Copeia, 1983(4), pp. 884–894

Genic and Morphological Differentiation in Mexican *Pseudoeurycea* (Caudata: Plethodontidae), with a Description of a New Species

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A new species of *pseudoeurycea* from the Transverse Volcanic Range of south-central Mexico is shown to be electrophoretically and morphologically distinct from *P. leprosa, P. robertsi* and *P. altamontana. Pseudoeurycea robertsi* and *P. altamontana*, although previously placed in different species groups, are shown to be closely similar genetically. *Pseudoeurycea leprosa*, the most widely distributed of the species that were studied, comprises a central "core" group of relatively undifferentiated populations, and a series of peripheral isolates that are genetically well-differentiated from one another and from the central group.

THE 23 recognized species of the plethodontid salamander genus *Pseudoeurycea* are widely distributed in humid montane areas of Mexico and Guatemala (Wake and Lynch, 1976). Although these salamanders are the most abundant vertebrates in many upland habitats, their ecology is virtually unstudied. In addition, the systematics of the genus is only imperfectly understood, for the dearth of taxonomically useful morphological characters has made it difficult to identify stable groupings of closely related species. In a previous paper (Lynch et al., 1977) we employed multivariate morphometry and starch gel electrophoresis to compare *P. smithi*, a well-known Oaxacan species, and *P. unguidentis*, an enigmatic sympatric form that earlier had been reduced to synonymy with *P. smithi* (Bogert, 1967). The results of our morphological and genetic comparisons agreed in indicating that *P. unguidentis* is a valid, well-differentiated species.

The present study applies similar methods to a group of populations of *Pseudoeurycea* that inhabits the Transverse Volcanic Range (Cordillera Volcanica) of south-central Mexico. The study began as a comparison of isolated populations of *P. leprosa*; we later included two nearby species whose systematic status was poorly understood, and the work received added impetus when our electrophoretic comparisons revealed the presence of an undescribed species of *Pseudoeurycea*. A continuing goal is to clarify the discrepancies between earlier attempts to delimit species groups within the genus (Taylor, 1944; Baird, 1951; Wake and Lynch, 1976) and a recent immunological study (Maxson and Wake, 1981) that indicated a radically different grouping of species.

METHODS

Electrophoresis.—Horizontal starch gel electrophoresis was used to analyze variation in samples from 7 populations currently referred to *P. leprosa*, and one population each of *P. altamontana*, *P. robertsi*, and a previously undescribed species (Table 1).

Salamanders were collected in 1975–1976, and were shipped alive to Berkeley, where they were killed by immersion in dilute chloretone. Samples of liver, kidney, spleen, stomach, heart and skeletal muscle were removed. Tissue extracts that were not used at once were stored at −70 C. Mixed tissue extracts were subjected to horizontal starch gel electrophoresis. We were able to consistently score 18 presumptive genetic loci. The enzymes, their abbreviations, and the appropriate gel/buffer systems (Selander et al., 1971) are given in Table 2. Nei's (1972) genetic distance (D_N) and Rogers' (1972) genetic distance (D_R) were calculated for each pairwise combination of the seven populations of *P. leprosa* and the new species, and for one population of *P. leprosa* versus the new species, *P. altamontana*, and *P. robertsi* (Tables 3, 4).

Morphometric methods.—Discriminant function analysis was employed to test the hypothesis that the four species of *Pseudoeurycea*, despite obvious similarities in size and overall body form, can be reliably distinguished on the basis of conventional external measurements and tooth counts. A related question is the extent of intraspecific, as opposed to interspecific, morphological differentiation in *Pseudoeurycea*. Because the fragmented geographic range of *P. leprosa* greatly exceeds the combined distributions of

TABLE 1. LOCALITY INFORMATION FOR SAMPLES OF *Pseudoeurycea* USED IN THE ELECTROPHORETIC ANALYSIS.

Sample number	Species	Locality	Elevation (m)
1	*P. leprosa*	6 km W Rio Frio, Mexico	
2	*P. leprosa*	La Malinche, 10 km N Canoa, Puebla	3,000
3	*P. leprosa*	12 km N Tlaxco, Puebla	2,900
4	*P. leprosa*	Xometla, Veracruz	2,600
5	*P. leprosa*	San Bernardino, Puebla	2,500
6	*P. leprosa*	12–14 km SW Las Vigas, Veracruz	2,950
7	*P. leprosa*	Zempoala District, 10 km N Tres Cumbres, Morelos	2,770
8	*P. longicauda*	23 km W Villa Victoria, Mexico	2,840–2,970
9	*P. robertsi*	Nevado de Toluca, 4 km N Raices, Mexico	3,320
10	*P. altamontana*	Zempoala District, 20 km N Tres Cumbres, Morelos	3,130

the other three species, it is of interest to compare the level of morphological divergence among isolated populations of *P. leprosa* to the divergence between these populations and *P. altamontana*, *P. robertsi*, and the undescribed *Pseudoeurycea*. The SPSS computer program for stepwise discriminant function analysis was used to generate discriminate functions, scores, and an *a posteriori* classification matrix for 134 salamanders assigned *a priori* to five populations of *P. leprosa*, and one population each of *P. altamontana*, *P. robertsi*, and the undescribed *Pseudoeurycea*.

All measurements were made on salamanders that had been killed by immersion in dilute cloretone solution, fixed in 10% neutral buffered Formalin, and stored in 70% ethanol. The following 8 characters were considered (see Lynch et al., 1977, for detailed description of the measurements): head width, head length, fore limb length, hind limb length, body length, tail length, combined number of maxillary and premaxillary teeth, and number of anterior vomerine teeth. Because previous studies had indicated the existence of marked ontogenetic changes in body proportions (Lynch et al., 1977) analysis was restricted to post-juveniles (combined head and body length >40 mm). We considered only individuals that possessed an intact tail, and rejected specimens that were contorted or mutilated in such a way that accurate measurement was impaired. It would have been

TABLE 2. ENZYMES, THEIR ABBREVIATIONS AND BUFFER SYSTEMS USED IN THE ELECTROPHORETIC ANALYSIS.

Enzyme	Abbreviation(s) of loci	Buffer	Voltage and time run
phosphogluconate dehydrogenase	Pgd	Tris maleate with NADP in gel	100 V (3.5 h)
peptidase (1-leucyl-alanine)	Pept-1, Pept-2	LiOH	300 V (3 h)
glutamate-oxaloacetate-transaminate	Got-1, Got-2		
mannose phosphate isomerase	Mpi	Tris citrate II	100 V (3.5 h)
indophenol oxidase	Ipo (from Mpi stain)		
isocitrate dehydrogenase	Icd-1, Icd-2		
sorbitol dehydrogenase	Sordh		
leucine aminopeptidase	Lap		
malate dehydrogenase	Mdh-1, Mdh-2	Tris citrate IV	180 V (3 h)
phosphate isomerase	Gpi		
phosphoglucomutase	Pgm-1, Pgm-2		
lactate dehydrogenase	Ldh-1, Ldh-2	Poulik	200 V (3 h)

TABLE 3. ALLOZYME FREQUENCIES AT 18 LOCI IN *Pseudoeurycea leprosa* (LOCALITIES 1–7) AND *P. longicauda* (LOCALITY 8). The most rapidly migrating band is indicated as "a." Numbered localities are identified in Table 1. Sample sizes are in parentheses.

	Locality							
Locus	1 (21)	2 (21)	3 (5)	4 (28)	5 (3)	6 (9)	7 (20)	8 (20)
Mpi	c (.95) d (.05)	c	c	a (.05) c (.91) d (.04)	c	b (.78) c (.22)	c	e (.78) f (.22)
Ipo	b	b	b	b	a (.83) b (.17)	b	b	c
Icd-1	a	a	a	a (.93) c (.07)	a (.33) c (.67)	a	a (.63) c (.37)	b
Icd-2	a	a	c	a	a	a (.78) c (.22)	a	b
Sordh	a (.88) e (.12)	a	e	a (.39) b (.15) c (.04) e (.42)	a (.67) b (.17) e (.16)	a (.67) b (.33)	a (.75) d (.25)	e
Pgd	c (.95) d (.05)	b (.02) c (.98)	c	c	c	b (.5) c (.5)	c	c
Gpi	b (.86) c (.14)	b	b	a (.12) b (.88)	a	b	b	a (.37) b (.63)
Pgm-1	a	a	a	a	a	a	a	a (.02) b (.98)
Pgm-2	b	b	b	b	b	b	b	a (.02) c (.98)
Mdh-1	c	c (.98) e (.02)	a	c	c	e	c	b (.02) d (.98)
Mdh-2	b	b	b	b	b	b	b	a
Lap	a	a	a	a (.98) c (.02)	a	a	a	b
Pept-1	d	d	c	b (.04) c (.04) d (.80) e (.12)	b (.67) c (.17) d (.16)	c	d	a
Pept-2	a	a	a	a	a	a	a	a
Got-1	b	a (.02) b (.98)	c	a (.13) b (.82) c (.05)	a (.50) b (.50)	b	b	d
Got-2	c	c	c	c (.98) d (.02)	c	c (.25) d (.75)	c	a (.02) b (.98)
Ldh-1	a	a	c	a (.93) c (.07)	a	a (.72) c (.28)	a	b
Ldh-2	c	c	c	c	c	c	c	a (.20) b (.80)
Mean heterozygosity	.039	.005	0	.079	.185	.056	.035	.064
Percent polymorphic loci	22	17	0	50	27	33	11	39
Alleles per locus (mean)	1.22	1.17	1.00	1.83	1.39	1.33	1.11	1.39

TABLE 4. ALLOZYME FREQUENCIES FOR LOCI IN *Pseudoeurycea altamontana* AND *P. robertsi*. These loci have been standardized relative to populations 1 and 8 in Table 2. Sample sizes are in parentheses.

Locus	Species	
	P. altamontana (7)	*P. robertsi* (13)
Mpi	e	e
Ipo	c (.72) d (.28)	a (.65) f (.35)
Icd-1	d	d (.60) e (.40)
Icd-2	a (.21) b (.79)	b
Sordh	e	e
Pgd	c	c (.40) d (.60)
Gpi	a (.14) b (.72) f (.14)	d (.25) e (.75)
Pgm-1	d	d
Pgm-2	c	a (.30) c (.70)
Mdh-1	f	f
Mdh-2	a	a
Lap	b	b
Pept-1	f (.21) g (.79)	g (.75) h (.25)
Pept-2	b	b
Got-1	d (.57)	c (.10)
Got-2	e	e
Ldh-1	d	d
Ldh-2	e	d (.10) e (.90)
Mean heterozygosity	.127	.167
Percent polymorphic loci	27	44
Alleles per locus (mean)	1.33	1.44

preferable to analyze males and females separately (Lynch et al., 1977), but this would have unacceptably reduced our sample sizes. The final morphometric analysis was based on samples of from 6 to 30 (mean = 17) intact post-juvenile salamanders from each of 8 populations.

RESULTS

In his analysis of the herpetofauna of Michoacan, Duellman (1961, 1965) reported high-elevation populations of *Pseudoeurycea* from the Sierra Temazcaltepec, near Michoacan's eastern border with the state of Mexico. Duellman referred these salamanders to *P. robertsi*, a species that had been known previously only from the Nevado de Toluca, a high volcanic peak some 60 km SE of the Sierra Temazcaltepec. In 1976 we collected a large series of *Pseudoeurycea* in the vicinity of the Michoacan-Mexico border, and were impressed by the differences in proportions and coloration between these animals and topotypic *P. robertsi*. Subsequent analysis of allozyme variation and external morphology confirmed the distinctness of this new form, which we name in reference to its unusually long tail.

Pseudoeurycea ***longicauda*** sp. nov.

Fig. 1

Holotype.—MVZ 137880. An adult female from the forested slope just South Mex. hwy 15, 23.1 km (by rd) W. Villa Victoria, State of Mexico, Mexico (elevation 2,850–2,970 m), collected 7 July 1976 by M. Feder, J. F. Lynch and D. B. Wake.

Paratypes.—MVZ 137879, 137881–921; 138418–36; 138518–19 (63 specimens) same data as holotype; Kansas University (KU) 59397–409, 59969 (14 specimens) Puerto Lengua de Vaca, Michoacan, Mexico (elevation 2860 m); University of Michigan Museum of Zoology (UMMZ) 115130 (5 specimens) 8 km E Macho de Agua, Michoacan, Mexico (elevation 2,880 m); UMMZ 115131 (4 specimens) 1.6 km E Macho de Agua, Michoacan, Mexico (elevation 2,800 m); UMMZ 115132 (3 specimens) Atzimba, Michoacan, Mexico (elevation 2,800 m).

Diagnosis.—*P. longicauda* exhibits a mosaic of morphological similarities and differences with reference to three other similar highland *Pseudoeurycea* that occur in the Cordillera Volcanica

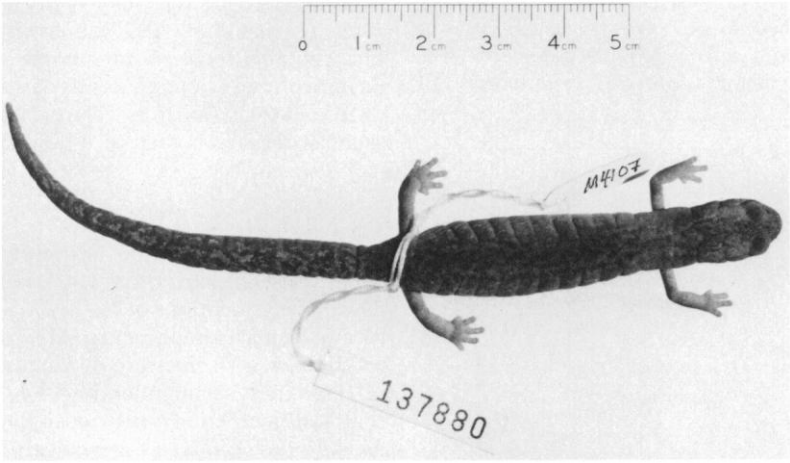

Fig. 1. Holotype of *Pseudoeurycea longicauda*.

east of the Mexico-Michoacan state border (Fig. 2). Adult *P. longicauda* can be distinguished from *P. altamontana*, *P. leprosa* and *P. robertsi* by the following combination of features, based on comparisons of individuals with standard length (SL), the distance from the snout to the posterior angle of the vent, greater than 40 mm: *P. longicauda* greatly exceeds all three of the other species in relative tail length (mean tail length/SL = .98 in *longicauda* vs .82 in *altamontana*, .80–.89 (mean .84) in 5 populations of *leprosa*, .80 in *robertsi*). *P. longicauda* also differs from *P. altamontana* in having a narrower head (mean head width/SL = .14 vs .16), shorter legs (mean combined length of hind limbs plus fore limbs/SL = .48 vs .54), fewer maxillary-premaxillary

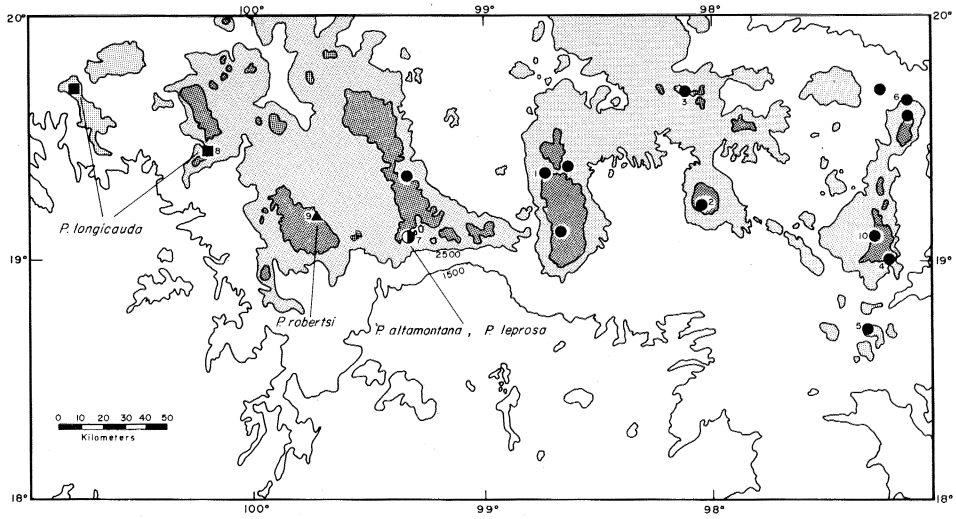

Fig. 2. Map of the Transverse Volcanic region of central Mexico, showing distribution of *Pseudoeurycea longicauda* (squares). *P. robertsi* (triangle), *P. altamontana* (open circle) and *P. leprosa* (solid circles). The latter species also has been reported from locality 9 and from Tianguistengo, Hidalgo, NE locality 3, but we have not examined material from these areas. The single known instance of sympatry involves the latter two species. Heavy Stippling: areas above 3,000 m elevation.

teeth (mean = 41 vs 47), fewer vomerine teeth (mean = 19 vs 25), and lighter pigmentation (see below, Variation). *P. longicauda* differs from *P. robertsi* in having a narrower head (mean head width/SL = .14 vs .16) and more maxillary and premaxillary teeth (mean = 41 vs 28). In *P. robertsi* the venter is uniform gray-black, and a striking tan to red-brown dorsal stripe is almost invariably present. *P. longicauda* is usually lighter than *P. robertsi* in overall color, the dorsal stripe is paler and more irregular, and the chin is distinctly lighter than the remainder of the venter.

Compared with *P. leprosa*, *P. longicauda* has longer legs (mean combined length of hind limb plus fore limb/SL = .48 vs .42) larger feet (mean foot width = .09 vs .07), fewer maxillary-premaxillary teeth (mean = 41 vs 67), and fewer vomerine teeth (mean = 19 vs 24). The two species are fairly similar in coloration, but *P. longicauda* tends to feature paler tones and a lightening of the mid-dorsal pigmentation into an irregular stripe. In both species the chin region is a lighter gray than is the belly, and there is a tendency toward dorsal lightening of the tail. However, *P. leprosa* is usually a dark brown-black salamander with obscure vermiculate brown markings on the dorsum; *P. longicauda* occasionally approach this coloration, but most individuals are a lighter gray-brown, and almost all show at least some development of an irregular light-colored mid-dorsal stripe. Whereas the venter is usually gray-black in *P. leprosa*, it is pale to medium gray in *P. longicauda*.

Variation.—The sexes broadly overlap in size, although females average slightly larger than males (mean SL for females >40 mm = 52.7, max. = 65.3 [N = 9]; mean SL for males = 51.0; max. = 61.6 [N = 16]). Adult males possess a prominent round mental gland. As noted by Duellman (1961), there is considerable variation in color pattern. Some individuals have a featureless gray-black dorsum, but most are much paler, with dorsal coloration that ranges from dark brown through various shades of gray-brown, to an almost pink hue. The dorsum may be mottled with obscure brown streaks and is frequently marked with a ragged mid-dorsal stripe of tan, yellow, orange, or brown. The light to medium gray lateral coloration is partially obscured by varying amounts of silvery-white iridophore "frosting." The color of the venter ranges from slaty to pale gray, but the chin is invariably lighter than the belly. Small white iridophores are scattered across the chin, and extend onto the chest in some specimens.

Measurements of the holotype (in mm).—Head width (HW) 9.1; snout to gular fold (HL) 14.2; head depth at posterior angle of jaw 5.5; eyelid length 3.8, anterior of orbit to snout 3.0; horizontal orbit diameter 2.0; interorbital distance 2.6; snout to insertion of fore limb 13.6; distance separating internal nares 1.7; distance separating external nares 2.8; projection of snout past mandible 1.0; snout to posterior angle of the vent (SL) 65.3; posterior to anterior angle of the vent 4.2; axilla to groin (AG) 36.9; posterior angle of vent to tip of tail (TL) 63.5; tail width at base 4.6; tail depth at base 4.8; axilla to tip of outstretched fore limb (FL) 14.5; groin to tip of outstretched hind limb (HL) 15.1; width of right hand 4.2; width of right foot (FW) 5.5. The holotype has 43 maxillary teeth, 5 premaxillary teeth, and 23 anterior vomerine teeth.

Habitat.—*P. longicauda* is restricted to pine-fir and pine-oak-fir forests at high elevations (2,850–3,000 m) in eastern Michoacan and westernmost Mexico. Specimens have been found on the ground beneath logs, wood chips and other debris, but most of the 64 individuals collected at the type locality in July 1976 were found off the ground, beneath the loose bark of large fir, pine, and cypress logs. Substrate temperatures ranged from 10.5–16.0 C (mean = 12.9 C; N = 14).

Sympatric salamanders.—The only other salamanders known to occur within the range of *P. longicauda* are *P. bellii* and *Ambystoma ordinarium*. All three species were collected by Duellman (1961) in pine-fir forest immediately to the west of the type locality, where we encountered only *P. longicauda*. The three species also occur in close proximity in the Atzimba National Park, some 50 km NW of the type locality (Duellman, 1961).

Range.—Known from two clusters of localities in the Cordillera Volcanica of eastern Michoacan and adjacent Mexico (Fig. 2), an area termed the "Sierra Temazcaltepec" by Duellman (1961). *P. longicauda* may occur in other isolated highland areas of pine-fir forest in the western Cordillera Volcanica (Duellman, 1961, 1965), but the species is absent from the Nevado de Toluca and the Zempoala area, some 50–75 km east of the type locality.

TABLE 5. COEFFICIENTS OF NEI'S DISTANCE (ABOVE DIAGONAL) AND ROGERS' SIMILARITY (BELOW DIAGONAL) FOR SEVEN POPULATIONS OF *Pseudoeurycea leprosa* AND ONE POPULATION OF *Pseudoeurycea longicauda* (SEE TABLE 1 FOR LOCALITIES).

	1	2	3	4	5	6	7	8
1	—	0.002	0.397	0.015	0.191	0.244	0.012	1.867
2	0.978	—	0.404	0.020	0.197	0.240	0.011	1.883
3	0.660	0.667	—	0.325	0.539	0.379	0.413	1.558
4	0.935	0.929	0.693	—	0.167	0.242	0.022	1.672
5	0.778	0.774	0.561	0.791	—	0.437	0.176	1.847
6	0.727	0.731	0.644	0.720	0.585	—	0.261	2.016
7	0.954	0.962	0.652	0.923	0.798	0.711	—	1.854
8	0.167	0.159	0.214	0.211	0.183	0.159	0.171	—

ELECTROPHORETIC COMPARISONS

Because we originally hypothesized that *P. leprosa* and *P. longicauda* were close genetic relatives, our initial electrophoretic survey compared the new species (Table 1, sample 8) with several populations of *P. leprosa*. When these two species proved to be strongly divergent genetically, we added two other local *Pseudoeurycea* (*P. altamontana* and *P. robertsi*) to our comparison. Thus, the second part of the survey compared one population each of *P. robertsi* (sample 9) and *P. altamontana* (sample 10) with *P. longicauda* and one population of *P. leprosa* (sample 1). Data are summarized in Tables 3, 4, and localities are indicated in Fig. 2. Readers are cautioned not to make any assumptions concerning homologies between allozymes that occurred in *P. robertsi* and *P. altamontana* as opposed to *P. leprosa* populations 2–7, unless those same allozymes were found in *P. leprosa* population 1.

Most of the populations we studied displayed substantial allozyme variation (Tables 3, 4). In *P. leprosa* heterozygosity ranged from 0 (population 5) to .079 (population 4) and .185 (population 5, a small sample). The other three *Pseudoeurycea* species exhibited high heterozygosity (maximum = .167 in *P. robertsi*). Polymorphism reached a maximum of 50% (population 4 of *P. leprosa*), and ranged from 27%–44% in the other species and populations. The average number of alleles per locus was also high, reaching a maximum of 1.83 in *P. leprosa* (population 4), and 1.33–1.44 in the remaining three species. Population 3 of *P. leprosa* displayed the lowest variability (fixed at all loci in five specimens).

There was substantial interpopulational variation within *P. leprosa* (Table 5), and Nei distances (D_N) for conspecific samples ranged from .002–.539 (mean .22). Four populations (1, 2, 4, 7) were closely similar genetically (maximum $D_N = .022$), and populations 1 and 2 were virtually identical ($D_N = .002$). However, the Xometla sample (population 4) was considerably more variable than the other three, having 33 alleles at 18 loci. The other three samples (Rio Frio, La Malinche, Zempoala) had 20 to 22 alleles at 18 loci, and were well differentiated from the first four samples, as well as from one another. The smallest genetic distance separating any of these three divergent populations from other *P. leprosa* populations was $D_N = .176$ (between samples 4 and 5, the Xometla and San Bernardino populations); the greatest genetic distance separated populations 3 and 5 ($D_N = .539$). The Tlaxco population (sample 3) appeared to be the most distinctive. The range of D_N values separating this population from the other *P. leprosa* populations was .325–.539 (mean .41). The sample size was sufficiently small (N = 5) that we may have failed to encounter infrequent alleles whose presence might have increased the resemblance of population 3 to other populations, but the Tlaxco population did show one unique, apparently fixed allele (*Mdh-1*, allele a). Sample 6 (Las Vigas population) was the next most distinctive ($D_N = .240–.437$, mean .30). For peripheral populations of *P. leprosa* genetic distance appeared to be a function of geographic separation. Thus, for population 5 (San Bernardino), D_N increased from .167 (vs population 4) to .437 (vs population 6) to .539 (vs population 3).

The Xometla population (sample 4) contained almost all of the alleles that occurred in any of the 7 *P. leprosa* populations. Even relatively well-differentiated populations (e.g., the

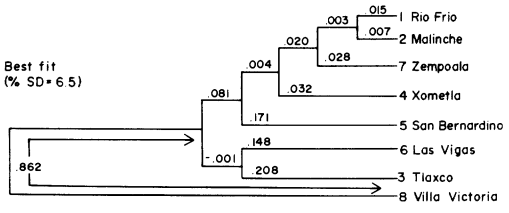

Fig. 3. "Best" Fitch-Margoliash trees constructed from electrophoretic data for seven populations of *P. leprosa* (1–7) and one population of *P. longicauda* (8).

TABLE 6. STANDARDIZED COEFFICIENTS FOR FIRST THREE MULTIPLE DISCIMINANT FUNCTIONS BASED ON 8 MORPHOMETRIC CHARACTERS

Variable*	Function 1	Function 2	Function 3
SL	−.471	−.765	−.984
TL	−.551	−1.178	−.972
HW	.385	.090	2.058
CL	1.537	1.763	−.685
AG	−1.071	−.304	.633
FW	.474	−.036	−.798
MP	−.556	.785	−.175
VT	.134	.199	.235

* See text for definition of variables.

Tlaxco population) differed from the Xometla population mainly in allozyme frequencies. The Tlaxco and Las Vigas populations (samples 3 and 6) shared a fixed allele for *Pept-1*, but showed no other clear-cut similarities in their patterns of allele distribution.

In view of the great genetic distances separating most species of *Pseudoeurycea* (Lynch et al., 1977), and the substantial genetic differentiation we found in *P. leprosa*, we were surprised at the relatively small genetic difference between *P. altamontana* and *P. robertsi* (D_N = .149). This value, which is well within the range of values for isolated populations of *P. leprosa*, is the smallest genetic distance yet recorded between any pair of species in the genus. To put this value into context, we can note that it is about the minimum genetic distance one encounters between sympatric species of the temperate plethodontid genus *Plethodon* (Highton and Larson, 1979).

Although *P. longicauda* previously had been referred to *P. robertsi* (Duellman, 1961, 1965), the two species are very distinct genetically (D_N = 1.41). *P. longicauda* is also very different from *P. altamontana* (D_N = 1.01). Based on gross morphological similarities, we initially had expected that *P. longicauda* would prove to be a close relative of *P. leprosa*, but the mean D_N between *P. longicauda* and the seven populations of *P. leprosa* is 1.81 (range 1.56–2.02). These distances match or exceed those which separate *P. robertsi* (D_N = 1.83) and *P. altamontana* (1.57) from population 1 of *P. leprosa*.

We used the EVOLVE program (provided by Walter Fitch) to generate Fitch-Margoliash trees from D_R values. These trees were rooted using *P. longicauda* as an outgroup. The "best" tree (i.e., the one with the lowest percent standard deviation [see Prager and Wilson, 1978]) is illustrated in Fig. 3. The second best tree differs in grouping population 6 with populations 1, 2, 4, 5 and 7, rather than with population 3. Both trees have a very small negative branch, an indication of some irregularity in the rate of molecular evolution in this group.

MORPHOMETRIC ANALYSIS

The results of the multiple discriminant function analysis (Fig. 4) were consistent with the electrophoretic analysis. The first discriminant axis (Table 6) accounted for 74% of the variation in morphology among populations. This dimension segregated *P. leprosa* from the other 3 species, and also separated *P. robertsi* from *P. longicauda*. The characters with the highest loadings on this axis were combined limb length and axilla-groin length. The second discriminant axis accounted for an additional 12% of

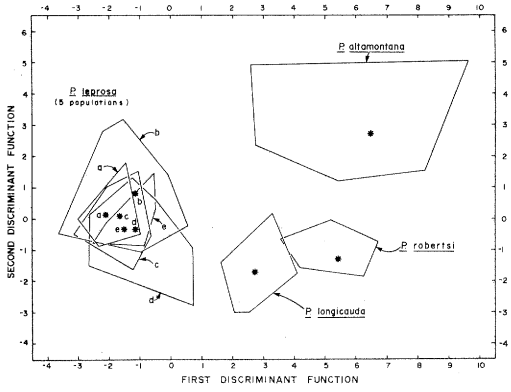

Fig. 4. Plot of first two discriminant functions for morphometric data from 6 populations of *P. leprosa* and one population each of *P. altamontana*, *P. longicauda* and *P. robertsi*.

TABLE 7. CLASSIFICATION MATRIX FOR EIGHT POPULATIONS OF *Pseudoeurycea*, BASED ON STEPWISE MULTIPLE DISCRIMINANT FUNCTION ANALYSIS OF 8 MORPHOMETRIC CHARACTERS. The Orizaba population (sample a) was collected on the NW flank of the Pico de Orizaba, near Sierra Negra, Puebla. Localities for other samples are given in Table 1.

Actual population	Locality	Predicted population							
		a	b	c	d	e	f	g	h
a. *P. leprosa*	Orizaba	14	4	3	2	4	0	0	0
b. *P. leprosa*	Xometla	6	16	0	3	5	0	0	0
c. *P. leprosa*	Las Vigas	0	1	4	1	0	0	0	0
d. *P. leprosa*	Malinche	0	0	4	21	2	0	0	0
e. *P. leprosa*	Zempoala	1	0	0	1	8	0	0	0
f. *P. altamontana*	Zempoala	0	0	0	0	0	9	0	0
g. *P. robertsi*	N. de Toluca	0	0	0	0	0	0	8	1
h. *P. longicauda*	Villa Victoria	0	0	0	0	0	0	0	16

the total among-population variation, and separated *P. altamontana* from *P. longicauda* and *P. robertsi*, mainly on the basis of tail length, combined limb length and tooth counts. Successive axes accounted for 14% of the total among-population variation. As indicated by the classification matrix (Table 7), the canonical analysis resulted in only one "mistaken" assignment of a specimen to the wrong species. Thus, more than 99% of the specimens could be assigned to the correct species solely on the basis of a few external characters. A number of individual *P. leprosa* were assigned to conspecific populations other than their own, but in no case was the assignment to a heterospecific population. In all, 56% of 107 *P. leprosa* could be assigned correctly not only to species, but also to local population.

DISCUSSION

Interspecific differentiation.—The results of the electrophoretic and morphological analyses agree in indicating that *P. longicauda* is well differentiated, both genetically and phenetically, relative to other plethodontid species that have been studied. Contrary to our original expectations, neither *P. leprosa* nor *P. robertsi* (nor *P. altamontana*) is a clear sister species to *P. longicauda*. The magnitude of the genetic distance between *P. longicauda* and *P. leprosa* is similar to that which separates many "large" and "small" eastern North American *Plethodon* species (Highton and Larson, 1979), and exceeds the genetic distance between most European and Californian species of *Hydromantes* (Wake et al., 1978). The genetic distances separating *P. longicauda* from *P. robertsi* or *P. altamontana* are somewhat smaller, but are nevertheless substantial (D_N = 1.41 and 1.00, respectively). Recently obtained electrophoretic data for 16 of the 24 known species of *Pseudoeurycea* indicate that *P. robertsi* and *P. altamontana* are more similar genetically to a number of Guatemalan and Oaxacan species of *Pseudoeurycea* (e.g., *P. rex, P. goebeli, P. smithi, P. unguidentis*) than either is to *P. longicauda*.

The status of species groups within *Pseudoeurycea* remains in a state of flux. The situation has been reviewed recently by Maxson and Wake (1981), who found that immunological data conflicted with a number of previous clusterings of species into groups. For example, although Maxson and Wake prepared five antisera and tested a total of 18 of the 23 *Pseudoeurycea* species that were then known, they discovered no close relatives of *P. leprosa*. Wake and Lynch (1976) had provisionally assigned *P. altamontana* to the *cephalica* group and *P. robertsi* to the *leprosa* groups, but Maxson and Wake (1981) found that both species were much more similar immunologically to *P. smithi* (*gadovii* group) than either was to *P. leprosa* or *P. cephalica*. Curiously, *P. altamontana* gave consistently lower immunological distances than *P. robertsi* to all antisera that were tested.

P. altamontana and *P. robertsi* are very similar genetically, and certainly warrant placement in the same species group. Based on the immunological comparisons of Maxson and Wake (1981), and on unpublished electrophoretic data, the *gadovii* species group seems the most reasonable provenance for these two species, although this group is admittedly a very hetero-

geneous assemblage. Because *P. altamontana* and *P. robertsi* are so similar genetically and are allopatrically distributed, a case could be made for synonymizing them. However, because the two species are distinct in color and body proportions we continue to recognize them as separate taxa for the present.

Intraspecific variation in P. leprosa.—*P. leprosa*, which has by far the most extensive geographical range of the four *Pseudoeurycea* species we studied, presently comprises numerous isolated populations (Fig. 2). Accordingly, we were not surprised to discover considerable morphological and genetic variation among populations of *P. leprosa*, although the extent and geographical pattern of this variation could not have been predicted. A core group of populations along the main E-W axis of the Cordillera Volcanica (Fig. 2) shows only slight genetic differentiation (Table 3, Fig. 3). From west to east these populations inhabit the Ajusco Range (Zempoala sample), the Popocatepetl-Iztaccihuatl volcanic massif (Rio Frio sample), La Malinche Volcano (Malinche sample), and the Pico de Orizaba area (Xometla sample). These populations are restricted to humid, high elevation (2,500–3,000 m) forested areas that today are separated by lower, somewhat drier country which is not inhabited by *P. leprosa*. However, during Pleistocene glacial epochs, elevational zones in this region were depressed by 800 m or more (Simpson, 1979; White, 1960). During pluvial periods continuous forest undoubtedly connected presently isolated areas of montane forest. Indeed, coniferous forest persisted in much of the valley of Mexico until historical times, when significant deforestation by Amerindian populations began. Deforestation of the foothills around the Valley of Mexico was completed in the 16th century when Cortes denuded the slopes of timber in order to construct present-day Mexico City. Clearly, the isolation of populations of *P. leprosa* along the main volcanic axis was a relatively recent event.

Along the Caribbean escarpment, an isolated population of *P. leprosa* approximately 40 km S Volcan Orizaba (San Bernardino) is moderately divergent from the "core" group ($D_N = .18$), but it is the Tlaxco population, some 60 km N Malinche, that shows the greatest degree of genetic differentiation ($D_N = .41$). The population on the flanks of Cofre de Perote (Las Vigas), at the NE periphery of the species range, is also well-differentiated from the core populations ($D_N = .20$), although there is no indication that the Las Vigas sample is particularly distinctive in morphology (Table 6). Indeed, in view of the absence of a major physiographic or climatic barrier between Pico de Orizaba and Cofre de Perote (Fig. 2), the genetic distinctiveness of this population is somewhat surprising. In contrast, the San Bernardino and Tlaxco populations are presently well isolated from the main Transverse Volcanics axis by climatic-topographic barriers.

Taylor (1938) believed that "*P. leprosa*" comprised two allopatric species. He restricted *P. leprosa* to salamanders from the vicinity of Volcan Orizaba (our Xometla and Sierra Negra samples fall within this geographic area), and referred all remaining populations of *P. leprosa*-like salamanders (including those from Rio Frio, Las Vigas, and the Zempoala lakes region) to *P. orizabensis*. Taylor and Smith (1945) later synonymized *P. orizabensis* and *P. leprosa*, a decision which is supported by the results of our study. If any populations currently referred to *P. leprosa* were to be recognized taxonomically, the northern and northeastern outliers (Tlaxco and Las Vigas) would be the most obvious candidates. However, in view of the considerable genetic and morphological overlap that exists among *leprosa* populations (Table 6, Fig. 4) any taxonomic change at this time would be unwarranted.

The results of the present study are in accordance with Wake's (1981) observation that both concordance and discordance between morphological and electrophoretic variation characterize salamander lineages. Populations of the widespread *P. leprosa* show moderate differentiation with respect to both external morphology and allozyme frequencies. In contrast, *P. robertsi* and *P. altamontana*, species which are sufficiently dissimilar in morphology to have been placed in different species groups by earlier workers, show only trivial genetic divergence. The frequent decoupling of morphological and genic variation in salamanders means that both morphometric and biochemical approaches will be indispensable in future attempts at systematic revision of this group.

Acknowledgments

We wish to thank Martin Feder, James Hanken, Theodore Papenfuss, Samuel Sweet and Thomas Wake for their help in collecting the

Pseudoeurycea for this study. Monica Frelow performed most of the electrophoretic work. Walter Fitch generously provided a copy of the EVOLVE program used in the phylogenetic analysis. Margaret McKim and Vicki Dedrickson typed the final manuscript. The illustrations are the work of Susan Strok. Financial support for the research came from the Museum of Vertebrate Zoology, the Smithsonian Institution and NSF Grant DEB78-03008 (D. B. Wake, principal investigator).

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