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Systematic Status of the San Marcos Salamander, *Eurycea nana* (Caudata: Plethodontidae)

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The San Marcos Pool of the Edwards Aquifer (Balcones Fault Zone) in Hays County, central Texas is a well-delineated hydrologic system characterized by several endemic species of vertebrates, invertebrates, and plants (Holsinger and Longley, 1980; Longley, 1986). Included among these endemics is the perennibranchiate hemidactyliine plethodontid salamander, *Eurycea nana* Bishop 1941, described from specimens collected from outlets of San Marcos Springs at the headwaters of the San Marcos River and now federally listed as threatened by the U.S. Fish and Wildlife Service.

Eurycea nana is generally regarded as a distinct species. Schmidt (1953), however, relegated it to a subspecies of the (supposedly) widespread species *Eurycea neotenes.* This taxonomic change was strongly protested by Brown (1967a). Bishop (1941) described the distribution of *E. nana* as the lake at the head of the San Marcos River in Hays County (fed by the outflows of San Marcos Springs); Baker (1961) misstated Bishop's description of the type locality as the "Comal Springs at San Marcos, Hays County." We believe that he intended to refer to San Marcos Springs, because Comal Springs are located approximately 30 km southwest of San Marcos Springs at the head of the Comal River in New Braunfels, Comal County, and because later in the same paper, Baker (1961) referred to the salamanders at Comal Springs in New Braunfels as *E. neotenes.* Sweet (1978) suggested informally that *E. nana* might occur at both San Marcos and Comal Springs but did not elaborate except to say that animals from the two localities were "very similar." The population of salamanders at Comal Springs was listed as *E. neotenes* in appendix 1 of Sweet (1978). Dixon (1987), citing a personal communication to him from S. Sweet, also extended the distribution of *E. nana* to include Comal County. R. J. Edwards, H. E. Beaty, G. Longley, D. H. Riskind, D. D. Tupa, and B. G. Whiteside (San Marcos River Recovery Plan for San Marcos Endangered and Threatened Species; USFWS, Albequerque, NM, 1984, unpubl.) cited Bogart (1967) as reporting that *E. nana* occurred at other sites in central Texas. A rereading of Bogart (1967) shows that this was a misinterpretation (verified by our personal communications with J. P. Bogart and D. Tupa). Bogart actually regarded only the San Marcos Springs population as *E. nana.* In this report, we review the confusion and controversy in the literature regarding the distribution of *E. nana* and attempt to resolve this issue based on molecular and morphological work on this taxon and other hemidactyliines in central Texas.

MATERIALS AND METHODS

We used standard methods of horizontal starch-gel electrophoresis (Murphy et al., 1996) to examine the products of 25 enzyme-encoding loci for 13 salamanders from San Marcos Springs and 12 from Comal Springs. This work was part of a much broader study that included a total of 357 *Eurycea* from sites throughout central Texas, plus eight hemidactyliine outgroup taxa (Chippindale, 1995). Electrophoretic conditions used to resolve the loci that distinguish populations from Comal and San Marcos Springs are listed in Table 1.

Descriptions of measurements for morphometric analysis are given in Chippindale et al. (1993), along with specimens, summary statistics, and descriptions of localities. HLB refers to the distance from the posterior margin of the eye to the posterior-most gill insertion, and HLC refers to the distance from the tip of the snout to the posterior-most gill insertion. We included 19 specimens from Comal Springs and 21 from San Marcos Springs in morphometric analysis. A forward stepwise discriminant function analysis was conducted using the program STATISTICA version 4.5 (Statsoft, Tulsa OK, 1993, unpubl.). An α level for the *F*-test of 0.05 was specified at each step. We also used this program to perform linear regressions of head width against axilla-groin length for both populations and to test for parallelism of resulting lines. An analysis of covariance (ANCOVA) was performed to determine whether head widths of salamanders from the two populations differed when effects of body size were removed.

RESULTS

We found the San Marcos Springs *Eurycea* to be extremely distinctive in allozyme composition. Of 25 enzyme-encoding loci examined for 357 specimens of central Texas hemidactyliines

TABLE 1. ALLOZYME LOCI THAT DISTINGUISH *Eurycea* sp. (COMAL SPRINGS) FROM *E. nana* (SAN MARCOS SPRINGS). Alleles indicated by an asterisk are unique among central Texas hemidactyliines and other hemidactyliine taxa examined. Numbers of individuals are indicated by N. Loci examined were aconitate hydratase 1 (Acoh-1; EC 4.2.1.3); adenylate kinase (Ak; EC 2.7.4.3); aspartate aminotransferase-cytosolic (Aats-S; EC 2.6.1.1); glutathione reductase (Gr; EC 1.6.4.2); peptidase B (L-leucylglycylglycine substrate; Pep-B; EC 3.4.?.?.); peptidase D (L-leucyl L-proline substrate; Pep-D; EC 3.4.13.9); and phosphogluconate dehydrogenase (Pgdh; EC 1.1.1.44). Electrophoretic conditions used are in parentheses; buffer systems were described by Murphy et al. (1996): 1) TC II; 2) Tris-citrate-EDTA pH 7.0; and 3) Tris-borate.

	Locus						
	Acoh-1 (1)	Ak (1,2)	Aat-S (3)	Gr (1)	Pep-B (1)	Pep-D (1)	Pgdh (2)
Comal	N = 12 b = 0.545 e = 0.455	N = 12 b = 1.000	N = 11 a = 0.591 b = 0.409	N = 11 a = 1.000	N = 11 b = 1.000	N = 12 c = 1.000	N = 11 b = 1.000
San Marcos	N = 12 d = 1.000*	N = 13 a = 1.000*	N = 13 e = 1.000*	N = 13 c = 1.000*	N = 13 d = 1.000	N = 12 h = 1.000*	N = 13 e = 1.000

(plus outgroups representing three genera and eight species of hemidactyliines), the populations from Comal Springs and San Marcos Springs differed from each other by apparently fixed or mutually exclusive differences at seven loci (Table 1; Chippindale, 1995). The population from San Marcos Springs exhibited fixed, unique alleles (i.e., not seen in other central Texas hemidactyliines or outgroups) at five loci, clear evidence of divergence of this taxon and the lack of gene flow between it and other *Eurycea* from central Texas. The population from Comal Springs exhibited no unique alleles but did possess an allele at aconitate hydratase 1 (45.5% frequency) otherwise seen only in the very distinct taxon, *Typhlomolge rathbuni* (Chippindale, 1995).

Eurycea nana from San Marcos were distinguishable morphologically from *Eurycea* at Comal Springs. Discriminant analysis of 11 external measures of morphology (Fig. 1) illustrated differences in body form between the two populations. The two were separable on the single canonical variate axis, save for slight overlap between a single individual in each population in the range where individual scores meet. Four of 11 variables examined contributed significantly to discrimination power of the analysis: axilla-groin length (AG), head width (HW), head length A (HLA), and head length B (HLB). The unstandardized discriminant function is −1.572HLB + 0.945AG − 1.942HW − 0.909HLA + 4.566. The standardized discriminant function is −0.639HLB + 1.383AG − 0.757HW − 0.459HLA. Reclassification of all 40 individuals (based on the function constructed using the same individuals) was 97.5% successful (one individual from San Marcos Springs was reclassified into the population from Comal Springs). Classification functions that can be used to identify individuals from one or the other population are as follows: Comal Springs: 17.326HLB + 1.455AG + 9.960HW + 1.733HLA − 79.954; and San Marcos Springs: 11.754HLB + 4.809AG + 3.067HW − 1.496HLA − 63.330. To use these functions, scores for an individual are substituted into both equations; the function that yields the highest score corresponds to the group to which the individual is classified. Finally, a bivariate plot of head width against axilla-groin length (Fig. 2) demonstrated that most animals examined from San Marcos Springs have proportionately narrower heads than those from Comal Springs, although there was some overlap between the two populations. Linear regressions of head width on axilla-groin length revealed that, for each population, the slope of the line describing the relationship differed significantly from zero. A test for parallelism, however, did not reject the null hypothesis that the slopes are the same ($F = 0.006$, $P = 0.938$). Results of ANCOVA indicated that head widths for salamanders from the two populations differed significantly when the effects of body size were removed ($F = 76.058$, $P < 0.001$).

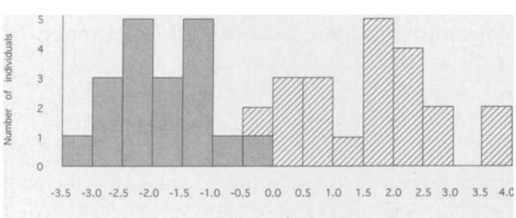

Fig. 1. Individual discriminant function scores for members of the populations from Comal Springs (dark bars) and San Marcos Springs (cross-hatched bars).

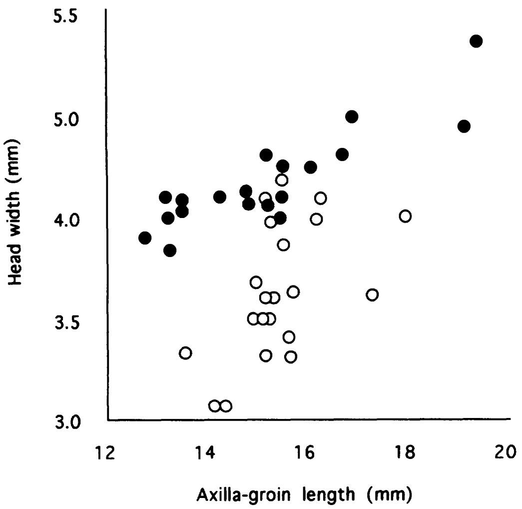

Fig. 2. Bivariate plot of head width versus axilla-groin length for members of the populations from Comal Springs (black circles) and San Marcos Springs (open circles).

Discussion

The allozyme data provided very strong evidence that populations of *Eurycea* from San Marcos and Comal Springs are highly diverged genetically from one another, with no gene flow occurring between them. Mitochondrial cytochrome *b* sequences also support this conclusion (Chippindale, 1995). The distinction between populations was reinforced by discriminant function analysis of morphology. Of four variables that contributed significantly to morphological discrimination, one (axilla-groin length) is a measure of body size; the other three (head width and head lengths A and B) describe head form. The positive loading of axilla-groin length, contrasted with negative loadings of head measures, suggests that this is not due to a simple correlation between head and body size. Our qualitative observations are that animals from San Marcos Springs have narrower heads than most other *Eurycea* from central Texas, consistent with results of discriminant function analysis, analysis of covariance, and the plot of head width versus axilla-groin length (Fig. 2).

In addition to body proportions, Brown (1967a) listed several features of coloration and dentition that distinguished the population of *Eurycea* at San Marcos Springs from other *Eurycea* of the Edwards Plateau. Brown's study included the population from Comal Springs, which he regarded as *E. n. neotenes* based on the distribution map in Brown (1967b). Although we have not examined dentition in these salamanders in detail, we agree with Brown that the combination of coloration characters seen in the population from San Marcos Springs (in particular the light reddish brown body color, coupled with a dark eye ring) is unique among *Eurycea* in central Texas.

Given that *E. nana* from San Marcos Springs can be differentiated by molecular markers from all other *Eurycea* in central Texas, and morphologically from at least the population at Comal Springs, we restrict the name *E. nana* to the population at San Marcos Springs. The status of the population at Comal Springs remains problematic, although it is clear that it is not *E. nana*. We believe that the population at Comal Springs may represent a distinct, undescribed species. Further study of this putative taxon is needed to determine its relationship to other *Eurycea* in central Texas and further clarify species boundaries in the central Texas clade of hemidactyliine plethodontid salamanders.

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