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Department of Biology, Pan American University, Edinburg, Texas 78539, *USA*

STUDIES OF NEOTROPICAL SALAMANDERS OF THE GENUS *PSEUDOEURYCEA*, I: SYSTEMATIC STATUS OF *PSEUDOEURYCEA UNGUIDENTIS*

JAMES F. LYNCH, SUH YUNG YANG, AND THEODORE J. PAPENFUSS

ABSTRACT: The systematic status of *Pseudoeurycea unguidentis*, an enigmatic Mexican plethodontid salamander described by Taylor (1941), is reviewed in the light of morphometric and biochemical analysis of recently collected material. It is concluded that *P. unguidentis* is a valid species which differs from *Pseudoeurycea smithi*, a superficially similar sympatric form, in numerous phenetic and genetic characters. Far from being conspecific, as suggested by an earlier investigator, these two salamanders are more different genetically than are most congeners in other animal groups.

SOME 35 yr ago, E. H. Taylor (1941) described *Bolitoglossa unguidentis* from a series of plethodontid salamanders collected in 1938 on Cerro San Felipe and nearby Cerro San Luis in central Oaxaca, Mexico. The type series of the new species (which subsequently was assigned to the genus *Pseudoeurycea*) was taken in local sympatry with *Pseudoeurycea smithi*, a closely similar salamander. Except for the mention of one additional specimen of *Pseudoeurycea unguidentis* by Taylor and Smith (1945), the species has not been reported since Taylor's original description. The lack of fresh comparative material, combined with the close resemblance between *P. unguidentis* and the common *P. smithi*, influenced Bogert (1967) to synonymize the two forms in his treatment of Oaxacan *Pseudoeurycea*.

Field parties from the Museum of Vertebrate Zoology, University of California, and the Smithsonian Institution visited Cerro San Felipe on four occasions in 1974 and 1975 in conjunction with studies of the salamander fauna of the region. On two of these trips (January and November 1974) *P. smithi* was the only *Pseudoeurycea* encountered, but in August 1974 and August 1975 one of us (TJP) obtained series of what appeared to be a different species in sympatry with *P. smithi*. Both of the latter collections were made at the same locality along the crest of a gently sloping ridge

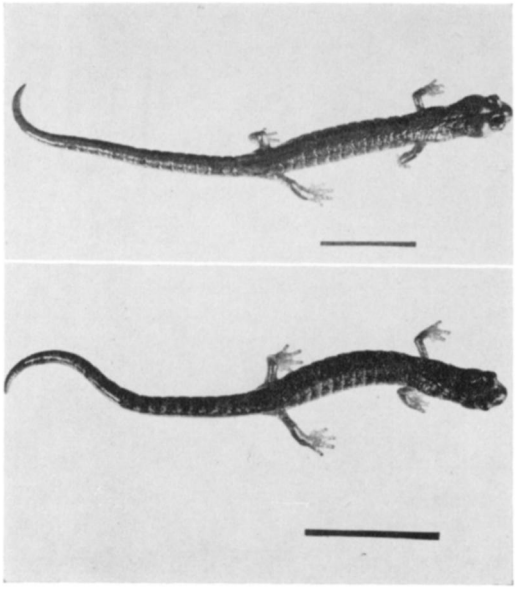

FIG. 1.—Dorsal view of living adult *P. smithi* (top) and *P. unguidentis* (bottom) ♂ ♂. Vertical bar = 25 mm. Photograph by James Hendel, Scientific Photographic Laboratory, University of California, Berkeley.

covered with humid, fairly open oak-pine forest. Based on several altimeter readings taken over a 2-yr period, the elevation of this locality is 3050 ± 25 m. Taylor (1941: 57) gives the elevation of the type locality of *P. unguidentis* as "about 2200 m", but in view of the rarity of specimens of any species of *Pseudoeurycea* at such low elevations in central Oaxaca, and given the restriction of the sympatric *P. smithi* to areas above 2800 m (Bogert, 1967), we believe Taylor's figure to be in error.

Relative to sympatric *P. smithi* (Fig. 1A), the second *Pseudoeurycea* (Fig. 1B) appeared to us to have a somewhat more slender habitus, longer legs, and lighter lateral and ventral pigmentation. These subtle differences appeared to be consistent, even though the overall similarity to *P. smithi* in size, build, and pigmentation was indeed striking. Collecting notes suggested the presence of behavioral and ecological differences as well. Relative to *P. smithi*, the second form was noticeably more vigorous in its attempts to escape and tended to occur off the ground more frequently, the loose bark of fallen logs being the favored microhabitat. The complex of morphological, ecological, and behavioral differences distinguishing the two *Pseudoeurycea* is reminiscent of that separating the slender, active, bark-dwelling *Aneides ferreus* from the more robust, sluggish, ground-dwelling *Aneides flavipunctatus* where the two species are sympatric in northwestern California (Stebbins, 1951; J. Lynch, *personal observation*).

In his description, Taylor stated (1941: 61) that *P. unguidentis* differs from *P. smithi* "... in having longer limbs ... and larger hands and feet. The tail is somewhat longer and narrower." Because these differences correspond to those we perceived in the *Pseudoeurycea* from Cerro San Felipe, we provisionally assigned the second form to *P. unguidentis*. To test our hypothesis that two distinct species (as opposed to mere morphological variants) were collected, we employed two independent modes of analysis: multivariate morphometrics and electrophoretic screening of isozymes.

METHODS

The specimens reported in this paper are deposited in the collection of the Museum of Vertebrate Zoology, Berkeley. A sample of living salamanders was shipped to Berkeley for biochemical analysis in August 1974; other specimens were killed by immersion in dilute chloretone solution within a few hours of capture, fixed in 10% Formalin, and later transferred to 70% ethanol for permanent storage.

Morphometric Analysis.—The morphological analysis is based on a detailed examination of 49 preserved *P. smithi* and 21 *P. unguidentis*. Discriminant function analysis was employed to specify patterns of interspecific differentiation. The following characters were considered (all measurements in millimetres): head length

(HL), the distance from tip of snout to center of gular fold; body length (BL), the distance from center of gular fold to posterior angle of vent; head width (HW), the distance across broadest part of head; tail length (TL), the distance from posterior angle of vent to tip of tail; coupling ratio (CR), the sum of the lengths of right fore limb and right hind limb divided by the axilla-groin distance; maxillary tooth count (MT), the total number of teeth on the maxillae; and vomerine tooth count (VT), the total number of teeth on the vomers. Standard length (SL), the distance from tip of snout to posterior angle of vent, was substituted for characters HL and BL in comparisons involving adult *P. unguidentis*, most of which had been dissected in the gular region for tissue removal.

Discriminant function is a multivariate statistical technique that defines the weighted combination of characters which best separates the members of two or more predefined groups. Each individual is assigned group membership *a priori*; the discriminant procedure then specifies the optimal set of weighted character coefficients that maximizes the ratio of between-group to within-group variance. The reader is referred to Blackith and Reyment (1971) for a general discussion of the application of this and related multivariate techniques to morphometric studies, and to Lynch and Wake (1975) for an example involving plethodontid salamanders.

Because ontogenetic and sexual variation in plethodontid salamanders can obscure interspecific differences, we made the following separate comparisons: (1) adult males, between species; (2) adult females, between species; (3) juveniles (both sexes combined), between species; (4) adult males vs. adult females, within species. The Smithsonian Institution's version of the SPSS program for stepwise discriminant analysis was used in all computations.

Electrophoresis.—Specimens of *P. smithi* (n = 26) and *P. unguidentis* (n = 21) were dissected and samples of blood and tissue were removed. Liver, kidney, spleen, stomach, heart, and skeletal muscle were homogenized using the methods of Selander et al., (1971), and extracts were stored at –76° C. Combined tissue extracts and hemolysate fractions of blood were subjected to horizontal starch-gel electrophoresis as described by Selander et al., (1971). The following gel buffer systems were used: (1) tris-hydrochloric acid (pH 8.5) for hemoglobin (Hb); (2) lithium hydroxide for albumin (ALB), transferrin (TRF), glutamate oxalate transaminases (GOT-1 and GOT-2), and peptidases (PEPT-1 and PEPT-2); (3) discontinuous tris-citrate (Poulik) for lactate dehydrogenases (LDH-1 and LDH-2); (4) continuous tris-citrate (pH 8.0) for sorbitol dehydrogenase (SDH), alpha-glycerophosphate dehydrogenase (α GPD), isocitrate dehydrogenases (IDH-1 and IDH-2), mannose phosphate isomerase (MPI), and superoxide dismutase (SOD); (5) continuous tris-citrate (pH 7.0) for leucine aminopeptidase (LAP), malate dehydrogenases (MDH-1 and MDH-2), phosphoglucomutases (PGM-1 and PGM-2), and phosphoglucose isomerase (PGI); and (6) tris-maleic EDTA for 6-phosphogluconate dehydrogenase (6PGD).

Genetic interpretations of allozymic variation are based on criteria elaborated by Selander et al. (1971). Estimates of heterozygosity are derived from actual counts of presumed heterozygotic genotypes. Mean heterozygosity ($\overline{H}$) is defined as the number of heterozygotic genotypes recorded in a sample divided by the product of the number of individuals and the number of loci surveyed. Estimates of polymorphism are based on the number of loci having more than one allele divided by the total number of loci. This estimate is highly conservative, as only loci showing more than one allele in our limited samples are considered to be polymorphic. The Rogers coefficient (Rogers, 1972) is used as an estimate of interspecific genic similarity.

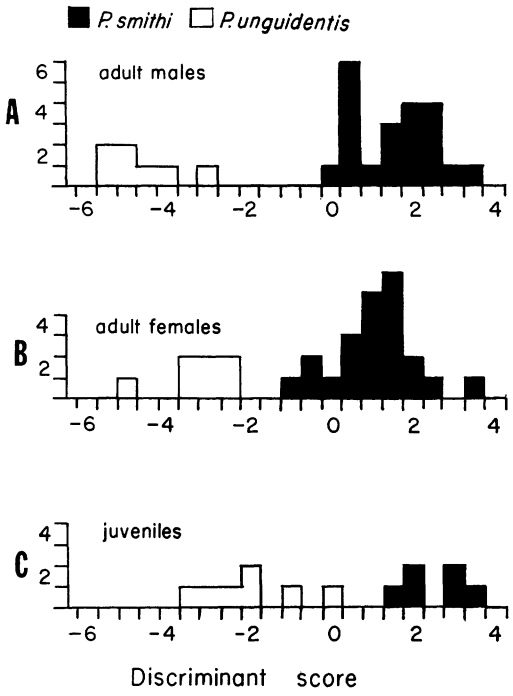

FIG. 2.—Histograms showing discriminant scores for interspecific morphometric comparisons of (A) adult ♂♂, (B) adult ♀♀, and (C) juveniles in *P. smithi* and *P. unguidentis*. The units are standard deviations relative to the mean for all cases. The absence of interspecific overlap in the scores for all three comparisons indicates that the discriminant function successfully separates the species in the three subgroups.

RESULTS

Interspecific Morphological Differentiation.—The discriminant analysis strongly supports our original judgment that two distinct populations of *Pseudoeurycea* coexist on Cerro San Felipe. Whether adult males (Fig. 2A), adult females (Fig. 2B), or juveniles (Fig. 2C) are compared, those individually we visually assigned to the *smithi* and *unguidentis* groups can be distinguished with virtually no "mistakes" on the basis of the six to seven morphological traits we utilized (Table 1). In interspecific comparisons of adults of either sex CR contributes the highest percentage of variation to the standardized discriminant function. *Pseudoeurycea unguidentis* has a higher CR value (i.e., relatively longer limbs) than *P. smithi*. Juveniles show less marked interspecific differentiation in CR; for the latter group HW is the most efficient single discriminator, *P. smithi* juveniles generally having broader heads.

Intraspecific Morphological Comparisons.—As summarized in Table 2, the discriminant function analysis was generally successful in distinguishing the sexes in both species. In some instances the best sexual discriminators were not the best interspecific discriminators. Among 14 adult *P. unguidentis* there were no misclassifications as to sex, and SL and HW proved to be the best discriminators. Males tend to exceed females in overall size (SL), but have relatively narrower heads. Of the 43 adult *P. smithi* examined, 38 (88%) were assigned to the appropriate sex on morphological grounds, with HW, BL, and CR being the main discriminators. Relative to females, male *P. smithi* tend to have shorter bodies, a broader head, and longer legs.

Regression analysis of individual characters as a function of SL (*see* Lynch and Wake, 1975, for explanation of methodology) confirms that the characters designated most important discriminants in the multivariate analysis do show statistically significant differences between species or between the sexes. As an example, there is a highly significant ($P < .01$) interspecific difference in the head width of adult males projected to a common body size. However, some interspecific overlap does occur in this and other single characters, and the discriminant function analysis allows a much cleaner separation of species or sexes than would be possible in any single-character analysis.

Electrophoretic Comparisons.—Sixteen proteins encoded by 22 presumptive loci were examined. Allelic frequencies for variable loci, proportion of polymorphic loci, and proportion of heterozygous loci are given in Table 3. These data constitute compelling evidence of profound genetic

TABLE 1.—Summary of results of discriminant analysis of interspecific morphological differentiation in adult ♂♂, adult ♀♀, and juveniles (sexes combined) of *Pseudoeurycea smithi* and *P. unguidentis*. The absolute magnitude of each standardized coefficient is proportional to the contribution of that variable to the discriminant function. For each comparison the most important coefficient is indicated by boldface type. See text for explanation of variables.

	Discriminant coefficient					
	♂♂		♀♀		Juveniles	
Variable	raw	standardized	raw	standardized	raw	standardized
SL	0.216	1.287	–0.096	–0.589	----------	----------
HL	----------	----------	----------	----------	–0.334	–0.313
BL	----------	----------	----------	----------	0.200	0.706
HW	–0.325	–0.403	0.726	0.661	–1.284	**–0.912**
TL	–0.059	–0.400	–0.048	–0.364	0.010	0.085
CR	–17.767	**–1.457**	–17.290	**–1.314**	–9.110	–0.583
MT	0.053	0.300	0.077	0.406	0.010	0.053
VT	–0.211	–0.830	–0.158	–0.686	–0.028	–0.157
No. and % *a priori* assignments to correct species	28 (100%)		29 (100%)		13 (100%)	
Incorrect assignments	0 (0%)		0 (0%)		0 (0%)	

differentiation between *P. smithi* and *P. unguidentis*. Of the 22 loci considered, 10 (IDH-1, α GPD, MDH-1, LAP, GOT-1, LDH-1, LDH-2, Hb, ALB, TRF) share no common alleles in our samples of the two *Pseudoeurycea*. Six of the 12 remaining loci share at least some polymorphisms between species, although frequency differences may exist in 5 of these (6GPD, PGI, PGM-2, PEPT-1, GOT-2). The remaining six loci are fixed identically in the two species.

TABLE 2.—Summary of results of discriminant analysis of intraspecific morphological differences between adult ♂♂ and adult ♀♀ in *P. smithi* and *P. unguidentis*. See text and legend to Table 1 for futher explanation.

	Discriminant coefficient			
	P. smithi		*P. unguidentis*	
Variable	raw	standardized	raw	standardized
SL	----------	----------	0.495	**2.672**
HL	–0.334	–0.384	----------	----------
BL	0.200	0.917	----------	----------
HW	–1.284	**–1.105**	–3.536	**2.627**
TL	0.010	0.058	0.016	0.142
CR	–9.110	–0.393	–4.086	–0.176
MT	0.010	0.050	0.102	0.637
VT	0.028	0.083	–0.190	–0.430
No. and % assignments to correct sex	38 (88.0%)		14 (100%)	
Incorrect assignments	5 (11.6%)		0 (0%)	

Although our sample sizes are too small to permit firm conclusions, genetic variability appears to be higher in *P. smithi* than in *P. unguidentis* whether the proportion of polymorphic loci (0.50 vs. 0.18) or the mean proportion of heterozygous loci per individual (0.09 vs. 0.04) is the criterion.

DISCUSSION

There can no longer be any serious doubt that *P. unguidentis* and *P. smithi* are distinct species. Even if the existing sympatry test were unavailable, the strong genetic and morphological differentiation demonstrated in the present analysis would justify retention of full specific status for *P. unguidentis*. In fact, by conventional genetic criteria the affinity between the latter species and the sympatric *P. smithi* is rather low for congeners. The computed level of genic similarity ($R = 0.42$) is lower than that found by Hedgecock and

TABLE 3.—Alleles and frequencies in each polymorphic locus in *P. smithi* and *P. unguidentis*. *h* is the proportion of individuals heterozygous at a given locus.

Locus	Allele	*P. smithi*	*h*	*P. unguidentis*	*h*
MPI	a	.154			
	b	.846		1.000	
			.231		0
IDH-1	a	.019			
	b	.981			
	c			1.000	
			.038		0
α GPD	a	1.000			
	b			1.000	
			0		0
6PGD	a	.058		.550	
	b	.904		.450	
	c	.038			
			.192		.400
PGI	a	.077			
	b	.923		.200	
	c			.800	
			.154		.300
PGM-2	a	.173		1.000	
	b	.827			
			.192		0
MDH-1	a	.058			
	b	.673			
	c	.269			
	d			1.000	
			.500		0
LAP	a	1.000			
	b			1.000	
			0		0
PEPT-1	a			.025	
	b			.675	
	c	.212			
	d	.788		.300	
			.231		.150
GOT-1	a	.846			
	b			1.000	
	c	.154			
			.231		0
GOT-2	a	.038		1.000	
	b	.962			
			.077		0
LDH-1	a	1.000			
	b			.975	
	c			.025	
			0		.050
LDH-2	a	1.000			
	b			1.000	
			0		0
Hb	a	.019			
	b	.981			
	c			1.000	
			.038		0
ALB	a	1.000			
	b			1.000	
			0		0
TRF	a	.904			
	b			1.000	
	c	.096			
			.115		0
Proportion of loci polymorphic		.500		.182	
Proportion of loci heterozygous			.091		.041

Ayala (1974) for three species of the salamandrid genus *Taricha*. Indeed, the two Oaxacan *Pseudoeurycea* fall toward the lower end of the range of genetic similarities found in congeneric animal species (*see review by* Avise, 1974). Far from being mere morphological variants, as suggested by Bogert (1967), or even incipient species, *P. unguidentis* and *P. smithi* are highly distinctive evolutionary units.

Work now in progress by two of the present authors (JFL and SYY) in collaboration with D. B. Wake suggests that low levels of genic similarity characterize most of the approximately 25 species that comprise the genus *Pseudoeurycea*. Because electrophoretic methods are relatively insensitive when the populations being compared share few alleles (Avise, 1974, gives 0.4 as the approximate lower limit of genic similarity for meaningful electrophoretic comparisons), allozymic data will have limited utility in our attempts to quantify evolutionary relationships across the entire genus. Webster et al. (1972) reached a similar conclusion with respect to the much larger lizard genus *Anolis*. On the other hand, electrophoretic methods have proved useful in establishing the evolutionary dis-

tinctness of superficially similar populations, as in the present study, and in assessing patterns of relationship within species groups of large genera such as *Anolis* (Yang et al., 1974).

Future papers in this series will further explore patterns of morphological, genetic, and ecological variation within the genus *Pseudoeurycea.*

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JFL: *Chesapeake Bay Center for Environmental Studies, Smithsonian Institution, Edgewater, Maryland* 21037, *USA*; SYY and TJP: *Museum of Vertebrate Zoology, University of California, Berkeley, Calfornia* 94720, *USA*