tridentifera

Eurycea tridentifera Mitchell and Reddell 1965:14.

Typhlomolge tridentifera: Wake 1966:64.

Holotype.—USNM 153780.

Type locality.—"Honey Creek Cave, Comal Co., Texas".

Comments.—Mitchell and Reddell (1965), Wake (1966), and Sweet (1977*a,* 1978*a,* 1984) regarded *Eurycea tridentifera* as exhibiting a cave-associated morphology second only to that of *E. rathbuni* (and *E. robusta*) in extremity among the Edwards Plateau hemidactyliines. The morphological features of this taxon led Wake (1966) to transfer it to the genus *Typhlomolge,* although later work by Mitchell and Smith (1972) suggested that the members of the genus *Typhlomolge* actually belong within *Eurycea,* a move that we also support based on the molecular evidence. The molecular evidence also indicates that *E. tridentifera* is not closely related to *E. rathbuni.* Sweet (1977*a,* 1978*a,* 1984) sug-

gested that the caves of northern Bexar Co. and the Cibolo Sinkhole Plain in which most known populations of this species occur are among the oldest in the plateau region, which would have allowed a long time for the evolution of cave-associated features. Sweet (1978*a*, 1984) demonstrated using morphometric analyses that populations from throughout the known range of the species cluster together, and recommended recognition of this taxon as a single species with a relatively wide subterranean range. The molecular evidence supports this view for the populations that we were able to examine; in addition, Bogart (1967) identified a chromosomal nondisjunction that appears to be unique to the two populations of *E. tridentifera* that he examined (Badweather Pit and Honey Creek Cave). Sweet (1977*a*) listed six known localities, and suggested that more likely would be found in the Cibolo Sinkhole Plain and northern Bexar Co. As he predicted, salamanders that appear to be this species have been seen at Genesis Cave in northern Bexar Co. (D. Pearson, personal communication; see Veni, 1988 for details of the cave), and Chippindale and A.G. Grubbs have collected this species at Ebert Cave in Comal Co. [near Sweet's (1977*a*, 1978*a*, 1984) Kappelman Salamander Cave locality]; *E. tridentifera* was first collected there by J. Reddell and M. Reyes. This species has also been found very recently in caves of the Camp Bullis army base, Comal Co. (J. Reddell and G. Veni, personal communication to PTC), and several specimens have been collected. Conant and Collins (1991) restricted the distribution of this species to the type locality, Honey Creek Cave, Comal Co., but this clearly was in error.

In addition to the distinctive morphological features exhibited by this taxon (see Mitchell and Reddell, 1965; Wake, 1966; Mitchell and Smith, 1972; Sweet, 1977*a*, 1978*a*, 1984) and the potential chromosomal autapomorphy described above, distinguishing molecular character states are as follows. Allozymes: At sAat, the *b* allele is at medium to high frequency (this allele otherwise is rare in the southeastern group, except in Comal Springs, Pedernales, and *Eurycea neotenes*); at G3pdh the *b* allele appears fixed in the Honey Creek Cave and Ebert Cave populations (otherwise this allele is seen at low frequency in *E. sosorum, E. rathbuni,* and members of the southwestern group; however, it does not appear to be present in Badweather Pit *E. tridentifera,* suggesting that this population may in fact be isolated); and all three populations examined possess the unique Mdhp *d* allele at very high or 100% frequency. Sequence: No diagnostic sequence substitutions were found.

Eurycea latitans Complex

The synonymy given below applies to *Eurycea latitans* from the type locality only. We include numerous populations in the *E. latitans* complex; all (except the previously unknown Less Ranch Spring, Cherry Creek Spring, and Cloud Hollow Spring populations) were assigned by Sweet (1978*a*, 1982) to *E. neotenes*. Some of these may have been assigned to species earlier by Baker (1961) or Brown (1967*c*), but for the populations in question these authors provided only maps, so the precise localities to which they referred are uncertain.

Eurycea neotenes Wright and Wright 1938:31 (in part) [assumed by Wright and Wright to be *E. neotenes* based on a second-hand report; location given only as "a cave near Boerne", assumed by Smith and Potter (1946) to be Cascade Caverns; Bishop (1943) reported the presence of *E. neotenes* in Cascade Caverns].

Eurycea latitans Smith and Potter 1946: 106.

Eurycea neotenes latitans: Schmidt 1953: 55.

Eurycea tridentifera: Sweet 1984:438 (in part) (Sweet regarded *E. latitans* as a junior synonym of *E. neotenes,* but believed that the population contained introgressed genes from *E. tridentifera*).

Holotype.—USNM 123594.

Type locality.—"the first large pool deep within the recesses of Cascade Cavern, 4.6

miles by road (3 ½ miles by airline) southeast of Boerne, Kendall County, Texas."

Comments.—The status of this taxon is problematic. Sweet (1978*a*, 1984) demonstrated substantial morphological variation in this population and showed that specimens from Cascade Caverns presented a morphological spectrum from surface-like morphologies to extreme troglobitic morphologies similar to those of *Eurycea tridentifera*. He hypothesized that this was the result of past introgression of genes from the advanced troglobite *E. tridentifera* into a cave population of *E. neotenes*. Our molecular data provide no evidence of a hybrid origin for this population. Because the population that lives in the underground system associated with Cascade Caverns also does not appear to represent *E. neotenes* based on the molecular evidence, it seems reasonable to resurrect the name *E. latitans* for salamanders in this cave system. Here we assign several other populations (Rebecca Creek Spring, Hays Co., Bear Creek Spring, Cibolo Creek Spring, Less Ranch Spring, and Kneedeep Cave Spring, all in Kendall Co., Cherry Creek and Cloud Hollow Springs, Kerr Co., and Honey Creek Cave Spring, Comal Co.) to this species based on similarity in allele frequencies and cytochrome *b* sequences. This group likely is a catch-all for members of the southeastern group whose affinities are uncertain, and definitely needs further investigation.

No molecular synapomorphies unite all members of the group; the Rebecca Creek population and topotypical *Eurycea latitans* each exhibit potential sequence autapomorphies. Specifically, Rebecca Creek exhibits a T at position 99 otherwise seen only in *Haideotriton*. Topotypical *E. latitans* have a T at position 82 (also seen in the southwestern Carson Cave population and outgroup members *E. bislineata* and *E. wilderae*).

Eurycea sp.—Comal Springs

Eurycea neotenes: Baker 1961:29 (in part).
Eurycea neotenes neotenes: Brown 1967*c*: 36.1 (in part; mapped locality only).
Eurycea nana: Dixon 1987:60 (in part).

Comments.—The many characters that distinguish this population from true *Eurycea nana* (from San Marcos Springs, Hays Co.) are discussed by Chippindale et al. (1998), and this population clearly does not represent *E. nana*. It may represent a distinct species, although additional study of this population and its relationship to others in the southeastern region is needed. Further study of this putative taxon is critical, because its spring habitat is threatened by human demands on the waters of the southern Edwards Aquifer.

Distinguishing molecular character states are as follows. Allozymes: At Acon-1, the *e* allele (otherwise seen only in *Eurycea rathbuni*) is at medium frequency; at sAat, the *b* allele (rare in the southeast except in *E. tridentifera*, *E. neotenes*, and Pedernales) is at medium frequency; and at Pgm, the *b* allele is at medium frequency (otherwise seen only in *E. neotenes*, Camp Mystic from the southwest, and the outgroup member *E. quadridigitata* from Texas). Sequence: No diagnostic sequence characters were found.

Eurycea sp.—Pedernales populations

No previous taxonomic history.

Comments.—Chippindale and Hillis found the first of two known populations of this salamander in 1989, in two small springs on the northeast side of the Pedernales River in extreme western Travis Co. These springs are located in an isolated band of Cow Creek limestone and are well separated geographically from all other known populations of central Texas *Eurycea*. Although no detailed morphological studies have yet been conducted, these salamanders appear to mature at a very small size, and exhibit a relatively high frequency of "gold" morphs (individuals in which the melanophores are widely separated, yielding a light yellowish-gold color). These salamanders possess unique combinations of allozyme and sequence character states and almost certainly represent a distinct species; we expect to formally describe them as such pending completion of additional molecular and morphological studies.

Distinguishing molecular character states are as follows. Allozymes: At Acon-1 the *c* allele (otherwise characteristic of the northern group) occurs at low frequency; at sAat, the *b* allele is at medium frequency (otherwise rare in the southeast except in *Eurycea tridentifera, E. neotenes,* and Comal Springs); at Ldh-A the *a* allele is at medium frequency (otherwise seen only in the T Cave population, a member of the *E. pterophila* group); and at Mdhp the unique *f* allele occurs at low frequency. Sequence: T at position 75 (shared only with Smith's Spring in the southwestern group); C at 195 (shared with *E. sosorum, E. nana,* and the southwestern Smith's Spring and Carson cave populations); unique G at 245; T at 246 (shared with some northern and southwestern group members as well as members of the outgroup); C at 282 (shared with *E. rathbuni* and members of the northern and southwestern groups and outgroup); and C at 312 (shared with members of the southwestern and northern groups, *E. rathbuni,* and outgroup members).

IC. Southwestern Region

This region encompasses southern Gillespie Co., most of Kerr Co. (except the easternmost extreme), Bandera, Real, and Edwards Counties, northern Medina, Uvalde, and probably Kinney Co., and likely the populations in Val Verde Co. Our molecular studies are only the beginning for discovery of diversity in this clade, and much more intensive sampling will be necessary to reliably identify species boundaries in this group. Most known populations in the southwestern region have been considered *Eurycea neotenes* (e.g., Baker, 1961; Brown, 1967*c*; Sweet, 1978*a*, 1982), and only one other species has been recognized in the southwestern region (*E. troglodytes* Baker 1957; see below). Both allozyme and sequence data suggest that many members of this group are isolated from gene flow and probably represent distinct species. Our recognition of a "Carson Cave group" is based on phenetic criteria and it may not represent a monophyletic group of populations or species. The southwestern group is strongly supported as monophyletic in phylogenetic analyses of the molecular data that include mitochondrial sequence.

Eurycea troglodytes Complex

The synonymy given below applies to *Eurycea troglodytes* from the type locality only. We include numerous populations in the *E. troglodytes* complex. All that we examined (except the previously unknown Greenwood Valley Ranch Springs and West Nueces Spring localities) were assigned by Sweet (1978*a*, 1982) to *E. neotenes.* Some of these may have been assigned to species earlier by Baker (1961) or Brown (1967*c*), but for the populations in question these authors provided only maps, so the precise localities to which they referred are uncertain.

E. troglodytes: Baker 1957:329.
E. neotenes: Sweet 1984:438 (in part).
E. tridentifera: Sweet 1984:438 (in part).

Holotype.—TNHC 21791.

Type locality.—"a pool approximately 600 feet from the entrance of the Valdina Farms Sinkhole, Valdina Farms, Medina County, Texas."

Comments.—Sweet (1978*a*, 1984) considered this taxon a hybrid swarm derived from *Eurycea tridentifera* and what he considered *E. neotenes* based on the morphological variability that it displays. However, this seems very unlikely based on molecular evidence and geographic and hydrologic considerations. Therefore, we recommend continued recognition of this species, especially because it is the only named member of the southwestern group. Unfortunately, the population at the type locality may have been destroyed by human modification of its habitat (Veni and Associates, 1987; G. Veni, personal communication to PTC).

Pending further investigation of relationships of the southwestern Edwards Plateau *Eurycea,* we recommend that all members of the southwestern group collectively be referred to as the *E. troglodytes* complex. This includes the following OTU's that we used in our phylogenetic analyses: Tucker Hollow Cave, Greenwood

Valley Ranch Springs, Sabinal group, Sutherland Hollow Spring, Trough Spring, and Carson Cave group (see Fig. 3 and Appendix I). This is a heterogeneous group that exhibits considerable molecular diversity, and probably includes several distinct species. However, the molecular evidence indicates that it probably is monophyletic, and it is clear that none of the members of the group that we have examined are conspecific with any other central Texas *Eurycea*. Below we offer a brief account of each of the OTUs from within this group that we used for phylogenetic analyses.

Distinguishing molecular character states for this species complex are as follows. Allozymes: At sAat the *b* allele, absent or at low frequency in many southeastern populations, appears fixed in all southwestern populations; in all populations the Pep-B *a* allele appears fixed or at very high frequency (otherwise seen only among the Texas *Eurycea* in topotypical *E. latitans, E. tridentifera,* and *E. sosorum,* and in the outgroup taxon *E. quadridigitata* from South Carolina). Sequence (characters and states listed are those that distinguish members of the *E. troglodytes* complex from members of the southeastern group, to which they are geographically adjacent): A at position 75 in all except Smith's Spring, which has a T (A shared with northern group and outgroup members); T at 126 (shared with members of the northern group, *E. rathbuni,* and some outgroup members); A at 198 (shared with some members of the northern group and outgroup); A at 219 (shared with *E. rathbuni,* some members of the northern group, and outgroup members); T at 240 (shared with *E. rathbuni,* some members of the northern group, and outgroup members); A at 291 (shared with *E. rathbuni,* members of the northern group, and members of the outgroup), G at 321 (shared with northern group); and A at 361 (shared with *E. nana, E. rathbuni,* and some outgroup members).

Eurycea troglodytes Complex—Tucker Hollow Cave

Eurycea neotenes: Sweet 1984:433 (in part)

Comments.—This is one of the few cave populations of *Eurycea* distant from the Balcones Fault Zone, and almost certainly is isolated from all or most other populations. These salamanders, known only from two shallow pools in a hillside cave, exhibit strong cave-associated morphologies, including reduced eyes and pigmentation and broadened heads. Sweet (1978*a*) provided a detailed morphological description of the animals, and Sweet (1984) included this population in a morphometric analysis of central Texas cave *Eurycea*. J. R. Reddell (personal communication to PTC) considered this population a distinct species, although a formal description was never published. Given the morphological and molecular distinctiveness of this population and its apparent isolation, this assessment may well be correct.

Distinguishing molecular character states are as follows. Allozymes: The unique *c* allele appears fixed at Idh-1; the *a* allele appears fixed at Gapdh (also seen at medium frequency in populations of the Sabinal group, below). Sequence: C at position 49 (shared with the southwestern Smith's Spring and Carson Cave populations, southeastern populations, and some outgroup members); C at 186 (shared with *E. rathbuni,* Smith's Spring, Carson Cave, and some outgroup members); unique G at 225.

Eurycea troglodytes Complex—Greenwood Valley Ranch Springs

No previous taxonomic history.

Comments.—Superficially, these salamanders appear similar to other southwestern spring populations. Additional sampling in this area of the range is very desirable, as the status of these populations is uncertain. Based on molecular divergence, they could represent an undescribed species.

Distinguishing molecular character states are as follows. Allozymes: Lack of detectable activity at Mdh-2 (treated as null allele). Sequence: C at position 174 (shared with *E. rathbuni*); C at 323 (shared with Carson Cave).

Eurycea troglodytes Complex—176 Spring

Eurycea neotenes: Sweet 1982:441 (in part).

Comments.—Like many southwestern populations, this population appears to be distinct based on molecular evidence, but sampling in the region is very limited and thus the status of this population is uncertain. Distinguishing molecular character states are as follows. Allozymes: Unique Ldh-A *e* allele at high (88%) frequency; fixed *a* allele at Pep-A (also seen in nearby Fessenden Springs). Sequence: Unique T at position 171; unique T at 196; C at 303 (shared with northern populations, *Eurycea rathbuni,* and some outgroup members).

Eurycea troglodytes Complex—Camp Mystic Springs

Eurycea neotenes: Sweet 1982:441 (in part).

Comments.—This is another southwestern spring population that does not appear morphologically distinct, but which has many molecular character states that indicate isolation from gene flow, at least from the other populations examined. Thus it may represent another distinct species. Several sequence characters suggest the possibility of close relationship with the Trough Spring population, here placed in the informal (and problematic) Carson Cave group (below). Distinguishing molecular character states are as follows. Allozymes: Unique *c* allele at 90% frequency at Mdh-1; fixed *a* allele at Pep-D (shared with some northern populations, *Eurycea sosorum,* and nearby Fessenden Springs); *b* allele fixed at Pk (an allele with the same mobility is seen in the outgroup member *Haideotriton*). Sequence: C at position 83 (shared with some outgroup members); T at 138 (shared with members of the northern group and the outgroup member *E. longicauda*); T at 161 (shared with members of the northern group, some outgroup members, and nearby Trough Springs); A at 203 (shared with Trough Springs, *E. rathbuni,* Cedar Breaks in the northern group, and some outgroup members); and C at 204 (shared with Testudo Tube in the northern group and Trough Springs).

Eurycea troglodytes Complex—Sabinal group

Eurycea neotenes: Sweet 1982:441 (in part).

Comments.—These populations include some of the few known naturally metamorphosing *Eurycea* in the Edwards Plateau region (discussed by Bruce, 1976; Sweet, 1977*b*). They may represent a distinct species.

Distinguishing molecular character states are as follows. Allozymes: At Gapdh the *a* allele (otherwise seen only in Tucker Hollow Cave) is at medium frequency; at Mdhp the unique *e* allele is at high frequency. Sequence: No diagnostic sequence substitutions were found.

Eurycea troglodytes complex—Carson Cave group

Eurycea neotenes: Sweet 1982:441 (in part).

Comments.—Here we omit earlier authors' taxonomic assignments of populations which may be closely related to members of this group, because the group's composition is highly problematic. Thus we cite only Sweet because he actually assigned most of the same populations that we examined to *Eurycea neotenes.* This assemblage (based on similarity in allozyme allele frequencies) definitely needs further study. Even the relationships suggested by the sequence data are at odds with this grouping in some respects (e.g., for Trough Spring and Camp Mystic Spring), and there may be several species involved. In future studies, the best strategy probably will be to address relationships within the southwestern group alone, treating as many populations as possible as separate units of analysis.

The population for which this group is named, Carson Cave, consists of morphologically distinctive troglobites similar in some respects to those from Tucker Hollow Cave (see Sweet, 1978*a,* 1984 for more detailed morphological information).

It may represent a distinct species (as believed by J. R. Reddell, personal communication to PTC), although sequence data suggest a close relationship between this population and the nearby but morphologically dissimilar Smith's Spring population. The populations included in this informal group range widely in the southwestern region and do not correspond to any single drainage. In this group we have included the populations from West Nueces Spring and Smith's Spring (Edwards Co.), Carson Cave and WB Spring (Uvalde Co.), Sutherland Hollow Spring (Bandera Co.), Robinson Creek Spring and Fessenden Spring (Kerr Co.), and Trough Spring (Gillespie Co.).

II. Northern Region

Molecular evidence for monophyly of the northern group is overwhelming. Despite their high level of divergence, no members of the group have been formally described as distinct species, mainly because animals from the northern surface populations known to previous workers appeared very similar based on external morphology to those from southern spring populations. Also, many of the northern populations were discovered during the course of this study, and thus their diversity and the extent of their range previously was very poorly known.

In addition to numerous allozyme and cytochrome *b* sequence characters, members of the northern group are characterized (among the central Texas *Eurycea*) by substantially larger genome sizes than all others [Chippindale and Lowcock, unpublished; Licht and Lowcock, 1991 (but see correction of Licht and Lowcock's data table above)], and all members examined so far exhibit a diagnostic ApaL1 restriction site near the end of the 28S nuclear ribosomal DNA repeat unit (Chippindale, unpublished). It is our subjective impression that the yellow stripe which occurs on the dorsal surface of the tail in mature animals from the northern group is usually wider and more vivid than that seen in members of the southern group in which this feature is present.

Identification of species boundaries in the northern group has proven difficult in some cases, especially with respect to the cave populations. We have treated most subterranean populations in the region as separate units for phylogenetic analysis because of the uncertainty of their placement; clearly additional sampling, and the use of new molecular markers, is desirable to further elucidate relationships and species boundaries. Here we provide formal descriptions of three members of the northern group that clearly warrant status as species distinct from one another, and identify additional populations whose status and affinities within the northern group remain problematic.

Eurycea ***tonkawae*** sp. nov.
Fig. 9A

Eurycea neotenes: Baker 1961:30 (in part).
Eurycea neotenes neotenes: Brown, 1967*c*: 36.1 (in part; mapped locality only).

Holotype.—TNHC 50952 (field number AHP 3240), an adult female collected by Andrew H. Price and Paul T. Chippindale 12 September 1991 at the primary outflows of Stillhouse Hollow Springs, Travis Co. Texas, 30° 22′ 28″ N, 97° N45′ 55″ W.

Paratypes.—TNHC 1802–816, 50933–951, 50953–954, 50956–962, 50964–969, 50972–990, 50992–993, 51169–172, 53465–473, 53475–505, 53857, 54225–226, 54546–549, 55132–134, 55136, 55139–157, 55387; MVZ 122695–703; UTA A-52989–52990. Cleared-and-stained: TNHC 50955, 50963, 50991, 52759, 53474.

Diagnosis.—The following diagnosis applies only to spring-dwelling populations of this species; see notes below under *Additional comments* regarding cave-dwelling

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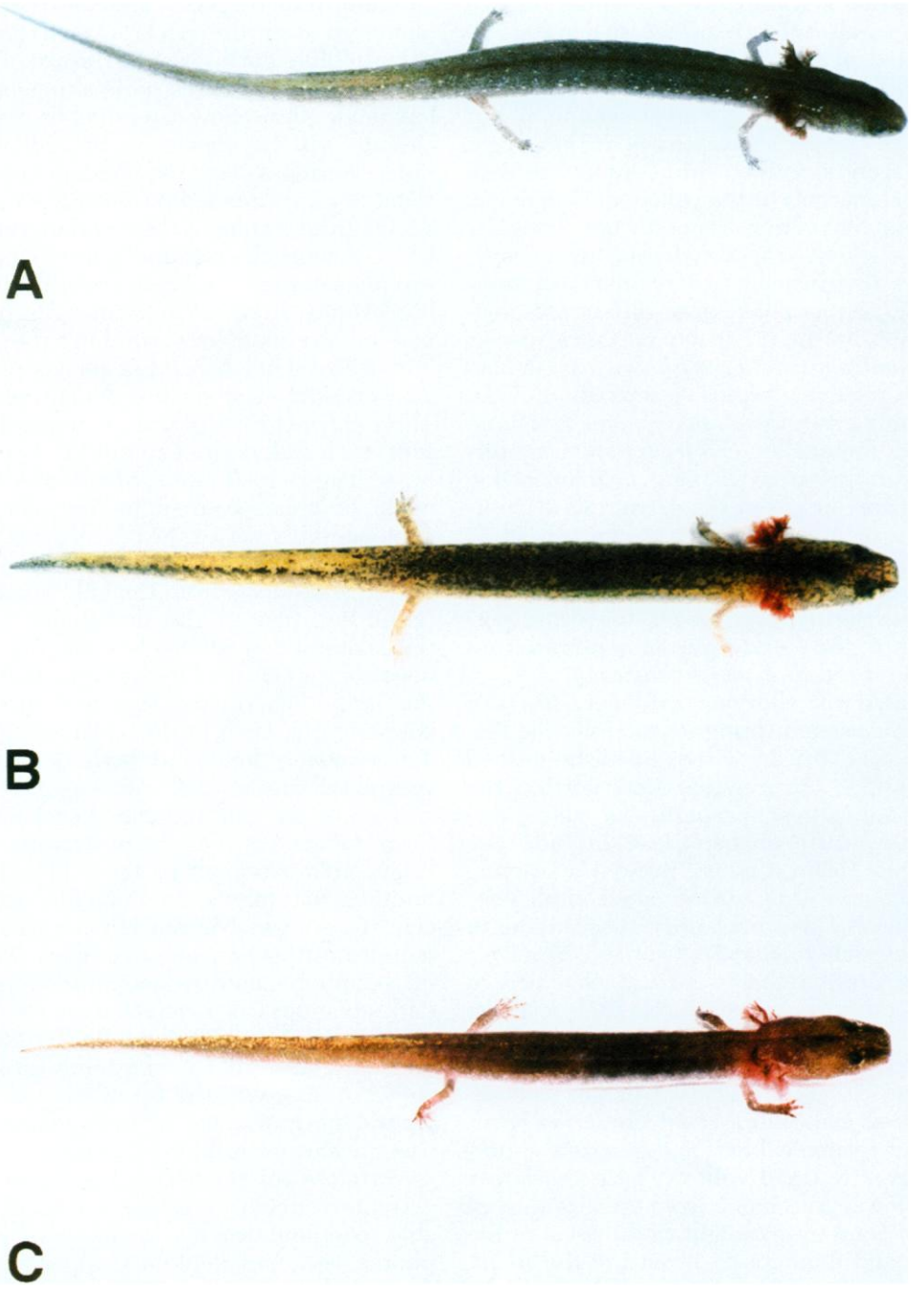
FIG. 9.—Holotypes of northern central Texas species of *Eurycea* in life. A. Holotype of *Eurycea tonkawae*, adult female, TNHC 50952. B. Holotype of *Eurycea naufragia*, adult male, TNHC 58860. C. Holotype of *Eurycea chisholmensis*, adult male, TNHC 58859.


A
B
C

populations in the region that we provisionally assign to this species. Based on external morphology, spring-dwelling specimens of *Eurycea tonkawae* appear very similar to *E. naufragia* (described below). However, the light areas surrounding each dorsolateral iridophore are square or oblong in all but two of the specimens examined in which this patterning was discernible; two exhibited the rosette or starburst shaped light areas seen in all but one of the 32 specimens of *E. naufragia* that were examined. In addition, the dark lateral margins of the yellow-orange dorsal tail fin coloration generally are irregular and chevron-shaped, whereas in *E. naufragia* they usually are regular and complete. *Eurycea tonkawae* differs osteologically from the other two northern species (*E. naufragia* and *E. chisholmensis*) in having a relatively extensive separation of the frontals posteriorly, and a larger frontoparietal fontanelle. *Eurycea tonkawae* further differs from *E. naufragia* in having the pars dorsalis of the premaxilla separated (in contact or abutting medially in *E. naufragia*) and from *E. chisholmensis* in having 16–17 presacral vertebrae (18 in *E. chisholmensis*) and having the distal portion of the ceratohyal mineralized (not mineralized in *E. chisholmensis*).

Based on allozyme evidence, *Eurycea tonkawae* from springs of the Jollyville Plateau and Brushy Creek is distinguished from other *Eurycea* that occur north of the Colorado River primarily by allele frequency differences; these include the unique Mdhp *a* allele, present at varying frequencies (but not observed in all populations). Three nucleotide substitutions in cytochrome *b* (C at 56, G at 198, T at 281) were present that were not observed in any other populations of the northern clade of central Texas *Eurycea*.

Description of holotype.—An adult female (TNHC 50952) with large, well-developed eyes and a relatively broad head. Snout relatively blunt and rounded in dorsal aspect. Head widest where the upper and lower jaws meet; from there outline of head continues straight to the level of the eyes and then curves inward to the snout. Three pairs of filamentous gills present on neck laterally just behind the angle of the jaws, deep rich red (in life) and increasing in size posteriorly. Gill insertions arranged in a stepped pattern with anterior pair farthest from and posterior pair closest to the dorsal midline; the anterior pair project laterally from the neck and the posterior pair arch over the neck. Dorsal ground color in life dark greenish-brown. Head and face anterior to the parietal region yellow in life, the yellow extending backwards laterally on the cheeks to the gill insertions, leaving a dark diamond-shaped parietal mark connected posteriorly with the dorsal ground color. A distinct dark canthal line connects the anterior corner of each eye to each naris, and another diffuse dark line extends from the anterior point of the parietal diamond forward along the midline of the skull. A band of melanophores is concentrated along the dorsal midline. Three distinct rows of iridophores are present, each iridophore centered in a square or oblong-shaped area which was dull-white or cream-colored in life. The two dorsolateral rows, the lateral-most just dorsal to the level of the limb insertions, extend posteriorly from the gills to the anterior insertion of the dorsal tail fin. A third lateral row at the level of the limb insertions is confined to the trunk between the limbs. There are 16 costal grooves, counting one each in the axilla and groin. Tail relatively long and both dorsal and ventral tail fins are well-developed. Dorsal surface of the tail and the dorsal tail fin from the level of the vent posteriorly were bright yellow-orange in life (color 18 of Smithe, Naturalist's Color Guide, American Museum of Natural History). Lateral margins of this area are a complex pattern of iridophores and melanophores forming dark chevrons, all connected as an irregular border. The ventral tail fin (bright yellow in life) is narrow, and extends from the vent posteriorly to the tip of the tail. The ventral body surface is immaculate and was translucent in life.

Variation (in alcohol).—The dorsal color pattern of different specimens is variable, compounded by the fact that iridophores and melanophores appear to be destroyed in some cases by initial formalin

fixation. Thus the holotype has faded to a very light color, and only the row of iridophores (more accurately, their enclosing light areas) along the lateral line is evident. The two dorsolateral rows of iridophores may be complete and distinct, one or the other may be incomplete or indistinct (e.g., TNHC 50972, 980–981), or both may be indistinct (TNHC 55142, 55144) or absent (TNHC 50962). In some cases there is a distinct row of melanophores concentrated along the lateral line with an irregular array of iridophores on either side (e.g., TNHC 55139). In two cases (TNHC 54226, 55154) the dorsolateral iridophores are scattered throughout the field and encompassed by starburst-shaped light areas. In many cases (e.g., TNHC 53465–473, 53475–478; 55152) no dorsal pattern remains, although iridophores may be scattered throughout the dorsal surface. Tail fins are not well-developed in two cases (TNHC 54230, 55134). Costal grooves are 14 (11 specimens), 15 (46 specimens), or 16 (32 specimens). (Specimens examined: 89; TNHC 6242; 50159; 50934–948, 50950–951, 50953–954, 50956–959, 50961–962, 50964–965, 50967, 50972–976, 50978–981, 50987–988, 50990, 50992–993; 51172; 53465–473, 53475–478; 54225–226, 54230–233; 55132–137, 55139–157).

Osteology.—Five cleared-and-stained specimens were examined; two from the type locality (TNHC 50955, 52759), one from Krienke Spring (TNHC 50963), and two from Brushy Creek Spring (TNHC 50991, 53474). Maxillae, septomaxillae, nasals and prefrontals are absent. Orbitosphenoids are present. The frontoparietal fontanelle is present and generally more extensive than in *Eurycea chisholmensis* or *E. naufragia,* and separation of the frontals is greater in *E. tonkawae* than in the other two taxa. The medial border of the parietals (forming the lateral margins of the frontoparietal fontanelle) is irregular in TNHC 50963, 50991, 53474, and one side of TNHC 52759 and is more straight-edged but scalloped at mid-length in 50955. The frontals are widely-separated and divergent posteriorly (but almost parallel in TNHC 50991) and approach each other closely at their anterior ends but do not contact. The frontals are relatively narrow, rounded posteriorly and overlapping the parietals and are pointed anteriorly and overlapped by the frontal processes of the premaxillae. The partes dentales of the premaxillae are fused. The frontal processes (pars dorsalis) are well-separated throughout their lengths. The premaxillae are dentate and bear 15 and 17 teeth in the two individuals from the type locality, and 11, 12, and 13 in the specimens from Brushy Creek. The vomer and palatopterygoid are present, dentate, and unfused. The parasphenoid lacks tooth-bearing patches, and the coronoid is present and dentate. The elements of the hyobranchial apparatus are primarily cartilaginous. All specimens have the posterior end of the second basibranchial ossified and triradiate (weakly ossified in TNHC 50991). Mineralization is also present on the distal ends of ceratobranchials I–III. The distal end of the ceratohyal is calcified only in the two specimens from Stillhouse Hollow. There are 17 presacral vertebrae in four specimens (not counting the atlas), and 16 in one (TNHC 53474). The sacral diapophyses are bicapitate in all individuals but one (TNHC 50963), in which they are fused. The carpals and tarsals are cartilaginous in all specimens. There are eight carpals in three specimens (50963, 50991, 53474) and one specimen appears to have seven carpals on one side through fusion of the prepollex and radiale. The cartilage on the other side of this specimen and in the other specimen from the type locality are poorly-stained and could not be scored for this feature. Four specimens were scored for variation in tarsal morphology. There are nine unfused elements present in TNHC 50955 and on one side of two other specimens (TNHC 50991 and 53474); on the other side of these two specimens and in TNHC 50963 the number of elements is seemingly reduced to eight by the fusion of tarsals four and five. Phalangeal formulae are: 1–2–3–2 (hand) and 1–2–3–3–2 (foot).

Distribution.—Surface populations of this species occur in springs of the Jollyville Plateau region of Travis and William-

son Counties, Texas, and springs of nearby Brushy Creek, which drains eastward from the Jollyville Plateau. The known range includes the Brushy Creek, Bull Creek, Cypress Creek, Long Hollow Creek, and Walnut Creek drainages; the Spicewood Springs population provisionally assigned to this species (see below) is located in the Shoal Creek drainage. Populations that we assign to this species include those from Balcones Community Park Spring, Barrow Hollow Springs, Bull Creek Spring, Brushy Creek Spring, Canyon Creek Spring, Canyon Vista Spring, Horsethief Hollow Spring, Krienke Spring, McDonald Well Spring (see comments below), New Bull Creek Spring, Schlumberger Springs, Stillhouse Hollow Spring, Spicewood Springs (see comments below), and Wheless Spring. We also provisionally assign populations from Kretschmarr Salamander Cave, Travis Co., Testudo Tube, Williamson Co., and caves of the Buttercup Creek system, Williamson Co. to this species, but see comments below regarding the status of these populations. Precise locations of these sites are given in Appendix I.

Etymology.—This species is named for the Tonkawa tribe, who inhabited central Texas until the 1850's, when the tribe was relocated to Indian Territory (now Oklahoma). The Tonkawa made camps around the springs that this salamander inhabits, and the stone tools and other artifacts of the Tonkawa are still much in evidence around many of these springs. Tonkawa is derived from a Waco Indian word meaning "they all stay together," a phrase which also describes the localization of *Eurycea tonkawae* around spring outflows. The name *tonkawae* is a noun in the genitive case (we treat Tonkawa as a latinized modern name).

Conservation status.—Virtually the entire range of this species is encompassed by a single USGS 7.5′ Topographic Quad Map (Jollyville; ca. 143 km^2). Successive editions show this area of northwest Austin undergoing tremendous development during the last two decades, which continues apace to this day. For example, an office building was recently built directly above the easternmost known locality of *Eurycea tonkawae* in Round Rock. Large quantities of foam of unknown chemical composition have been observed issuing from Stillhouse Hollow Springs (the type locality), and some individuals from this locality examined recently exhibited spinal deformities. City of Austin personnel have been monitoring populations of *E. tonkawae* since January, 1997, and have found that salamander density and abundance decrease as water quality parameters associated with urbanization increase (Beth Davis, personal communication). In general, the aquifers upon which this species depends are small and localized, and thus are very susceptible to pollution, drying, or draining. Despite the location of some populations within City of Austin preserves and the existence of what may represent this species on the Travis County Audubon Sanctuary, the future of *Eurycea tonkawae* remains problematic.

Additional comments.—We assign two spring populations, McDonald Well Spring and Spicewood Springs (both in Travis Co.) to *Eurycea tonkawae.* Although neither has yet been examined for molecular markers, both occur within the known range of *E. tonkawae* and appear morphologically similar to other specimens of this species. Specimens examined for the latter two localities are MVZ 122705–711 (McDonald Well Spring) and TNHC 54230–233 (Spicewood Springs). Four specimens (TNHC 21640–643) collected in 1956 by J. K. Baker are listed as from "Travis: Dodd City: Jack Dies Ranch". According to Mary Helen Bunton, who owns the property on which McDonald Well Spring is located (personal communication to AHP and PTC), the Jack Dies Ranch was adjacent to this site (across FM 2769); thus, salamanders from this locality probably represent *E. tonkawae.*

We provisionally assign the population of *Eurycea* from Kretschmarr Salamander Cave to *E. tonkawae,* based primarily on geographic distribution. This population occurs in a tiny stream cave on the Jollyville Plateau in the vicinity of known spring localities for *E. tonkawae.* Salamanders from this locality appear superficially

similar to animals from nearby spring localities. However, they are somewhat distinct based on allozymes and may be isolated from gene flow with other populations; further study is necessary.

We provisionally assign *Eurycea* from Testudo Tube Cave, and caves of the Buttercup Creek Cave system (Williamson Co.) to *E. tonkawae*, but note that the systematic status of these recently-discovered cave populations is problematic. The few known specimens from Testudo Tube appear similar to surface animals, and salamanders which probably represent *E. tonkawae* are known from springs on the nearby Audubon Preserve (we were unable to collect at the Audubon locality although we have examined specimens in the field). Testudo Tube may be separated hydrologically from the nearby Buttercup Creek system (Russell, 1993) and thus salamanders from Testudo Tube may not be conspecific with those of the Buttercup Creek caves. The recently discovered Buttercup Creek Cave system, in the Cedar Park area of Williamson Co., is a relatively extensive and probably hydrologically interconnected subterranean system (e.g., see Russell, 1993). However, the *Eurycea* that inhabit these caves display considerable variability in allozymes; this could simply be an artifact of limited sampling. Most salamanders that have been observed in the Buttercup Creek caves exhibit strong cave-associated morphologies including depigmentation, eye reduction, and broadening and flattening of the head (personal observation and J. Reddell, personal communication to PTC), but so few specimens are available that generalizations about the morphologies of these salamanders are difficult. One potential sequence synapomorphy unites the Testudo Tube population with that from Ilex Cave, the single member of the Buttercup Creek caves group for which sequence data are available. Combined parsimony analysis of all molecular data (Fig. 7) indicates a sister relationship between Testudo Tube and the Buttercup Creek Caves, with Kretschmarr salamander cave sister to this pair, and this group in turn sister to spring-dwelling populations of *E. tonkawae* from the Jollyville Plateau and Round Rock areas.

We strongly suspect that salamanders of the Buttercup Creek Caves system, and perhaps Testudo Tube, represent a distinct evolutionary lineage, but additional study is needed.

Eurycea ***naufragia*** sp. nov.
Fig. 9B

Eurycea neotenes: Sweet 1982:442 (in part).

Holotype.—TNHC 58860 (field number PC 1998-10), an adult male collected by David M. Hillis and Laurie A. Dries, 14 August 1998 from the headsprings of Buford Hollow, a small tributary of the South San Gabriel River below Lake Georgetown, 30° 39′ 39″ N, 97° 43′ 36″ W.

Paratypes.—TNHC 50999–51008, 51010–018, 51023–025, 51027–031, 55386, 58861; MVZ 122775. Cleared-and-stained: TNHC 51026, 51009.

Diagnosis.—Based on external morphology, specimens of *Eurycea naufragia* appear very similar to individuals from spring-dwelling populations of *E. tonkawae*. However, the light areas surrounding each dorsolateral iridophore are rosette or starburst shaped in all but one of the specimens examined, unlike those of nearly all specimens of *E. tonkawae* examined. In addition, the dark lateral margins of the yellow dorsal tail fin coloration are regular and complete, whereas in *E. tonkawae* they usually are irregular.

Osteologically, *Eurycea naufragia* differs from *Eurycea tonkawae* and *E. chisholmensis* in having the pars dorsalis of the premaxillae in contact or abutting medially (although these processes are close in one individual of *E. chisholmensis*). *Eurycea naufragia* further differs from *E. tonkawae* in having less extensive separation of the frontals posteriorly and a smaller frontoparietal fontanelle, and from *E. chisholmensis* in having 17 presacral vertebrae (18 in *E. chisholmensis*), the distal portion of the ceratohyal mineralized (not mineralized in *E. chisholmensis*), distal tarsals 4 and 5 fused (unfused in *E. chisholmensis*), and relatively irregular medial borders of

the parietal (relatively straight in *E. chisholmensis*).

Based on allozyme evidence, *E. naufragia* can be distinguished from other northern central Texas *Eurycea* by possession of the unique Acon-1 *f* allele at medium allele frequency in all populations examined except Avant's Spring and occurrence of the unique Ck-1 *c* allele at medium frequency in all populations examined. It is distinguished from *E. tonkawae* by high frequency of the Pep-D *a* allele (also seen in *E. chisholmensis*), and from *E. tonkawae* and *E. chisholmensis* by apparent fixation of the G3pdh *d* allele (this allele also was seen in one individual from the Buttercup Creek cave group that we provisionally assign to *E. tonkawae*). In *E. naufragia* from the Lake Georgetown area, we also found five nucleotide substitutions in cytochrome *b* that were not observed in any members of the northern clade of central Texas *Eurycea,* specifically T at 62, T at 88, G at 179, C at 213, and G at 300. *Eurycea naufragia* from springs of the Georgetown area is quite distinct from *E. tonkawae* and *E. chisholmensis* based on cyt *b* sequence (more so than is either of these latter species from one another), despite its geographic occurrence between the ranges of these two species.

Description of holotype.—An adult male with a gray dorsal ground color in life, except for a light-brown streak extending posteriorly from a diamond-shaped mark in the parietal area of the head along the midline to the base of the tail. There are melanophores scattered throughout the dorsal and lateral surfaces of the body, including the limbs and digits, leading to the formation of a black, finely-reticulated, netlike pattern. There are a few scattered melanophores on the palms of all four feet, and along the margin of the lower jaw. Head broad but relatively short, widest where the upper and lower jaws meet; snout slightly rounded and short. Eyes large; iris gold in life. There are distinct melanophore concentrations forming a black circle around each eye and a black canthal line from the anterior corner of the eye through each naris. Three pairs of filamentous gills present on neck laterally just behind the angle of the jaws, deep rich red (in life), increasing in size posteriorly. Gill insertions arranged in a stepped pattern so that the anterior pair is farthest and the posterior pair closest to the dorsal midline; the anterior pair project laterally from the neck and the posterior pair arch over the neck. Their stalks were gray (in life) with scattered melanophores. There are three more-or-less well-defined longitudinal rows of iridophores on the body, one along the lateral line and two beginning just dorsal to the anterior limb insertions and extending onto the tail. Each iridophore sits at the center of a pale "rosette" or starburst-shaped area, light cream- or flesh-colored in life, with the irregular margin of each defined by the unique interaction of its pigment and the encroaching melanin. The row along the lateral line may more appropriately be termed a field, as iridophores are dense and scattered throughout. Of the two remaining rows, the rosettes of the superior row are indistinct, especially anteriorly, whereas those of the inferior row are more distinct, almost rectangular, and almost form a continuous longitudinal streak. Skin pigment ceases below the level of the lateral line, and the ventral surface is immaculate and (in life) translucent. There are 16 costal grooves, counting one each in the axilla and groin. The dorsal tail fin, which begins at the level of the fourth caudal vertebra, is poorly developed. It is translucent along the free margin and cream-colored towards its base, with melanophores scattered throughout. In life, the base of the fin was extensively suffused with a rich golden-yellow color which extended laterally on to the tail itself, encompassing some of the iridophores there. Melanophores are densely concentrated along the lateral margins of this yellow field, forming a distinct black border. The ventral surface of the tail is very finely mottled with melanophores on either side of a weakly-developed ventral tail fin, which begins at the level of the third caudal vertebra and is pigmented similarly to the dorsal fin.

Description of paratopotype in life.—An adult male (TNHC 58861) which differs from the holotype in the following

ways: an overall darker animal as the result of a greater concentration of melanophores and a coarser dorsal pattern; denser melanophores on the palms of the feet but none on the lower jaw; iridophores along the lateral line more distinct and in a single row anteriorly; iridophores of dorsolateral rows distinct within their rosettes only anteriorly; dorsal tail fin small but distinct and beginning at level of sixth caudal vertebra; basal tail fin coloration dull orange in life, not extending laterally on to the tail, and lateral melanophore border less dense.

Variation (in alcohol).—The dorsal pattern varies from an overall light appearance due to a paucity of melanophores (e.g., TNHC 54556) to a very dense concentration of melanophores with only scattered iridophores present in no apparent order (e.g., TNHC 51014, 54560). The dark eye ring remains in specimens of the former type. One specimen (TNHC 54558) has the margins of iridophore fields forming more regular ovoid or rectangular outlines rather than a rosette or starburst shape. Costal grooves are 14 (5), 15 (16), or 16 (3). (Specimens examined: 24; TNHC 50999–51005, 51007–008, 51010–017, 51023–025, 51027–028, 51030; 55386). Of eight additional specimens from Cowan Creek Spring (TNHC 54555–562), tentatively assigned to this species, three possess 15 costal grooves and five possess 16.

Osteology.—Two cleared-and-stained specimens were examined, TNHC 51009 and 51026. Maxillae, septomaxillae, nasals and prefrontals are absent. Orbitosphenoids are present. The frontoparietal fontanelle is present. The medial border of the parietals is irregular with short, finger-like projections extending into the frontoparietal fontanelle. The frontals diverge slightly posteriorly but are nearly parallel for most of their lengths. They are in medial contact anteriorly in TNHC 51026 and almost in contact in TNHC 51009. The frontals are rounded posteriorly and overlapping the parietals and are pointed anteriorly and overlapped by the frontal processes of the premaxillae. The partes dentales of the premaxillae are fused. The frontal processes are in contact in TNHC 51026 and are abutting in TNHC 51009. The premaxillae are dentate with 14 (TNHC 51026) and 17 (TNHC 51009) teeth. The vomer and palatopterygoid are present, dentate, and unfused. The parasphenoid lacks tooth-bearing patches, and the coronoid is present and dentate. The elements of the hyobranchial apparatus are primarily cartilaginous. Both specimens have the posterior end of the second basibranchial ossified and triradiate. Mineralization is also present on the distal ends of the ceratohyal and ceratobranchials I–III, with mineralization at least three times more extensive in TNHC 51026. There are 17 presacral vertebrae, not counting the atlas. The sacral ribs are bicapitate. The carpals and tarsals are cartilaginous in all specimens. There are eight carpals and eight tarsals; the number of tarsals is seemingly reduced to eight by the fusion of tarsals 4 and 5. Phalangeal formulae are: 1–2–3–2 (hand) and 1–2–3–3–2 (foot).

Distribution.—This species is known only from springs and possibly one cave in Williamson County, Texas, associated with drainages of the south, middle and north forks of the San Gabriel river. We also provisionally assign populations from the Cowan Creek drainage to this species. Cowan Creek drains into Berry Creek, which drains into the San Gabriel River below the city of Georgetown. The population from Bat Well, which we also provisionally assign to this species, is located in the Berry Creek drainage.

Etymology.—The name of this species is derived from the Latin *naufragium,* which means "remnants" or "remains." The name refers to the springs in which this species lives, several of which have been destroyed or are threatened by human development.

Conservation status.—Based on our observations, the populations of this species within the City of Georgetown proper probably are on the brink of extirpation. The recently discovered populations on Cowan Creek that we provisionally assign to this species lie within Sun City Georgetown, a new leisure and retirement com-

munity designed to accommodate 9,000 homes (about 18,000 people) at build-out. It is our understanding that management of Sun City Georgetown are aware of the springs within their tract, and have no plans to physically impact them. The several springs harboring *Eurycea naufragia* along the Middle Fork of the San Gabriel River are in the immediate vicinity and just downstream of a large quarrying operation on leased land. The lessee, Capitol Aggregates, has consulted with one of us (AHP) after commissioning separate hydrogeological and salamander surveys of the area, and it seems probable under current conditions that the quarrying activity will not jeopardize recharge and springflow. We believe there probably are undiscovered localities for *E. naufragia* within the San Gabriel River watershed west of Georgetown; therefore it is difficult to project the conservation status of this species. The realizable range of *E. naufragia* probably is no greater than that of the Jollyville Plateau Salamander, however, and the development pressure on the area that its range encompasses likely will soon catch up with that around Austin. We assume that salamanders reported by Sweet (1978*a*, 1984) from springs in the city park at Georgetown are conspecific with those that we examined from nearby springs in the vicinity of Lake Georgetown. A single individual that we found at the Georgetown Park site in 1991 was a tiny juvenile that failed to yield successful results for key molecular markers. Here we provisionally assign salamanders from the Bat Well and Cowan Creek Springs populations to *E. naufragia*, based largely on geographic proximity. The single individual available from Bat Well differs with respect to some molecular markers from other *E. naufragia* examined, although in most respects it appears similar morphologically. The Cowan Creek Spring population was discovered late in the course of this study, and animals from this locality have not yet been examined for key molecular markers. Eight specimens are available (TNHC 54555–562). Two of these exhibit eye diameter : axilla-groin length ratios within the range of those seen in salamanders from Salado Springs (although in other respects they appear to be specimens of *E. naufragia*); the others appear in all respects (based on external morphology) to be specimens of *E. naufragia*. Given this situation, and the problematic placement of the Bat Well population, further study of these and other populations of salamanders in the region between Georgetown and Salado is needed to verify their taxonomic status.

Eurycea ***chisholmensis*** sp. nov.
Fig. 9C

Eurycea neotenes: Sweet 1982:441 (in part).

Holotype.—TNHC 58859 (field number PC 1998–9), an adult male collected by Paul T. Chippindale on 1 August 1998, in approximately 10 cm of water at side spring immediately adjacent to Main (= Salado, Big Boiling, or Siren) Springs, Salado, Bell Co., Texas, 30° 56′ 37″ N, 97° 32′ 31″ W., at approximately 1030 h.

Paratypes.—TNHC 51139–146. Cleared-and-stained: TNHC 52770–771.

Diagnosis.—*Eurycea chisholmensis* generally exhibits reduced eyes in comparison to other spring-dwelling north central Texas *Eurycea* (see Fig. 10). The dorsolateral body fields lack well-defined iridophores or melanophores, in contrast to *E. tonkawae* and *E. naufragia*. The dark eye ring seen in most individuals of *E. naufragia* and *E. tonkawae* is absent, and the upper lip between the posterior border of the eye and the naris lacks dark pigment altogether. Dorsal coloration typically is dark, with a series of fine, lighter reticulations; in contrast, other surface-dwelling north central Texas *Eurycea* usually exhibit varying densities of scattered melanophores on a lighter background. Dark lateral margins of yellow dorsal tail fin coloration are absent.

Morphometric differentiation between *E. chisholmensis* and the other new northern species with respect to the ratio of eye diameter to axilla-groin length is illustrated in Fig. 10.

Eurycea chisholmensis differs osteologically from *E. tonkawae* and *E. naufragia*

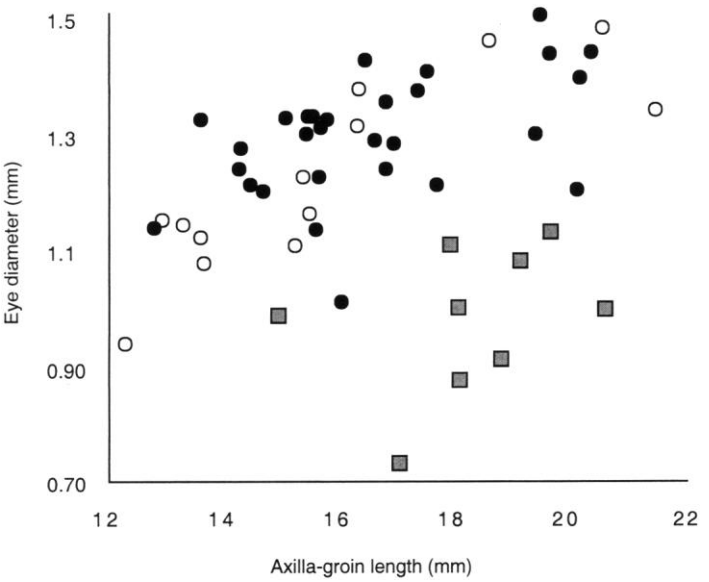


FIG. 10.—The relationship between eye diameter and axilla-groin length in *Eurycea tonkawae* (solid circles), *E. naufragia* (open circles), and *E. chisholmensis* (grey squares).

in having 18 presacral vertebra (as opposed to the usual 17 in *E. tonkawae* and *E. naufragia*; one individual of *E. tonkawae* examined has 16), and by the absence of mineralization on the distal portion of the ceratohyal. *Eurycea chisholmensis* further differs from *E. naufragia* in having distal tarsals 4 and 5 unfused (fused in *E. naufragia*), relatively straight medial borders of the parietal (irregular in *E. naufragia*), and medial separation of the pars dorsalis of the premaxilla (in contact or abutting in *E. naufragia*). *Eurycea chisholmensis* further differs from *E. tonkawae* in having less extensive separation of the frontals posteriorly and a smaller frontoparietal fontanelle.

Based on allozyme evidence, *Eurycea chisholmensis* can be distinguished from *E. tonkawae* and nearly all other northern central Texas *Eurycea* provisionally assigned to this species by apparently fixed occurrence of the Pep-D *a* allele. This species appears to lack any of the unique alleles found in *E. naufragia*, the nearest taxon geographically. One nucleotide substitution in cytochrome *b* (T at 252) also distinguishes this taxon from other members of the northern clade of central Texas *Eurycea* examined, and it appears to lack the unique sequence substitutions that characterize *E. naufragia*, spring populations of *E. tonkawae*, or cave populations provisionally assigned to *E. tonkawae*.

Description of holotype.—An adult male (TNHC 58859). Eyes are reduced and head is flat; in lateral profile eyes do not project above the level of the head. Head is relatively long, and snout is blunt and rounded in dorsal aspect. Head is broadest where the two jaws meet, and narrows very gradually to the level of the eyes and then slightly more to the snout. There are three pairs of filamentous gills on the neck laterally just behind the angle of the jaws; these were reddish-brown (in life) and increase in size posteriorly; the most posterior arch slightly over the neck. Gill insertions are arranged in a stepped pattern so that the anterior pair are farthest and the posterior pair closest to the dorsal midline. Dorsal coloration nearly uniform grayish brown in life with a slight cinnamon tinge, with occasional tiny, lighter flecks present at irregular intervals. Under magnification, dorsal pigmentation appears as a series of fine reticulations. Laterally, the coloration becomes a fine speckling on a cream (in

life) background, and the ventral surface is largely translucent. Large, dark testes were readily visible through the skin of the ventral surface in life. Lateral iridophores are nearly indistinguishable and are uncountable; there is an ill-defined, faint series of marks slightly paler than the surrounding areas (this may correspond to iridophores) present between the front and hind legs. Front and hind legs cream-colored in life, densely speckled with brown above; the undersides of the front legs are nearly free of this speckling, and it is restricted primarily to the bases of the undersides of the hind legs. The top of the head has widely scattered flecks, cream-colored in life; the area immediately surrounding the gill insertions was pink in life. The undersides of the eyes are outlined in cream; this is not visible from directly above because the dorsal portions of the eyes are partly covered with skin. Iris of eye in life pale gold with dark flecking. Very faint brown canthal lines are visible but indistinct. The upper labial region lacks dark pigmentation from the posterior margin of the eye to the naris, while the lower labial region exhibits slight dark speckling at the posterior edge, decreasing anteriorly. A prominent gold stripe was present in life on the dorsal part of the tail, narrow at the base and widening toward the middle; by about ⅔ of the way along the tail, the gold color became increasingly fragmented, changing to speckling along the caudal fin on the posterior part of the tail. Sides of the tail were also speckled with yellow in life. In life, the underside of the tail exhibited a pale yellow line, extending from the posterior opening of the vent along and onto the ventral caudal fin. Both dorsal and ventral caudal fins are well developed on the posterior portion of the tail.

Description of paratopotype.—An adult, sex uncertain (TNHC 51146) with a drab ground color in life (color 27 of Smithe, Naturalist's Color Guide, American Museum of Natural History). There is a light ring around each eye due to the absence of melanophores in this area. Descriptions of head and gills are as for the holotype, except that in this specimen the gills are reduced and were bright red in life; the anterior pair project laterally from the neck but the proximal pair to the midline do not arch over the neck. A dark parietal diamond-shaped mark is present but indistinct, as are the canthal lines connecting the anterior corners of the eyes with the nares. Dorsolateral fields of the trunk light-brown with faint light lines in life, and lack obvious iridophores or melanophores. The dorsal tail fin is well-developed posteriorly but small anteriorly, and ends abruptly before it reaches the vent. The ventral tail fin is weakly developed and occupies only the posterior one-third of the tail. Both tail fins translucent in life except for a scattering of melanophores. Total circumference of the surface of the anterior half of the tail yellow in life, but this area lacks a defining border of any kind.

Variation (in alcohol).—The degree of patterning of white lines within the brown to gray dorsolateral fields is variable, from very few (TNHC 51141) to extremely dense (TNHC 51145). There may occasionally be a few larger, irregularly-shaped, light areas superimposed on this basic pattern (TNHC 51146). The dark canthal line, which is usually poorly defined, may be absent (TNHC 51143). The dorsal tail fin may be essentially absent (TNHC 51142). Costal grooves are 15 (5 specimens) or 16 (2 specimens). (Specimens examined: 7; TNHC 51139, 51141–146).

Osteology.—Two cleared-and-stained specimens were examined: TNHC 52770 and 52771. Maxillae, septomaxillae, nasals and prefrontals are absent. Orbitosphenoids are present but are very small in TNHC 52770. The frontoparietal fontanelle is present. The medial border of the parietals is more-or-less straight and regular, but less so in TNHC 52771. The frontals are in contact medially near their articulation with the premaxillae in TNHC 52771, and are slightly separated in TNHC 52770. They are parallel for most of their lengths in TNHC 52770 and are parallel for their anterior half in TNHC 52771. The frontals are rounded posteriorly and overlapping the parietals and are pointed and somewhat irregular anteriorly and overlapped by the frontal processes of the

premaxillae. The partes dentales of the premaxillae are fused. The frontal processes are separate but in close proximity in TNHC 52770. The premaxillae are dentate with 17 (TNHC 52771) and 18 (TNHC 52770) teeth. The vomer and palatopterygoid are present, dentate, and unfused. The parasphenoid lacks tooth-bearing patches, and the coronoid is present and dentate. The elements of the hyobranchial apparatus are primarily cartilaginous. Both specimens have the posterior end of the second basibranchial ossified and triradiate. TNHC 52771 also has limited mineralization near the posterior end of ceratobranchials I and III. There are 18 presacral vertebrae, not counting the atlas. The two heads and corresponding diapophyses of the presacral ribs are fused but still distinguishable in TNHC 52770 whereas in TNHC 52771 two heads are distinct on one side and completely fused on the other. The carpals and tarsals are cartilaginous in all specimens, and there are eight carpals and nine tarsals. Phalangeal formulae are: 1–2–3–2 (hand) and 1–2–3–3–2 (foot).

Distribution.—This species is known only from Big Boiling (= Main, Salado, or Siren) Springs and Robertson Springs at Salado, Bell Co., Texas. Recently, salamanders that could represent this species have been found in springs of nearby Buttermilk Creek (G. Longley, personal communication to PTC) but specimens are not yet available for examination.

Etymology.—Jesse Chisholm laid out a trail from the Canadian River in Indian Territory to Wichita, Kansas in 1865, and started driving cattle up the trail in 1866. By 1867, the Chisholm Trail had been extended south into Texas, and Salado was an important stop on the trail because of its clean, clear springs, which are also the habitat of this species. The name *chisholmensis* is an adjective referring to the Chisholm Trail.

Conservation status.—Determining the conservation status of this species is problematical, given the difficulty with which specimens have been acquired and lack of knowledge of the extent of its range. Most of the spring outlets at Salado have been modified to some extent during the past 150 years, and the type locality is on the south bank of Salado Creek in a municipal park. Several groundwater contamination incidents have occurred in the recent past (Price et al., 1995) and the potential for more still exists. As we have suggested, however, additional localities may yet be discovered, and we believe it is still possible to plan and implement conservation measures for this species.

Additional comments.—This is the northeasternmost known population of *Eurycea* in the Edwards Plateau region, and salamanders from these springs are very elusive. Prior to this study, only a single juvenile specimen was known (private collection of B. C. Brown, Baylor University); we obtained most of the known specimens in 1989–1991 but (despite more than 20 additional visits to the type locality between 1991 and 1998) found no others until 1998, when the holotype was collected.

CONCLUSIONS

Many problems remain in the the taxonomic allocation of the central Texas *Eurycea.* This is not surprising, given the large number of populations, extreme geographic fragmentation, and the complex mixture of morphological conservatism and apparent parallelism or convergence. Our findings are consistent with a general pattern in plethodontid salamanders: in almost every wide-ranging "species" that has been studied using molecular techniques, numerous morphologically cryptic species have been revealed (reviewed by Larson and Chippindale, 1993). We see the work described here as the basis for more detailed studies of relationships and species boundaries within and among the taxa that we have recognized. The identification of the major monophyletic groups in this assemblage will facilitate future systematic and taxonomic work, as it will be possible to focus on particular subsets of the Texas *Eurycea* that represent historical (monophyletic) units. Previously, for example, a study of "*E. neotenes*" would necessarily have had to encompass the whole region and what have proven to be numerous dis-

tinct species. Future work on the group should include both morphological study and use of rapidly-evolving molecular markers, and such work is in progress by the authors of this paper. We are optimistic that further study will clarify many of the systematic and taxonomic problems that remain in the Texas *Eurycea,* and hope that it will be possible to characterize and preserve the diversity in the group before much of it is lost due to human activities.

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APPENDIX I

Localities for populations of central Texas hemidactyliine salamanders examined in this study

Watersheds in which each locality is located are given where not obvious based on the locality name. In cases in which formal taxon names have been assigned to populations from particular localities, those names are used. Informal groups to which populations have been assigned based on this study are listed where appropriate.

Bandera County.—Murphy's Spring (Wedgeworth Creek South Spring), Sabinal River watershed, 29° 48′ 00″ N, 98° 33′ 31″ W. (Southwest, *E. troglodytes* complex, Sabinal group); Sabinal Canyon Spring, Sabinal River watershed, 29° 49′ 26″ N, 99° 34′ 01″ W. (Southwest, *E. troglodytes* complex, Sabinal group); Sutherland Hollow Spring, west prong Medina River, 29° 44′ 58″ N, 99° 25′ 36″ W. (Southwest, *E. troglodytes* complex, Carson Cave group).

Bell County.—Salado (Big Boiling, Main, or Siren) Springs, Salado Creek, 30° 56′ 37″ N, 97° 32′ 31″ W. (North, *E. chisholmensis*–type locality); Salado (Robertson) Springs, Salado Creek, 30° 56′ 37″ N, 97° 32′ 39″ W. (North, *E. chisholmensis*).

Bexar County.—Helotes Creek Spring, Medina River watershed, 29° 38′ 15″ N, 98° 41′ 40″ W. (Southeast, *E. neotenes*–type locality); Leon Springs, Leon Creek, Medina River watershed, 29° 39′ 46″ N, 98° 38′ 14″ W. (Southeast, *E. neotenes*).

Blanco County.—Boardhouse Springs, Blanco River watershed, 30° 06′ 40″ N, 98° 18′ 07″ W. (Southeast, *E. pterophila*); T-Cave, Blanco River watershed, 30° 04′ 36″ N, 98° 19′ 46″ W. (Southeast, *E. pterophila*); Zercher Spring, Blanco River watershed, 30° 06′ 10″ N, 98° 27′ 25″ W. (Southeast, *E. pterophila*).

Comal County.—Badweather Pit, Cibolo Creek watershed, 29° 45′ 21″ N, 98° 37′ 13″ W. (Southeast, *E. tridentifera*); Comal Springs, headwaters of the Comal River, 29° 42′ 49″ N, 98° 08′ 13″ W. (Southeast, *E.* sp.); Ebert Cave, Cibolo Creek watershed, 29° 45′ 06″ N, 98° 23′ 28″ W. (Southeast, *E. tridentifera*); Honey Creek Cave, Guadalupe River watershed, 29° 50′ 50″ N, 98° 29′ 30″ W. (Southeast, *E. tridentifera*–type locality); Rebecca Creek Spring, Guadalupe River watershed, 29° 55′ 28″ N, 98° 22′ 22″ W. (Southeast, *E. latitans* complex).

Edwards County.—Smith's (= Dutch Creek) Spring, Nueces River watershed, 29° 39′ 09″ N, 100° 06′ 12″ W. (Southwest, *E. troglodytes* complex, Carson Cave group); West Nueces River Spring, 29° 43′ 20″ N, 100° 24′ 51″ W. (Southwest, *E. troglodytes* complex, Carson Cave group).

Gillespie County.—Trough Spring, Pedernales River watershed, 30° 08′ 36″ N, 99° 04′ 40″ W. (Southwest, *E. troglodytes* complex, Carson Cave group).

Hays County.—Ezell's Cave, San Marcos River watershed, 29° 52′ 27″ N, 97° 57′ 34″ W. (San Marcos, *E. rathbuni*); Fern Bank (Little Arkansas) Springs, Blanco River watershed, 29° 59′ 00″ N, 98° 00′ 49″ W. (Southeast, *E. pterophila*–type locality); Grapevine Cave, Blanco River watershed, approximately 30° 02′ 30″ N, 98° 12′ 45″ W. (Southeast, *E. pterophila*); Rattlesnake Cave, San Marcos River watershed, 29° 54′ 07″ N, 97° 55′ 17″ W. (San Marcos, *E. rathbuni*); San Marcos (Aquarena) Springs, pipe outflow at submarine theater, headwaters of the San Marcos River, 29° 53′ 35″ N, 97° 55′ 50″ W. (San Marcos, *E. rathbuni*); San Marcos (Aquarena) Springs, headwaters of the San Marcos River, 29° 53′ 35″ N, 97° 55′ 50″ W. (San Marcos, *E. nana*–type locality).

Kendall County.—Bear Creek Spring, Medina River watershed, 29° 48′ 15″ N, 98° 52′ 10″ W. (Southeast, *E. latitans* complex); Cibolo Creek Tributary Spring, Cibolo Creek watershed, 29° 49′ 03″ N, 98° 51′ 43″ W. (Southeast, *E. latitans* complex); Kneedeep Cave Spring, Guadalupe River State Park, 29° 52′ 31″ N, 98° 29′ 05″ W. (Southeast, *E. latitans* complex); Less Ranch Spring, Guadalupe River water-

shed,29° 46′ 40″ N, 98° 50′ 52″ W. (Southeast, *E. latitans* complex); Mueller's Spring, Medina River watershed, approximately 29° 44′ N, 98° 47′ 30″ W. (Southeast, *E. neotenes*); Peavey's Springs, headwaters of the Blanco River, approximately 30° 05′ 30″ N, 98° 39′ 30″ W. (Southeast, *E. pterophila*); Pfeiffer's Water Cave, Guadalupe River watershed, 29° 45′ 44″ N, 98° 39′ 59″ W. (Southeast, *E. latitans*–subterranean extension of type locality).

Kerr County.—176 Spring, Guadalupe River watershed, 30° 05′ 18″ N, 99° 19′ 14″ W. (Southwest, *E. troglodytes* complex); Cherry Creek Spring, Guadalupe River watershed, 29° 50′ 41″ N, 98° 56′ 52″ W. (Southeast, *E. latitans* complex); Cloud Hollow Spring, Medina River watershed, 29° 50′ 36″ N, 98° 57′ 14″ W (Southeast, *E. latitans* complex); Edmunson Creek (Camp Mystic) Springs, Guadalupe River watershed, 30° 00′ 21–3″ N, 99° 21′ 43–54″ W. (Southwest, *E. troglodytes* complex); Fessenden Springs, Guadalupe River watershed, 30° 10′ 00″ N, 99° 20′ 32″ W. (Southwest, *E. troglodytes* complex, Carson Cave group); Robinson Creek (Highway 16 South) Spring, north prong Medina River watershed, 29° 54′ 55″ N, 99° 15′ 08″ W. (Southwest, *E. troglodytes* complex, Carson Cave group).

Real County.—Greenwood Valley Ranch Spring #1, east prong Nueces River, 29° 57′ 20″ N, 99° 58′ 17″ W. (Southwest, *E. troglodytes* complex); Greenwood Valley Ranch Spring #2, east prong Nueces River, 29° 59′ 11″ N, 99° 57′ 51″ W. (Southwest, *E. troglodytes* complex); Greenwood Valley Ranch Spring #3, east prong Nueces River, 29° 59′ 22″ N, 99° 57′ 13″ W. (Southwest, *E. troglodytes* complex); Tucker Hollow Cave, Frio River watershed, 29° 44′ 33″ N, 99° 46′ 42″ W. (Southwest, *E. troglodytes* complex).

Travis County.—Balcones Community Park Spring, Walnut Creek watershed, 30° 24′ 45″ N, 97° 43′ 02″ W. (North, *E. tonkawae*); Barrow Hollow Spring, Bull Creek watershed, 30° 22′ 33″ N, 97° 46′ 02″ W. (North, *E. tonkawae*); Barton Springs, Barton Creek, 30° 15′ 49″ N, 97° 46′ 14″ W. (Southeast, *E. sosorum*–type locality); Bull Creek (Hanks Tract) Spring, north fork Bull Creek, 30° 25′ 38″ N, 97° 49′ 08″ W. (North, *E. tonkawae*); Bull Creek Spring Pool (New Bull Creek Spring), west fork Bull Creek, 30° 24′ 59″ N, 97° 49′ 00″ W. (North, *E. tonkawae*); Canyon Creek Spring, north fork Bull Creek, 30° 25′ 33″ N, 97° 48′ 51″ W. (North, *E. tonkawae*); Canyon Vista Spring, Bull Creek watershed, 30° 25′ 51″ N, 97° 46′ 55″ W. (North, *E. tonkawae*); Hammett's Crossing Spring #1 (Pedernales Spring #1), Pedernales River, 30° 20′ 28″ N, 98° 08′ 14″ W. (Southeast, *E.* sp.); Hammett's Crossing Spring #2 (Pedernales Spring #2), Pedernales River, 30° 20′ 23″ N, 98° 08′ 15″ W. (Southeast, *E.* sp); Horsethief Hollow Spring, Bull Creek watershed, 30° 24′ 31″ N, 97° 49′ 00″ W. (North, *E. tonkawae*); Kretschmarr Cave, Colorado River watershed, 30° 24′ 47″ N, 97° 51′ 10″ W. (North, provisionally *E. tonkawae*); Schlumberger Spring, headwaters west fork Bull Creek, 30° 25′ 15″ N, 97° 50′ 24″ W. (North, *E. tonkawae*); Stillhouse Hollow Springs, Bull Creek watershed, 30° 22′ 28″ N, 97° 45′ 55″ W. (North, *E. tonkawae*–type locality); Wheless Springs, Long Hollow Creek, Colorado River watershed, 30° 27′ 42″ N, 97° 52′ 28″ W. (North, *E. tonkawae*).

Uvalde County.—Carson Cave, West Nueces River watershed, 29° 28′ 50″ N, 100° 04′ 44″ W. (Southwest, *E. troglodytes* complex, Carson Cave group); WB (= Wetback) Spring, Sabinal River watershed, 29° 35′ 12″ N, 99° 36′ 14″ W. (Southwest, *E. troglodytes* complex, Carson Cave group).

Williamson County.—Avant's (Capitol Aggregates) Spring, middle fork San Gabriel River, 30° 38′ 44″ N, 97° 44′ 11″ W. (North, *E. naufragia*); Bat Well, Cowan Creek watershed, San Gabriel River drainage, 30° 42′ 10″ N, 97° 42′ 59″ W. (North, provisionally *E. naufragia*); Brushy Creek (Round Rock) Spring, 30° 31′ 00″ N, 97° 39′ 38″ W. (North, *E. tonkawae*); Buford Hollow Springs, just below Lake Georgetown Dam, north fork San Gabriel River, 30° 39′ 39″ N, 97° 43′ 36″ W. (North, *E. naufragia*–type locality); Buttercup Creek Cave (Buttercup River Cave), Buttercup Creek Karst, Brushy Creek watershed, approximately 30° 29′ 33″ N, 97° 50′ 44″ W. (North, provisionally *E. tonkawae*); Cedar Breaks Hiking Trail Spring, south shore of Lake Georgetown, north fork San Gabriel River, 30° 39′ 36″ N, 97° 45′ 02″ W. (North, *E. naufragia*); Ilex Cave, Buttercup Creek Karst, Brushy Creek watershed, approximately 30° 29′ 28″ N, 97° 50′ 50″ N. (North, provisionally *E. tonkawae*); Knight (Crockett Garden) Spring, south shore of Lake Georgetown, north fork San Gabriel River, 30° 39′ 50″ N, 97° 45′ 04″ W. (North, *E. naufragia*); T.W.A.S.A. Cave, Buttercup Creek Karst, Brushy Creek watershed, approximately 30° 29′ 49″ N, 97° 50′ 48″ W. (North, provisionally *E. tonkawae*); Testudo Tube, Buttercup Creek Karst, Brushy Creek watershed, approximately 30° 29′ 35″ N, 97° 51′ 23″ W. (North, provisionally *E. tonkawae*); Treehouse Cave, Buttercup Creek Karst, Brushy Creek watershed, approximately 30° 29′ 55″ N, 97° 50′ 07″ W. (North, provisionally *E. tonkawae*).

APPENDIX II

Part A

Allele Frequencies in Populations of Central Texas Eurycea *and Outgroup Members*

N is the number of individuals from a given population in which a particular locus was resolved. Loci are numbered from least to most anodally migrating, and alleles are designated by letters that in most cases increase with increasing anodal mobility. H = direct-count heterozygosity per locus per individual, P = percentage polymorphic loci, and A = number of alleles/locus. Standard errors are in brackets. NC = not calculated.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
NORTH											
Jollyville Plateau											
E. tonkawae											
Balcones Park Spring	N=	5	5	5	5	5	5	4	4	4	5
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.625	b: 1.000	a: 1.000
									b: 0.375		
Barrow Hollow Spring	N=	5	5	5	4	4	4	5	5	4	5
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Bull Creek Spring	N=	5	5	5	4	5	5	5	5	5	5
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Canyon Creek Spring	N=	3	3	3	3	3	3	3	3	3	3
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Canyon Vista Spring	N=	4	4	4	3	3	3	4	4	2	4
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Horsethief Hollow Spring	N=	1	1	1	1	1	1	1	1	1	1
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
New Bull Creek Spring	N=	2	2	2	2	2	2	2	2	1	1
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Schlumberger Spring	N=	6	6	6	6	6	6	6	6	6	6
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Stillhouse Hollow Springs	N=	14	14	14	13	14	14	14	14	14	14
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Wheless Springs	N=	5	5	5	5	5	5	5	5	5	5
		c: 1.000	b: 1.000	b: 0.900	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
				c: 0.100							
Round Rock Spring	N=	9	9	9	9	9	9	9	9	9	9
E. tonkawae		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Lake Georgetown Area	N=	4	4	5	5	4	5	3	4	3	4
E. naufragia											
Avant's Springs		c: 1.000	b: 1.000	b: 1.000	a: 0.700	a: 1.000	a: 1.000	a: 0.667	a: 0.375	b: 1.000	d: 1.000
					c: 0.300			b: 0.333	b: 0.625		
Buford Hollow Springs	N=	8	8	9	8	9	9	9	7	7	9
		c: 0.688	b: 1.000	b: 1.000	a: 0.688	a: 1.000	a: 1.000	a: 0.944	a: 0.929	b: 1.000	d: 1.000
		f: 0.313			c: 0.313			b: 0.056	b: 0.071		

APPENDIX II

Part A—Continued.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
Cedar Breaks Spring	N=	5	5	5	4	5	5	5	5	4	5
		c: 0.900	b: 1.000	b: 1.000	a: 0.625	a: 1.000	a: 1.000	a: 1.000	a: 0.100	b: 1.000	d: 1.000
		f: 0.100			c: 0.375				b: 0.900		
Knight Spring	N=	5	5	5	5	5	5	5	4	4	5
		c: 0.200	b: 1.000	b: 1.000	a: 0.500	a: 1.000	a: 1.000	a: 1.000	a: 0.375	b: 1.000	d: 1.000
		f: 0.800			c: 0.500				b: 0.625		
Salado Springs	N=	8	8	8	8	8	8	8	8	8	8
E. chisholmensis		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 0.875	a: 1.000
										c: 0.125	
Bat Well	N=	1	1	1	1	1	1	1	1	1	1
E. naufragia		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	a: 1.000
Testudo Tube	N=	2	2	2	2	2	2	2	2	2	2
E. tonkawae		b: 1.000	b: 1.000	b: 0.250	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
				c: 0.750							
Kretschmarr Cave	N=	4	4	4	4	4	4	3	4	4	4
E. tonkawae		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000
Buttercup Creek Caves											
E. tonkawae											
Buttercup Creek Cave	N=	3	3	3	3	3	3	3	3	2	3
		b: 0.167	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
		c: 0.833									
Ilex Cave	N=	2	2	2	2	2	2	2	2	2	2
		c: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
Treehouse Cave	N=	1	1	1	1	1	1	1	1	1	1
		b: 1.000	b: 1.000	b: 0.500	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 1.000
				c: 0.500							
T.W.A.S.A. Cave	N=	1	1	1	1	1	1	1	1	1	1
		c: 1.000	b: 1.000	b: 0.500	a: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 0.500
				c: 0.500							d: 0.500
SAN MARCOS											
E. nana	N=	12	13	13	13	13	13	13	13	11	13
		d: 1.000	a: 1.000	e: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	c: 1.000
E. rathbuni	N=	5	5	5	4	5	5	5	0	5	5
		e: 1.000	b: 1.000	b: 0.600	b: 1.000	b: 1.000	a: 1.000	a: 1.000		c: 1.000	b: 0.700
				c: 0.400							c: 0.300

APPENDIX II

Part A—Continued.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
SOUTHEAST											
E. latitans complex											
E. latitans (type locality)	N=	3	4	5	4	4	3	5	5	2	2
		b: 1.000	b: 1.000	a: 0.900 b: 0.100	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Bear Creek Spring	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Cherry Creek Spring	N=	2	2	2	2	2	1	2	2	2	2
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Cloud Hollow Spring	N=	5	5	5	5	5	5	4	5	4	5
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Cibolo Creek Spring	N=	8	8	8	8	8	8	7	8	8	8
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Kneedeep Cave Spring	N=	6	5	6	6	6	5	5	6	6	6
		a: 0.083 b: 0.917	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.900 b: 0.100	a: 1.000	c: 1.000	c: 1.000
Honey Creek Cave Spring	N=	1	1	1	1	1	1	1	1	1	1
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Less Ranch Spring	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Rebecca Creek Spring	N=	6	6	3	5	5	6	5	6	5	6
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	b: 1.000	a: 1.000	c: 1.000	c: 1.000
E. neotenes											
Helotes Spring (type locality)	N=	11	9	11	11	10	9	10	11	6	9
		b: 1.000	b: 1.000	a: 0.136 b: 0.864	b: 1.000	a: 1.000	a: 1.000	a: 0.700 c: 0.100 d: 0.200	b: 1.000	c: 1.000	c: 1.000
Leon Springs	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.500 b: 0.500	b: 1.000	c: 1.000	c: 1.000
Mueller's Spring	N=	4	4	4	4	4	4	4	4	4	3
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.875 d: 0.125	b: 1.000	c: 1.000	c: 1.000

APPENDIX II

Part A—Continued.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
E. pterophila											
Fern Bank Spring (type loc.)	N=	10	10	10	10	10	10	10	10	10	10
		b: 1.000	b: 1.000	a: 0.950 b: 0.050	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
Boardhouse Springs	N=	11	11	11	11	11	11	11	11	11	11
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.955 b: 0.045	b: 1.000	c: 1.000	c: 1.000
Grapevine Cave	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
Peavey's Springs	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
T Cave	N=	5	5	5	5	5	4	5	5	5	5
		b: 1.000	b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
E. sosorum	N=	13	14	14	14	14	14	14	13	12	14
		b: 0.923 c: 0.077	b: 1.000	a: 0.964 b: 0.036	b: 1.000	a: 1.000	a: 1.000	a: 0.464 b: 0.536	a: 1.000	c: 1.000	b: 0.107 c: 0.893
E. tridentifera											
Honey Creek Cave (type locality)	N=	5	5	5	5	5	4	5	5	5	5
		b: 1.000	b: 1.000	a: 0.300 b: 0.700	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 1.000
Badweather Pit	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	b: 1.000	a: 0.400 b: 0.600	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.900 b: 0.100	c: 1.000	c: 1.000
Ebert Cave	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	b: 1.000	a: 0.500 b: 0.500	b: 1.000	a: 1.000	a: 1.000	a: 0.583 b: 0.417	a: 1.000	c: 1.000	b: 1.000
Comal Springs	N=	11	12	11	11	11	12	11	11	9	9
		b: 0.545 e: 0.455	b: 1.000	a: 0.591 b: 0.409	b: 1.000	a: 1.000	a: 1.000	a: 0.545 b: 0.455	a: 1.000	c: 1.000	c: 1.000
Pedernales											
Spring 1	N=	7	7	6	7	7	6	5	7	7	7
		b: 0.857 c: 0.143	b: 1.000	a: 0.333 b: 0.667	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000
Spring 2	N=	7	7	7	7	7	7	7	7	7	7
		b: 0.857 c: 0.143	b: 1.000	a: 0.714 b: 0.286	b: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000

APPENDIX II

Part A—Continued.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
SOUTHWEST											
(*E. troglodytes* complex)											
Carson Cave Group											
Carson Cave	N=	5	5	5	5	5	5	5	5	4	5
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 1.000
Fessenden Spring	N=	8	8	8	8	8	8	8	8	8	8
		a: 0.063	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
		b: 0.938									
Robinson Creek Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
Smith's Spring	N=	14	14	14	14	14	14	14	14	14	14
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 0.536
											c: 0.464
Sutherland Hollow Spring	N=	11	11	11	11	11	11	11	11	11	11
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.955	b: 1.000	c: 1.000	b: 0.455
								b: 0.045			c: 0.545
Trough Spring	N=	10	10	10	10	10	10	10	10	10	10
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
West Nueces Spring	N=	1	1	1	1	1	1	1	1	1	1
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 1.000
WB Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.750	b: 1.000	c: 1.000	c: 1.000
								b: 0.250			
Camp Mystic Spring	N=	10	10	10	10	10	10	10	10	10	10
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000
Greenwood Valley Ranch Springs	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 1.000
176 Spring	N=	4	3	4	4	4	4	4	4	4	4
		a: 0.125	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 1.000
		b: 0.875									
Sabinal Group											
Murphy's Spring	N=	4	4	4	4	4	2	4	4	4	4
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	b: 1.000	b: 1.000	a: 0.500	b: 1.000
										c: 0.500	
Sabinal Canyon Spring	N=	11	11	11	11	11	11	11	11	10	11
		b: 1.000	b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.091	b: 1.000	a: 0.950	b: 0.955
								b: 0.909		c: 0.050	c: 0.045

APPENDIX II

Part A—Continued.

		Locus									
		Acon-1	Ak	sAat	Ck-1	Ck-2	Cap	Gpi	Gr	Gapdh	G3pdh
Tucker Hollow Cave	N=	6 b: 1.000	5 b: 1.000	6 b: 1.000	6 b: 1.000	6 a: 1.000	6 a: 1.000	6 a: 0.667 b: 0.333	6 b: 1.000	6 a: 1.000	6 b: 1.000
OUTGROUP											
Eurycea m. multiplicata	N=	2 c: 1.000	2 b: 1.000	2 l: 1.000	2 d: 1.000	2 c: 1.000	2 c: 1.000	2 b: 1.000	2 e: 1.000	2 x: 1.000	2 a: 1.000
E. l. longicauda	N=	2 x: 0.500 y: 0.500	2 b: 1.000	2 y: 1.000	2 d: 1.000	2 e: 1.000	2 a: 1.000	2 b: 1.000	2 a: 1.000	2 y: 1.000	2 a: 1.000
E. wilderae	N=	3 e: 1.000	3 b: 1.000	0	0	2 f: 1.000	3 a: 1.000	3 b: 1.000	3 a: 1.000	3 y: 1.000	3 a: 1.000
E. bislineata	N=	1 i: 0.500 k: 0.500	1 b: 1.000	1 y: 1.000	0	1 e: 1.000	1 a: 1.000	1 b: 1.000	1 a: 1.000	1 y: 1.000	1 a: 1.000
E. quadridigitata TX	N=	2 c: 1.000	2 b: 1.000	2 f: 0.500 g: 0.500	2 c: 1.000	2 a: 1.000	2 a: 1.000	2 b: 1.000	2 e: 1.000	2 y: 1.000	2 a: 1.000
E. quadridigitata SC	N=	2 k: 1.000	2 b: 1.000	2 b: 1.000	2 y: 1.000	2 a: 1.000	2 a: 1.000	2 b: 1.000	2 e: 1.000	2 y: 1.000	2 b: 1.000
Haideotriton wallacei	N=	1 d: 1.000	1 b: 1.000	1 x: 1.000	1 d: 1.000	1 c: 1.000	0	0	1 a: 1.000	1 j: 1.000	0
Typhlotriton spelaeus	N=	2 d: 0.500 e: 0.500	2 b: 1.000	2 b: 1.000	0	2 c: 1.000	2 c: 1.000	2 b: 1.000	2 b: 0.750 c: 0.250	2 d: 1.000	2 x: 1.000

APPENDIX II

Part B

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
NORTH											
Jollyville Plateau											
E. tonkawae											
Balcones Park Spring	N=	5	5	5	5	5	5	5	5	5	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Barrow Hollow Spring	N=	5	5	5	5	4	4	5	4	5	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Bull Creek Spring	N=	5	5	5	5	5	5	5	5	5	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.100 b: 0.900	c: 1.000	d: 1.000	b: 1.000
Canyon Creek Spring	N=	3	3	3	3	3	3	3	3	2	3
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.333 b: 0.667	c: 1.000	d: 1.000	b: 1.000
Canyon Vista Spring	N=	4	4	4	4	4	4	4	4	4	4
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.875 b: 0.125	c: 1.000	d: 1.000	b: 1.000
Horsethief Hollow	N=	1	1	1	1	1	1	1	1	1	1
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
New Bull Creek Spring	N=	2	2	2	2	2	2	2	2	2	2
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Schlumberger Spring	N=	6	6	6	6	6	6	6	6	6	6
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Stillhouse Hollow	N=	14	14	14	14	14	14	14	14	14	14
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.214 b: 0.786	c: 1.000	d: 1.000	b: 1.000
Wheless Springs	N=	5	5	5	5	4	4	5	5	2	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Round Rock Spring	N=	9	9	9	9	9	9	9	9	9	9
E. tonkawae		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	a: 0.063 b: 0.938	c: 1.000	d: 1.000	b: 1.000
Lake Georgetown Area											
E. naufragia											
Avant's Spring	N=	5	5	5	5	5	5	4	5	4	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Buford Hollow Springs	N=	9	9	9	9	9	9	9	9	9	9
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 0.944 c: 0.056	b: 1.000	c: 1.000	d: 1.000	b: 1.000

APPENDIX II

Part B—Continued.

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
Cedar Breaks Spring	N=	5	5	5	5	5	5	5	5	5	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Knight Spring	N=	5	5	5	5	5	5	5	5	4	5
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Salado Springs	N=	8	8	8	8	8	8	8	8	8	8
E. chisholmensis		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Bat Well	N=	1	1	1	1	1	1	1	1	1	1
E. naufragia		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Testudo Tube	N=	2	2	2	2	2	2	2	2	2	2
E. tonkawae		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 1.000	b: 1.000
Kretschmarr Cave	N=	4	4	4	4	4	4	3	4	4	4
E. tonkawae		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Buttercup Creek Caves											
E. tonkawae											
Buttercup Creek Cave	N=	1	3	3	3	3	3	3	3	3	3
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Ilex Cave	N=	2	2	2	2	2	2	2	2	2	2
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
Treehouse Cave	N=	1	1	1	1	1	1	1	1	1	1
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	d: 1.000	b: 1.000
T.W.A.S.A. Cave	N=	1	1	1	1	1	1	1	1	1	1
		a: 1.000	b: 1.000	c: 1.000	a: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	b: 0.500	b: 1.000
										d: 0.500	
SAN MARCOS											
E. nana	N=	13	13	13	13	13	13	13	12	12	13
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 0.375	d: 1.000
										d: 0.625	
E. rathbuni	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	b: 1.000	b: 1.000	a: 1.000	a: 1.000	b: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 0.900
											d: 0.100
SOUTHEAST											
E. latitans complex											
E. latitans (type locality)	N=	3	5	5	5	5	5	5	4	2	3
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	a: 1.000
Bear Creek Spring	N=	5	6	6	6	6	6	6	6	6	6
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000

APPENDIX II

Part B—Continued.

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
Cherry Creek Spring	N=	2	2	2	2	2	1	2	1	2	2
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Cloud Hollow Spring	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Cibolo Creek Spring	N=	8	8	8	8	8	8	8	8	8	8
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Kneedeep Cave Spring	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 0.667	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
						d: 0.333					
Honey Creek Cave Spring	N=	1	1	1	1	1	1	1	1	1	1
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Less Ranch Spring	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Rebecca Creek Spring	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
E. neotenes											
Helotes Spring (type locality)	N=	9	11	11	11	9	11	10	11	11	11
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 1.000	b: 1.000	b: 1.000
Leon Springs	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 0.570	b: 1.000	b: 1.000
									c: 0.250		
Mueller's Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 1.000	b: 1.000	b: 1.000
E. pterophila											
Fern Bank Spring (type locality)	N=	10	10	10	10	10	10	10	10	10	10
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 0.600	b: 1.000	b: 1.000
									c: 0.400		
Boardhouse Springs	N=	11	11	11	11	11	11	11	11	11	11
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Grapevine Cave	N=	2	2	2	2	2	2	2	2	2	2
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 1.000	b: 1.000	b: 1.000
Peavey's Springs	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
T Cave	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	a: 1.000	a: 0.500	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
				d: 0.500							

APPENDIX II

Part B—Continued.

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
E. sosorum	N=	12	14	14	14	14	14	14	14	12	14
		b: 1.000	a: 1.000	b: 0.964	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	d: 0.167	a: 0.071
				d: 0.036						e: 0.833	b: 0.929
E. tridentifera											
Honey Creek Cave (type locality)	N=	4	4	5	5	5	5	5	5	3	4
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	d: 1.000	c: 1.000	b: 1.000	a: 0.750
											b: 0.250
Badweather Pit	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 0.900	d: 1.000	c: 1.000	b: 1.000	a: 0.400
							c: 0.100				b: 0.600
Ebert Cave	N=	6	6	6	6	6	6	6	6	6	6
		b: 1.000	a: 1.000	b: 0.500	a: 1.000	a: 1.000	a: 1.000	c: 0.083	c: 1.000	b: 1.000	a: 1.000
								d: 0.917			
Comal Springs	N=	11	12	12	11	11	12	12	11	12	11
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	b: 1.000
Pedernales											
Spring 1	N=	7	7	7	7	7	7	7	7	6	7
		b: 1.000	a: 1.000	a: 0.429	a: 1.000	a: 1.000	a: 1.000	c: 0.929	b: 0.500	b: 0.917	b: 1.000
				b: 0.571				f: 0.071	c: 0.500	d: 0.083	
Spring 2	N=	7	7	7	7	7	7	7	7	7	6
		b: 1.000	a: 1.000	a: 0.429	a: 1.000	a: 1.000	a: 1.000	c: 0.929	b: 0.429	b: 1.000	b: 1.000
				b: 0.571				f: 0.071	c: 0.571		
SOUTHWEST											
(*E. troglodytes complex*)											
Carson Cave Group											
Carson Cave	N=	5	5	5	5	5	5	5	5	5	5
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 0.900	a: 1.000
										c: 0.100	
Fessenden Spring	N=	8	8	8	8	8	8	7	8	7	8
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 0.063	a: 0.929	a: 1.000
									c: 0.938	b: 0.071	
Robinson Creek Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	a: 1.000
Smith's Spring	N=	12	14	14	14	14	14	14	14	13	14
		b: 1.000	a: 0.857	b: 1.000	a: 1.000	a: 0.393	a: 1.000	c: 1.000	c: 1.000	b: 0.654	a: 1.000
			b: 0.143			b: 0.607				c: 0.346	

APPENDIX II

Part B—Continued.

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
Sutherland Hollow Spring	N=	11	11	10	11	11	11	11	11	11	11
		b: 1.000	a: 1.000	b: 0.650	a: 1.000	a: 1.000	a: 1.000	c: 0.909	c: 1.000	b: 1.000	a: 1.000
				d: 0.350				e: 0.091			
Trough Spring	N=	10	10	10	10	10	10	10	10	9	9
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	d: 1.000	b: 1.000	a: 1.000
West Nueces Spring	N=	1	1	1	1	1	1	1	1	1	1
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	a: 1.000
WB Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	b: 1.000	a: 0.875
											b: 0.125
Camp Mystic Spring	N=	10	10	10	10	10	10	10	10	10	10
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 0.100	a: 1.000	c: 1.000	c: 1.000	b: 1.000	a: 1.000
						c: 0.900					
Greenwood Valley Ranch Springs	N=	6	6	6	6	6	0	6	6	6	6
		d: 1.000	a: 1.000	b: 1.000	a: 1.000	e: 1.000		c: 1.000	c: 1.000	c: 1.000	a: 1.000
176 Spring	N=	4	4	4	3	4	4	4	4	3	4
		b: 1.000	a: 1.000	b: 0.125	a: 1.000	a: 1.000	a: 1.000	c: 1.000	b: 1.000	a: 1.000	a: 1.000
				e: 0.875							
Sabinal Group											
Murphy's Spring	N=	4	4	4	4	4	4	4	4	4	4
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 0.125	c: 1.000	b: 1.000	a: 1.000
								e: 0.875			
Sabinal Canyon Spring	N=	11	11	11	11	11	11	11	11	11	11
		b: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 0.409	a: 0.045	b: 1.000	a: 1.000
								e: 0.591	c: 0.955		
Tucker Hollow Cave	N=	5	6	6	6	6	6	6	6	6	4
		c: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	a: 1.000	c: 1.000	c: 1.000	c: 1.000	a: 1.000
OUTGROUP											
Eurycea m. multiplicata	N=	2	2	2	2	2	2	2	2	0	2
		b: 1.000	e: 1.000	b: 1.000	b: 1.000	a: 1.000	e: 1.000	x: 1.000	d: 0.750		p: 1.000
									i: 0.250		
E. l. longicauda	N=	2	2	2	2	2	2	2	2	0	2
		b: 1.000	e: 1.000	x: 1.000	a: 0.500	a: 1.000	g: 1.000	k: 0.750	e: 1.000		l: 1.000
					c: 0.500			m: 0.250			

APPENDIX II

Part B—Continued.

		Locus									
		Idh-1	Idh-2	Ldh-A	Ldh-B	Mdh-1	Mdh-2	Mdhp	Mpi	Pep-A	Pep-B
E. wilderae	N=	3	3	3	3	3	3	3	3	0	3
		b: 1.000	e: 1.000	f: 1.000	a: 1.000	a: 1.000	g: 1.000	m: 0.670	c: 0.250		b: 0.250
								n: 0.330	y: 0.750		d: 0.750
E. bislineata	N=	1	1	1	0	1	1	1	1	0	1
		b: 1.000	e: 1.000	f: 1.000		a: 1.000	g: 1.000	g: 1.000	e: 1.000		b: 1.000
E. quadridigitata TX	N=	2	2	2	2	2	2	2	2	0	2
		e: 1.000	e: 1.000	f: 1.000	a: 1.000	a: 1.000	g: 1.000	g: 1.000	c: 0.500		j: 0.500
									e: 0.500		l: 0.500
E. quadridigitata SC	N=	2	2	2	2	2	2	2	2	0	2
		b: 1.000	x: 1.000	f: 1.000	c: 1.000	x: 1.000	d: 1.000	g: 1.000	d: 1.000		a: 1.000
Haideotriton wallacei	N=	1	1	1	0	1	1	1	1	0	1
		x: 1.000	b: 1.000	f: 1.000		a: 1.000	k: 1.000	m: 1.000	c: 1.000		i: 0.500
											k: 0.500
Typhlotriton spelaeus	N=	2	2	2	2	2	2	2	2	0	2
		b: 0.750	e: 1.000	f: 1.000	x: 1.000	a: 1.000	i: 1.000	x: 0.750	z: 1.000		l: 1.000
		g: 0.250						g: 0.250			

APPENDIX II

Part C

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
NORTH									
Jollyville Plateau									
E. tonkawae									
Balcones Park Spring	N=	5	5	5	5	5			
		e: 0.900	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.018	8.0	1.1
		f: 0.100					[0.013]		[0.1]
Barrow Hollow Spring	N=	4	4	5	4	5			
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
Bull Creek Spring	N=	5	5	5	5	5			
		e: 0.900	a: 0.900	e: 1.000	a: 1.000	a: 1.000	0.024	12.0	1.1
		f: 0.100	c: 0.100				[0.013]		[0.1]
Canyon Creek Spring	N=	2	3	3	3	3			
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.027	4.0	1.0
							[0.027]		[0.0]
Canyon Vista Spring	N=	4	4	4	4	4		4.0	1.0
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.010		[0.0]
							[0.01]		
Horsethief Hollow Spring	N=	1	1	1	1	1		0.0	1.0
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000		[0.0]
							[0.000]		
New Bull Creek Spring	N=	1	2	2	2	2			
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
Schlumberger Spring	N=	6	6	6	6	6			
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
Stillhouse Hollow Springs	N=	14	14	14	14	14			
		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.006	4.0	1.0
							[0.006]		[0.0]
Wheless Springs	N=	4	4	5	5	5			
		e: 0.875	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.018	8.0	1.1
		f: 0.125					[0.013]		[0.1]
Round Rock Spring	N=	9	9	9	9	9			
E. tonkawae		c: 0.944	a: 1.000	b: 0.056	a: 1.000	a: 1.000	0.014	12.0	1.1
		e: 0.056		e: 0.944			[0.008]		[0.1]

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
Lake Georgetown Area	N=	2	5	5	3	5			
E. naufragia									
Avant's Springs		a: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.038 [0.031]	12.0	1.1 [0.1]
Buford Hollow Springs	N=	9	9	9	9	9		24.0	1.2 [0.1]
		a: 0.944 e: 0.056	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.049 [0.022]		
Cedar Breaks Spring	N=	4	5	5	5	5			
		a: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.026 [0.014]	12.0	1.1 [0.1]
Knight Spring	N=	5	5	5	5	5			
		a: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.034 [0.020]	12.0	1.1 [0.1]
Salado Springs	N=	8	8	8	7	8			
E. chisholmensis		a: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.010 [0.010]	4.0	1.0 [0.0]
Bat Well	N=	1	1	1	1	1			
E. naufragia		a: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000 [0.000]	0.0	1.0 [0.0]
Testudo Tube	N=	2	2	2	2	2			
E. tonkawae		e: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.020 [0.020]	4.0	1.0 [0.0]
Kretschmarr Cave	N=	3	4	4	4	3			
E. tonkawae		f: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000 [0.000]	0.0	1.0 [0.0]
Buttercup Creek Caves									
E. tonkawae									
Buttercup Creek Cave	N=	3	3	3	3	3			
		e: 0.167 f: 0.833	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.027 [0.018]	8.0	1.1 [0.1]
Ilex Cave	N=	2	2	2	2	2			
		e: 0.250 f: 0.750	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.020 [0.020]	4.0	1.0 [0.0]
Treehouse Cave	N=	1	1	1	1	1			
		f: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.040 [0.040]	4.0	1.0 [0.0]

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
T.W.A.S.A. Cave	N=	1	1	1	1	1			
		f: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.120	12.0	1.1
							[0.066]		[0.1]
SAN MARCOS									
E. nana	N=	12	11	13	8	12			
		h: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.017	4.0	1.0
							[0.017]		[0.0]
E. rathbuni	N=	4	5	5	4	4			
		d: 0.200	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.048	16.0	1.2
		g: 0.800					[0.024]		[0.1]
SOUTHEAST									
E. latitans complex									
E. latitans (type locality)	N=	4	5	5	5	4			
		c: 0.625	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.018	8.0	1.1
		d: 0.375					[0.013]		[0.1]
Bear Creek Spring	N=	6	6	6	6	6			
		c: 1.000	a: 0.917	d: 1.000	a: 1.000	a: 1.000	0.007	4.0	1.0
			c: 0.083				[0.007]		[0.0]
Cherry Creek Spring	N=	2	2	2	2	1			
		c: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
Cloud Hollow Spring	N=	4	5	5	4	5			
		c: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
Cibolo Creek Spring	N=	8	8	8	8	7			
		c: 1.000	a: 1.000	a: 0.063	a: 1.000	a: 1.000	0.015	4.0	1.2
				b: 0.063			[0.015]		[0.2]
				c: 0.063					
				d: 0.250					
				e: 0.563					
Kneedeep Cave Spring	N=	6	6	6	5	5			
		c: 1.000	a: 1.000	b: 0.250	a: 1.000	a: 1.000	0.048	16.0	1.2
				e: 0.750			[0.028]		[0.1]
Honey Creek Cave Spring	N=	1	1	1	1	1			
		c: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
Less Ranch Spring	N=	2	2	2	2	2			
		c: 0.750	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.020	4.0	1.0
		d: 0.250					[0.020]		[0.0]
Rebecca Creek Spring	N=	5	6	6	6	6			
		c: 0.900	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.008	4.0	1.0
		d: 0.100					[0.008]		[0.000]
E. neotenes									
Helotes Spring (type locality)	N=	9	10	10	10	11			
		c: 0.667	a: 0.400	c: 0.650	a: 1.000	a: 1.000	0.081	20.0	1.2
		d: 0.333	b: 0.600	e: 0.350			[0.036]		[0.1]
Leon Springs	N=	2	2	2	2	2			
		c: 1.000	a: 0.500	c: 0.750	a: 1.000	a: 1.000	0.040	16.0	1.2
			b: 0.500	e: 0.250			[0.028]		[0.1]
Mueller's Spring	N=	3	4	4	4	4			
		c: 1.000	a: 0.125	b: 0.500	a: 1.000	a: 1.000	0.020	12.0	1.1
			b: 0.875	e: 0.500			[0.014]		[0.1]
E. pterophila									
Fern Bank Spring (type locality)	N=	10	10	10	10	7			
		c: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.028	8.0	1.1
							[0.024]		[0.1]
Boardhouse Springs	N=	11	11	11	11	11			
		c: 0.955	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.007	8.0	1.1
		d: 0.045					[0.005]		[0.1]
Grapevine Cave	N=	2	2	2	2	2			
		c: 0.250	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.020	4.0	1.0
		d: 0.750					[0.02]		[0.0]
Peavey's Springs	N=	5	5	5	4	5			
		c: 1.000	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.000	0.0	1.0
							[0.000]		[0.0]
T Cave	N=	5	5	5	5	5			
		c: 0.500	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.032	8.0	1.1
		d: 0.500					[0.025]		[0.1]
E. sosorum	N=	11	14	14	12	11			
		a: 1.000	a: 1.000	b: 0.214	a: 1.000	a: 1.000	0.053	32.0	1.3
				e: 0.786			[0.023]		[0.1]

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
E. tridentifera									
Honey Creek Cave (type locality)	N=	5	5	5	5	4			
		a: 0.100	a: 1.000	b: 0.200	a: 1.000	a: 1.000	0.032	16.0	1.2
		d: 0.900		e: 0.800			[0.019]		[0.1]
Badweather Pit	N=	4	5	5	5	5			
		c: 0.250	a: 1.000	b: 0.700	a: 1.000	a: 1.000	0.108	24.0	1.2
		d: 0.750		e: 0.300			[0.040]		[0.1]
Ebert Cave	N=	4	6	6	6	6			
		d: 0.625	a: 1.000	b: 0.833	a: 1.000	a: 1.000	0.097	20.0	1.2
		e: 0.375		e: 0.167			[0.046]		[0.1]
Comal Springs		12	12	11	11	12			
		c: 1.000	a: 0.375	b: 1.000	a: 1.000	a: 1.000	0.057	16.0	1.2
			b: 0.625				[0.030]		[0.1]
Pedernales									
Spring 1		7	7	7	5	6			
		c: 0.857	a: 1.000	b: 1.000	a: 1.000	a: 1.000	0.090	28.0	1.3
		d: 0.143					[0.038]		[0.1]
Spring 2		7	7	7	6	7			
		c: 1.000	a: 1.000	b: 0.929	a: 1.000	a: 1.000	0.057	24.0	1.2
				e: 0.071			[0.027]		[0.1]
SOUTHWEST									
(*E. troglodytes* complex)									
Carson Cave Group									
Carson Cave	N=	5	5	5	5	5			
		c: 1.000	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.008	4.0	1.0
							[0.008]		[0.0]
Fessenden Spring	N=	8	8	8	8	8			
		a: 0.063	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.026	16.0	1.2
		d: 0.875					[0.0131		[0.1]
		e: 0.063							
Robinson Creek Spring	N=	3	4	4	4	4			
		d: 0.333	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.027	4.0	1.0
		e: 0.667					[0.027]		[0.0]
Smith's Spring	N=	14	14	14	14	14			
		c: 0.929	a: 1.000	e: 1.000	a: 1.000	a: 1.000	0.044	20.0	1.2
		d: 0.071					[0.018]		[0.1]

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
Sutherland Hollow Spring	N=	10 c: 1.000	11 a: 1.000	11 e: 1.000	11 a: 1.000	11 a: 1.000	0.046 [0.029]	16.0	1.2 [0.1]
Trough Spring	N=	9 c: 0.778 d: 0.222	10 a: 1.000	10 d: 0.050 e: 0.950	10 a: 1.000	10 a: 1.000	0.013 [0.010]	8.0	1.1 [0.1]
West Nueces Spring	N=	1 c: 1.000	1 a: 1.000	1 e: 1.000	1 a: 1.000	1 a: 1.000	0.000 [0.000]	0.0	1.0 [0.0]
WB Spring	N=	4 c: 0.625 d: 0.375	4 a: 1.000	4 e: 1.000	4 a: 1.000	4 a: 1.000	0.060 [0.036]	12.0	1.1 [0.1]
Camp Mystic Spring	N=	4 a: 1.000	10 a: 0.900 b: 0.100	10 e: 1.000	10 b: 1.000	10 a: 1.000	0.008 [0.008]	8.0	1.1 [0.1]
Greenwood Valley Ranch Springs	N=	4 c: 0.250 d: 0.750	6 a: 1.000	6 e: 1.000	6 a: 1.000	6 a: 1.000	0.020 [0.020]	4.0	1.0 [0.0]
176 Spring	N=	2 d: 0.750 e: 0.250	4 a: 1.000	4 e: 1.000	4 a: 1.000	4 a: 1.000	0.040 [0.024]	12.0	1.1 [0.1]
Sabinal Group Murphy's Spring	N=	4 c: 0.875 d: 0.125	4 a: 1.000	4 e: 1.000	4 a: 1.000	4 a: 1.000	0.040 [0.024]	12.0	1.1 [0.1]
Sabinal Canyon Spring	N=	11 c: 0.909 d: 0.091	10 a: 1.000	11 e: 1.000	11 a: 1.000	11 a: 1.000	0.037 [0.020]	24.0	1.2 [0.1]
Tucker Hollow Cave	N=	6 c: 0.500 d: 0.500	4 a: 1.000	6 e: 1.000	6 a: 1.000	6 a: 1.000	0.040 [0.029]	8.0	1.1 [0.1]
OUTGROUP *Eurycea m. multiplicata*	N=	0	2 a: 0.750 x: 0.250	2 c: 1.000	2 x: 0.75 d: 0.25	2 f: 1.000	NC	NC	NC
E. l. longicauda	N=	0	2 a: 1.000	2 o: 1.000	2 d: 1.000	2 f: 1.000	NC	NC	NC

APPENDIX II

Part C—Continued.

		Locus							
		Pep-D	Pgm	Pgdh	Pk	sSod	H	P	A
E. wilderae	N=	0	3 a: 1.000	3 e: 0.500 x: 0.333 y: 0.167	3 d: 1.000	3 f: 1.000	NC	NC	NC
E. bislineata	N=	0	1 a: 1.000	1 b: 1.000	1 d: 1.000	1 f: 1.000	NC	NC	NC
E. quadridigitata TX	N=	0	2 b: 1.000	2 e: 1.000	2 e: 1.000	2 f: 1.000	NC	NC	NC
E. quadridigitata SC	N=	0	2 a: 1.000	2 b: 1.000	2 x: 1.000	2 f: 1.000	NC	NC	NC
Haideotriton wallacei	N=	0	1 a: 1.000	1 e: 1.000	1 b: 1.000	1 f: 1.000	NC	NC	NC
Typhlotriton spelaeus	N=	0	2 a: 1.000	2 d: 0.250 e: 0.750	2 d: 1.000	2 f: 1.000	NC	NC	NC

APPENDIX III

Partial cytochrome b sequence for central Texas Eurycea *and outgroup members*

Positions shown are 43 to 398 within the gene. Texas group members are listed by major geographic region of occurrence.

Northern	
E. tonkawae (Testudo Tube)	?TTAACAACTCATTTATTGATCTCCCAGCCCCTTCTACCTTATCCTACT
E. tonkawae (Ilex Cave)	????.....
E. tonkawae (Kretschmarr Cave)	???????.....N.....
E. tonkawae (Stillhouse Hollow Springs)	???????.....G.....N.G.....
E. tonkawae (Horsethief Hollow Spring)	???????.....NNG.....
E. tonkawae (Round Rock Spring)	?????.....NN.G.....
E. naufragia (Cedar Breaks Spring)	?????.....C.....G.....
E. naufragia (Bat Well)	?????.....C.... .....NNNG.....
E. chisholmensis (Salado Springs)	????.....N.G.....
San Marcos	
E. rathbuni	?????...T..C.....C..A...A....C..C.A...N..T..T.
E. nana	?????.....C.....C..T...N....G..A.A.C.N..T....
Southeastern	
E. sosorum (Barton Springs)	???.....C.....C.....G..A.AN.....T...C
E. tridentifera (Badweather Pit)	?????.....C.....C.....N.....C.NNNNT.....T...C
E. tridentifera (Honey Creek Cave)	???????....C.....C.....C..A.NNN....T...C
E. tridentifera (Ebert Cave)	??????.....C.....C.....C..A.ANN....T...C
E. pterophila (Boardhouse Spring)	?????.....C.....C.....N.....C..A.ATN....T...C
E. latitans (Pfeiffer's Water Cave)	??????.....C.....C.....C..A.AT.....T...C
E. latitans complex (Cibolo Creek Spring)	???????....C.....C.....G..A.NN.....T...C
E. latitans complex (Cloud Hollow Spring)	?????????..NN.....C..N.....C..A.AT.....T...C
E. latitans complex (Honey Creek Cave Spring)	?????..?...C.....C.....C..A.AT.....T...C
E. latitans complex (Rebecca Creek Spring)	???????....C.....C.....C.NANNN.....T...C
E. neotenes (Helotes Creek Spring)	????????...C.....C.....G..ANNN.....T...C
E. sp. (Comal Springs)	?????.....C.....C.....G..A.ATN....T...C

APPENDIX III

Continued.

E. sp. (Pedernales Springs)	??????????.C........C....NN.N...G.NA.ANNNN..T...C
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	????????...C........C.....C.....A..A.ANN....T....
E. troglodytes complex (176 Spring)	???????....T........C.....N.....G..A.ANC....T....
E. troglodytes complex (Camp Mystic Spring)	?????......C........C..........G..A.A.C....T....
E. troglodytes complex (Carson Cave)	????????...C........C.....C.....G..A.ATC.N......C
E. troglodytes complex (Greenwood Valley Ranch)	????????...C........C..........G..A.ANC....T....
E. troglodytes complex (Sabinal Canyon Spring)	??????.....C........C.....C.....A..A.A.C....T....
E. troglodytes complex (Smith's Spring)	?????????..C........C.....N.N..NG.NA.NNN.N......C
E. troglodytes complex (Sutherland Hollow Spr.)	???????....C........C.....N....NA..A.ANN....T....
E. troglodytes complex (Trough Spring)	???????....C........C..........G..A.ANC....T....
E. troglodytes complex (Tucker Hollow Cave)	?????????..C........C.....C.....A..A.NNC....T...C
Outgroup	
E. bislineata	?????......T........C.....C.....A....A.C.N..T...C
E. longicauda	?????T.....C..............C.....A..A.GN....C....C
E. multiplicata	?????......C........C.....C.....A..A.G.C.G.C....C
E. quadridigitata SC	?????......C........C.....C.....A..A.G.C.G.C....C
E. quadridigitata TX	?????T.....T..............CA....A....GTC.G.C.....
E. wilderae	???????....T........C.....C.....A...NGNN.N..T....
Haideotriton wallacei	A.C..T.....T..............CA....A....GTC.G.C.....
Typhlotriton spelaeus	??????..T..........C.....CA....G..A.G.C.G.CT..T.
Northern	
E. tonkawae (Testudo Tube)	TATGAAATTTTGGCTCTCTCTTAGGAGTCTGCCTAATTTCACAAATTCT
E. tonkawae (Ilex Cave)	.....
E. tonkawae (Kretschmarr Cave)	.....
E. tonkawae (Stillhouse Hollow Springs)	.......C.....
E. tonkawae (Horsethief Hollow Spring)	.......C.....
E. tonkawae (Round Rock Spring)	.......C.....
E. naufragia (Cedar Breaks Spring)	............T.....................T.....
E. naufragia (Bat Well)	.....
E. chisholmensis (Salado Springs)	.....
San Marcos	
E. rathbuni	.......C.....A..C..A......C.T.........A........T.

APPENDIX III

Continued.

E. nana	.....C.....A.....T.....CA.....A.....T.
Southeastern	
E. sosorum (Barton Springs)	.....C.....A.....T.....CA.....A.....T.
E. tridentifera (Badweather Pit)	.....C.....A.....T.....CA.....A.....T.
E. tridentifera (Honey Creek Cave)	.....C.....A.....T.....CA.....A.....T.
E. tridentifera (Ebert Cave)	.....C.....A.....T.....CA.....A.....T.
E. pterophila (Boardhouse Spring)	.....C.....A.....T.....CA.....A.....T.
E. latitans (Pfeiffer's Water Cave)	.....C.....A.....T.....CA.....T.....A.....T.
E. latitans complex (Cibolo Creek Spring)	.....C.....A.....T.....CA.....A.....T.
E. latitans complex (Cloud Hollow Spring)	.....C.....A.....T.....CA.....A.....T.
E. latitans complex (Honey Creek Cave Spring)	.....C.....A.....T.....CA.....A.....T.
E. latitans complex (Rebecca Creek Spring)	.....C.....A.....T.....CA.....A.....T.
E. neotenes (Helotes Creek Spring)	.....C.....A.....T.....CA.....A.....T.
E. sp. (Comal Springs)	.....C.....A.....T.....CA.....A.....T.
E. sp. (Pedernales Springs)	.N..N..C.....A.....T.....TA.....A.....T.
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (176 Spring)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (Camp Mystic Spring)	.....C.....A..C..T.....CA.....T.
E. troglodytes complex (Carson Cave)	.....C.....A..C..T.....TC....A.....T.
E. troglodytes complex (Greenwood Valley Ranch)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (Sabinal Canyon Spring)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (Smith's Spring)	.....C.....A..C..T.....T.NNNN.....A.....T.
E. troglodytes complex (Sutherland Hollow Spr.)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (Trough Spring)	.....C.....A..C..T.....A.....T.
E. troglodytes complex (Tucker Hollow Cave)	.....C.....A..C..T.....A.....T.
Outgroup	
E. bislineata	.....C.....G..CC.T.....T.....A.....CT.
E. longicauda	.....C.....A..CC.....A.T.....CA.....CT.
E. multiplicata	.....A..CC.T.....CA....G..CT.
E. quadridigitata SC	.....A..CC.T.....CA....G..CT.
E. quadridigitata TX	.....C.....A..CC.T.....A.T.....A.....T.
E. wilderae	.....C.....G..CA.T.....A...T.....A...N...CT.
Haideotriton wallacei	.....C.....A..CC.T.....A.T.....A.....CT.
Typhlotriton spelaeus	.....C..C..A...C.A.....A.....A.....C..

APPENDIX III

Continued.

Northern	
E. tonkawae (Testudo Tube)	AACAGGCCTATTTCTCGCAATACACTATACTGCAGATACTACTTCCGCA
E. tonkawae (Ilex Cave)	.....
E. tonkawae (Kretschmarr Cave)	.....C..C..C.....
E. tonkawae (Stillhouse Hollow Springs)	.....
E. tonkawae (Horsethief Hollow Spring)	.....
E. tonkawae (Round Rock Spring)	.....
E. naufragia (Cedar Breaks Spring)	.....C.....
E. naufragia (Bat Well)	.....G.
E. chisholmensis (Salado Springs)	.....
San Marcos	
E. rathbuni	.....T.....T.....C..C..C..A...
E. nana	...G...T.....T.....T..C.....C..C..C..A...
Southeastern	
E. sosorum (Barton Springs)	...G...T.....T.....T..C.....C..C..C..A...
E. tridentifera (Badweather Pit)	...G...T.....T.....T..C.....C..C..C..A...
E. tridentifera (Honey Creek Cave)	...G...T.....T.....T..C.....C..C..C..A...
E. tridentifera (Ebert Cave)	...G...T.....T.....T..C.....C..C..C..A...
E. pterophila (Boardhouse Spring)	...G...T.....T.....T..C.....C..C..C..A...
E. latitans (Pfeiffer's Water Cave)	...G...T.....T.....T..C.....C..C..C..A...
E. latitans complex (Cibolo Creek Spring)	...G...T.....T.....T..C.....C..C..C..A...
E. latitans complex (Cloud Hollow Spring)	...G...T.....T.....T..C.....C..C..C..A...
E. latitans complex (Honey Creek Cave Spring)	...G...T.....T.....T..C.....C..C..C..A...
E. latitans complex (Rebecca Creek Spring)	T..G...T.....T.....T..C.....C..C..C..A...
E. neotenes (Helotes Creek Spring)	...G...T.....T.....T..C.....C..C..C..A...
E. sp. (Comal Springs)	...G...T.....T.....T..C.....C..C..C..A...
E. sp. (Pedernales Springs)	...G...T.....T.....T..C.....C..C..C..A...
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	...G...T.....T.....T.....C..C..C..A...
E. troglodytes complex (176 Spring)	...G...T.....T.....T.....C..C..C..A...
E. troglodytes complex (Camp Mystic Spring)	...G...T.....T.....T.....C.....C..A...
E. troglodytes complex (Carson Cave)	.....T.....T.....T.....C..C..C..A...
E. troglodytes complex (Greenwood Valley Ranch)	...G...T.....T.....T.....C..C..C..A...

APPENDIX III

Continued.

E. troglodytes complex (Sabinal Canyon Spring)	...G...T.....T.....T.....C..C..C..A...
E. troglodytes complex (Smith's Spring)	.....T.....T.....T.....C..C..C..A...
E. troglodytes complex (Sutherland Hollow Spr.)	...G...T.....T.....T.....C..C..C..A...
E. troglodytes complex (Trough Spring)	...G...T.....T.....T.....C..C..C..A...
E. troglodytes complex (Tucker Hollow Cave)	...G...T.....T.....T.....C..C..C..A...
Outgroup	
E. bislineata	.....A.....C..T.....T.....G.....C.....T...
E. longicauda	.....AT.....T.....T..C.....C.....C.....
E. multiplicata	.....GT....C..T.....C..C.....C..CGTC..A...
E. quadridigitata SC	.....GT....C..T.....C..C.....C..CT.C..A...
E. quadridigitata TX	.....T.....T.....C..C.....C..C..C.....
E. wilderae	.....A.....C..T.....G..C..C..C.....
Haideotriton wallacei	T.....AT.....T..T.....C..C..G..C..CT.C..T...
Typhlotriton spelaeus	.....C..T.....C..C.....C..CTC.....
Northern	
E. tonkawae (Testudo Tube)	TTTTCCTCCGTGGCCCACATTTGCCGTGATGTAAATTATGGCTGACTTG
E. tonkawae (Ilex Cave)	.....
E. tonkawae (Kretschmarr Cave)	.....
E. tonkawae (Stillhouse Hollow Springs)	.....
E. tonkawae (Horsethief Hollow Spring)	.....
E. tonkawae (Round Rock Spring)	.....
E. naufragia (Cedar Breaks Spring)	.....G.....
E. naufragia (Bat Well)	.....N.....
E. chisholmensis (Salado Springs)	.....
San Marcos	
E. rathbuni	..C.....T..A.....C.....C.....A
E. nana	..C.....T..A.....C.
Southeastern	
E. sosorum (Barton Springs)	..C.....T..A.....C.
E. tridentifera (Badweather Pit)	..C.....T..A.....
E. tridentifera (Honey Creek Cave)	..C.....T..A.....
E. tridentifera (Ebert Cave)	..C.....T..A.....
E. pterophila (Boardhouse Spring)	..C.....T..A.....

APPENDIX III

Continued.

E. latitans (Pfeiffer's Water Cave)	..C.....T..A.....
E. latitans complex (Cibolo Creek Spring)	..C.....T..A.....
E. latitans complex (Cloud Hollow Spring)	..C.....T..A.....
E. latitans complex (Honey Creek Cave Spring)	..C.....T..A.....
E. latitans complex (Rebecca Creek Spring)	..C.....T..A.....
E. neotenes (Helotes Creek Spring)	..C.....T..A.....N.
E. sp. (Comal Springs)	..C.....T..A.....
E. sp. (Pedernales Springs)	..C.....T..A.....C.
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	..C.....T.....C.....
E. troglodytes complex (176 Spring)	..C.....T..A.....T.....T
E. troglodytes complex (Camp Mystic Spring)	.....T..AAT.....G.....
E. troglodytes complex (Carson Cave)	..C.....T..A.....C.....C.
E. troglodytes complex (Greenwood Valley Ranch)	..C.....T.....T.....C.....C.....
E. troglodytes complex (Sabinal Canyon Spring)	..C.....T.....T.....C.....
E. troglodytes complex (Smith's Spring)	..C.....T..A.....C..N.....C.
E. troglodytes complex (Sutherland Hollow Spr.)	..C.....T.....T.....C.....
E. troglodytes complex (Trough Spring)	.....T..AAT.....
E. troglodytes complex (Tucker Hollow Cave)	..C.....T.....T.....C..N.....
Outgroup	
E. bislineata	..C.....T..A..T..T.....A..C.....C.....A.....G.
E. longicauda	..C.....T..A.....T..C.....A..C..T.....T.....A.
E. multiplicata	..C..T..T..A.....T..C.....A..C.....A.
E. quadridigitata SC	..C.....T..A.....T..C.....A..C.....A.
E. quadridigitata TX	.....A..T.....C.....A.....C..T.....
E. wilderae	..C.....A.....T.....A.....GGN.....T.....
Haideotriton wallacei	.....T..T..T.....A.....T.....A.
Typhlotriton spelaeus	.....T..A..A..A..T..C.....A.....C..A.....G.
Northern	
E. tonkawae (Testudo Tube)	TACGCAGCATTCACACTAATGGAGCATCACTATTCTTTATTTGTATGTA
E. tonkawae (Ilex Cave)	.....
E. tonkawae (Kretschmarr Cave)	.....A..

APPENDIX III

Continued.

E. tonkawae (Stillhouse Hollow Springs)	.G.....T.....G.....N..C.....A..
E. tonkawae (Horsethief Hollow Spring)	.G.....T.....G.....N..C.....A..
E. tonkawae (Round Rock Spring)	.G.....T.....C.....A..
E. naufragia (Cedar Breaks Spring)	.....AT.....C.....C.....A..
E. naufragia (Bat Well)	.....T.....A..
E. chisholmensis (Salado Springs)	.....T.....C.....A..
San Marcos	
E. rathbuni	.G..T.AT.....C.....G...A.C.....C.....T..
E. nana	.G.....T.....CA.C.....CA.C.....C..C..T..
Southeastern	
E. sosorum (Barton Springs)	.G.....T.....C..C.....CA.C.....C..C..T..
E. tridentifera (Badweather Pit)	.G.....T.....C..C.....CA.C.....C..C..T..
E. tridentifera (Honey Creek Cave)	.G.....T.....C..C.....CA.C.....C..C..T..
E. tridentifera (Ebert Cave)	.G.....T.....C..C.....CA.C.....C..C..T..
E. pterophila (Boardhouse Spring)	.G.....T.....C..C.....CA.C.....C..C..T..
E. latitans (Pfeiffer's Water Cave)	.G.....T.....C..C.....CA.C.....C..C..T..
E. latitans complex (Cibolo Creek Spring)	.G.....T.....C..C.....CA.C.....C..C..T.N
E. latitans complex (Cloud Hollow Spring)	.G.....T.....C..C.....CA.C.....C..C..T..
E. latitans complex (Honey Creek Cave Spring)	.G.....T.....C..C.....CA.C.....C..C..T..
E. latitans complex (Rebecca Creek Spring)	.G.....T.....C..C.....CA.C.....C..C..T..
E. neotenes (Helotes Creek Spring)	.G.....T.....C..C.....CA.C.....C..C..T..
E. sp. (Comal Springs)	.G.....?.....C..C.....CA.C.....C..C..T..
E. sp. (Pedernales Springs)	.G.....T.....C..C.....CA.C.....C..C..T.G
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	.....T.....CA.C.....T..
E. troglodytes complex (176 Spring)	.....T.....CA.C.....T..
E. troglodytes complex (Camp Mystic Spring)	.....A.....T.....CA.C.....T..
E. troglodytes complex (Carson Cave)	.....T.....C.....G..CACC.....C.....T..
E. troglodytes complex (Greenwood Valley Ranch)	.....T.....CA.C.....T..
E. troglodytes complex (Sabinal Canyon Spring)	.....T.....CA.C.....T..
E. troglodytes complex (Smith's Spring)	.....T.....C.....G..CACC.....C.....T..
E. troglodytes complex (Sutherland Hollow Spr.)	.....T.....CA.C.CT.....T..
E. troglodytes complex (Trough Spring)	.....A.....T.....CA.C.CT.....T..
E. troglodytes complex (Tucker Hollow Cave)	.....T.....CG.C.....T..
Outgroup	

APPENDIX III

Continued.

E. bislineata	.G..T.AT.....T..........C..TA.T..T..........T..
E. longicauda	.G..........T..........T..TA...CT..N.....C..T..
E. multiplicata	..............C..C.....C...A..............T..
E. quadridigitata SC	..............C..C.....C...A..............T..
E. quadridigitata TX	......AT..........C.....C..TA.T..T..C.....C..T..
E. wilderae	......AT.....T.....C.....C..TA.T..T..........T..
Haideotriton wallacei	.G....AT..............C..TA.T..T..C.....C..T..
Typhlotriton spelaeus	......ATG.........C.....C...A.T............T..
Northern	
E. tonkawae (Testudo Tube)	TCTACACATTGGACGGGGCCTATATTATGGCTCTTACATATTTAAAGAA
E. tonkawae (Ilex Cave)	.....
E. tonkawae (Kretschmarr Cave)	.....
E. tonkawae (Stillhouse Hollow Springs)	.A.....T.....
E. tonkawae (Horsethief Hollow Spring)	.A.....T.....
E. tonkawae (Round Rock Spring)	.A.....T.....
E. naufragia (Cedar Breaks Spring)	C.....
E. naufragia (Bat Well)	.T.....
E. chisholmensis (Salado Springs)	......T.....
San Marcos	
E. rathbuni	C..T..T.....T.....G.....A.....A.....
E. nana	C..T..T.....T.....A..T..G.A...G...
Southeastern	
E. sosorum (Barton Springs)	C..T..T.....T.....A..T..G.A...G...
E. tridentifera (Badweather Pit)	C..T..T.....T.....A..T..G.A...G...
E. tridentifera (Honey Creek Cave)	C..T..T.....T.....A..T..G.A...G...
E. tridentifera (Ebert Cave)	C..T..T.....T.....A..T..G.A...G...
E. pterophila (Boardhouse Spring)	C..T..T.....T.....A..T..G.A...G...
E. latitans (Pfeiffer's Water Cave)	C..T..T.....T.....A..T..G.A...G...
E. latitans complex (Cibolo Creek Spring)	C..T..T.....T.....A..T..G.A...G...
E. latitans complex (Cloud Hollow Spring)	C..T..T.....T.....A..T..G.A...G...
E. latitans complex (Honey Creek Cave Spring)	C..T..T.....T.....A..T..G.A...G...
E. latitans complex (Rebecca Creek Spring)	C..T..T.....T.....A..T..G.A...G...

APPENDIX III

Continued.

E. neotenes (Helotes Creek Spring)	C..T..T.....T.....A..T..G.A...G...
E. sp. (Comal Springs)	C..T..T.....T.....A..T..G.A...G...
E. sp. (Pedernales Springs)	...T..T.....T.....A.....G.A...G...
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	...T..T.....G.....G.....A..T....A.....
E. troglodytes complex (176 Spring)	C..T..T.....G.....A.....A.....
E. troglodytes complex (Camp Mystic Spring)	C..T..T.....G.....G.....A.....A.....
E. troglodytes complex (Carson Cave)	C..T..T.....A..T....A.....
E. troglodytes complex (Greenwood Valley Ranch)	C..T..T.....G.....G.....A..T....A.....
E. troglodytes complex (Sabinal Canyon Spring)	...T..T.....G.....G.....A.....A.....
E. troglodytes complex (Smith's Spring)	C..T..T.....A.....A.....
E. troglodytes complex (Sutherland Hollow Spr.)	...T..T.....G.....G.....A..T....A.....
E. troglodytes complex (Trough Spring)	C..T..T.....G.....G.....A.....A.....
E. troglodytes complex (Tucker Hollow Cave)	C..T..T.....G.....G.....A.....A.....
Outgroup	
E. bislineata	...T..T.....C..A.....T..G..T.....
E. longicauda	...T..T.....A...T....C.....A.....G..C.....
E. multiplicata	...T.....T.....A..T....A.....
E. quadridigitata SC	...T.....A.....N..A..T.....
E. quadridigitata TX	...G.....C.....A.....A..T.....N.....
E. wilderae	...C..T.....C..A....N...C.....T..A.....N.....G
Haideotriton wallacei	...T..T....CG..A.....G.....T.....G
Typhlotriton spelaeus	...T.....A.....A..T....A.....G
Northern	
E. tonkawae (Testudo Tube)	ACCTGAAACATCGGAGTTATTCTTTTGTTTTTAGTAATAGCGACAGCAT
E. tonkawae (Ilex Cave)	.....G.....N.....
E. tonkawae (Kretschmarr Cave)	.....G.....N.....N
E. tonkawae (Stillhouse Hollow Springs)	.....G.....N.....
E. tonkawae (Horsethief Hollow Spring)	.....G.....N.....
E. tonkawae (Round Rock Spring)	.....G.....
E. naufragia (Cedar Breaks Spring)	.....G.....N...N...
E. naufragia (Bat Well)	.....NNNN

APPENDIX III

Continued.

E. chisholmensis (Salado Springs)	.....G.....N.....N...
San Marcos	
E. rathbuni	..T.....T.....C.....A..A.....T.....A.....
E. nana	..T.....T..T.....A..A.....T.....A.....
Southeastern	
E. sosorum (Barton Springs)	..T.....T..T.....G..A.....T.....A.....
E. tridentifera (Badweather Pit)	..T.....T..T.....G..A.....T...N.A...N..N
E. tridentifera (Honey Creek Cave)	..T.....T..T.....G..A.....T.....A...N.??
E. tridentifera (Ebert Cave)	..T.....T..T.....G..A.....T...N.A.....C
E. pterophila (Boardhouse Spring)	..T.....T..T.....G..A.....T.....A...????
E. latitans (Pfeiffer's Water Cave)	..T.....T..T.....G..A.....T...N.A...N..?
E. latitans complex (Cibolo Creek Spring)	..T.....T..T.....G..A.....T.....?.....
E. latitans complex (Cloud Hollow Spring)	..T.....T..T.....G..A.....T...N.A.....C
E. latitans complex (Honey Creek Cave Spring)	..T.....T..T.....G..A.....T.....A.....
E. latitans complex (Rebecca Creek Spring)	..T.....T..T.....G..A.....T.....A.....??
E. neotenes (Helotes Creek Spring)	..T.....T..T.....G..A.....T.....A..NN.??
E. sp. (Comal Springs)	..T.....T..T.....G..A.....T.....A.....
E. sp. (Pedernales Springs)	..T.....T..T.....C.....G..A.....T.....A.....
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	..T.....T..T.....C...T.A.....C...NNN.....
E. troglodytes complex (176 Spring)	..T.....T.....C...T.A.....A.C.....A.....
E. troglodytes complex (Camp Mystic Spring)	..T.....T..T.....C...T.A.....T.....A...NN.N
E. troglodytes complex (Carson Cave)	..T.....T..T.....C...T.AN..C.....T.....A.....
E. troglodytes complex (Greenwood Valley Ranch)	..T.....T..T.....C...T.A...C.....C...N.A.....
E. troglodytes complex (Sabinal Canyon Spring)	..T.....T..T.....C...T.A.....C.....A.....
E. troglodytes complex (Smith's Spring)	..T.....T..T.....C...T.A.....T....NN.....
E. troglodytes complex (Sutherland Hollow Spr.)	..T.....T..T.....C...T.A.....C.....A.....
E. troglodytes complex (Trough Spring)	..T.....T..T.....C...T.A.....T.....A...N..?
E. troglodytes complex (Tucker Hollow Cave)	..T.....T..T.....C...T.A.....C.....A.....
Outgroup	
E. bislineata	.....T.....A.....C.A.....A.....
E. longicauda	.....T.....CN.AC.A.....G.....A....???
E. multiplicata	.....T..T.....C..C..CC.A.....C..G..T.....
E. quadridigitata SC	.....T..T.....C..C...C.A.....A.....
E. quadridigitata TX	.....T..T.....C..C..NC.A.....A.....

APPENDIX III

Continued.

E. wilderae	..........T.........N.C.A.............A.....
Haideotriton wallacei	........T..T.....C.....CC.............A....N..
Typhlotriton spelaeus	..T.....................A.....CC....C.....A.....
Northern	
E. tonkawae (Testudo Tube)	TTGTTGGGTA???
E. tonkawae (Ilex Cave)	........?????
E. tonkawae (Kretschmarr Cave)	????????????
E. tonkawae (Stillhouse Hollow Springs)	..........TGT
E. tonkawae (Horsethief Hollow Spring)	..........TGT
E. tonkawae (Round Rock Spring)	........?????
E. naufragia (Cedar Breaks Spring)	.????????????
E. naufragia (Bat Well)	????????????
E. chisholmensis (Salado Springs)	????????????
San Marcos	
E. rathbuni	.C....???????
E. nana	.N...????????
Southeastern	
E. sosorum (Barton Springs)	....?????????
E. tridentifera (Badweather Pit)	NC???????????
E. tridentifera (Honey Creek Cave)	????????????
E. tridentifera (Ebert Cave)	????????????
E. pterophila (Boardhouse Spring)	????????????
E. latitans (Pfeiffer's Water Cave)	NC???????????
E. latitans complex (Cibolo Creek Spring)	.C???????????
E. latitans complex (Cloud Hollow Spring)	????????????
E. latitans complex (Honey Creek Cave Spring)	.C..C.???????
E. latitans complex (Rebecca Creek Spring)	????????????
E. neotenes (Helotes Creek Spring)	NC..?????????
E. sp. (Comal Springs)	.C....???????
E. sp. (Pedernales Springs)	.C..C..A..TG?
Southwestern	
E. troglodytes (Valdina Farms Sinkhole)	????????????
E. troglodytes complex (176 Spring)	.C.??????????

APPENDIX III

Continued.

E. troglodytes complex (Camp Mystic Spring)	NC.??????????
E. troglodytes complex (Carson Cave)	.C..C..A?????
E. troglodytes complex (Greenwood Valley Ranch)	????????????
E. troglodytes complex (Sabinal Canyon Spring)	.C..C.???????
E. troglodytes complex (Smith's Spring)	.C..C..??????
E. troglodytes complex (Sutherland Hollow Spr.)	.C????????????
E. troglodytes complex (Trough Spring)	????????????
E. troglodytes complex (Tucker Hollow Cave)	????????????
Outgroup	
E. bislineata	......???????
E. longicauda	????????????
E. multiplicata	....C????????
E. quadridigitata SC	N.......?????
E. quadridigitata TX	.C.....??????
E. wilderae	????????????
Haideotriton wallacei	.C..A.....???
Typhlotriton spelaeus	.C..A.....TG?