

A New Species of Frog of the *Rana tarahumarae* Group from Southwestern Mexico

DAVID M. HILLIS, JOHN S. FROST AND ROBERT G. WEBB

Rana zweifeli, a new species of the *Rana tarahumarae* group, is named from southwestern Mexico. This species occurs from southern Jalisco through parts of Colima, Michoacan, Mexico (state), Morelos, Guerrero and Oaxaca. *R. zweifeli* is electrophoretically distinct from the other members of the *R. tarahumarae* group that occur in western Mexico (*R. pustulosa* and *R. tarahumarae*), and it is distinct in several morphometric and pattern characteristics from all the described species in the group.

THE *Rana tarahumarae* species group (as defined by Webb, 1978) has caused considerable confusion among systematists. Several authors (e.g., Blair, 1947; Webb, 1966; Webb and Korky, 1977; Zweifel, 1955, 1968) have noted the problems associated with distinguishing among the species in this group that inhabit western Mexico. The incorrect allocation of names to various species has contributed to the problem (Hillis et al., 1983a; Webb, 1984). Because of the confusing array of morphological manifestations of members of the *R. tarahumarae* group, this study was initiated in order to analyze this group electrophoretically, as well as to reevaluate the morphological differentiation of the various species.

Boulenger (1883) described *Rana pustulosa* from Ventanas, Durango. This name has been applied to *Rana* from Sonora to Oaxaca (Smith and Taylor, 1948; Zweifel, 1955), although most recent systematists have followed Zweifel (1955) in considering *R. pustulosa* to occupy only the southern part of this range. Mocquard (1899) described *R. trilobata* from the vicinity of Guadalajara, Jalisco, and stated that its relationships were with *R. maculata* (of the *R. palmipes* group). However, Boulenger (1920) placed *R. trilobata* in synonymy with *Rana halecina* (= *Rana pipiens*), and that view was generally accepted until Hillis et al. (1983a) examined the holotype and determined it to be a member of the *R. tarahumarae* group. *Rana tarahumarae* was described by Boulenger (1917) from "Ioquiuro" and Barranca del Cobre, Chihuahua, making it the northern-most representative of the group. Blair (1947) described *Rana moorei* (= *R. johni* because of homonymy with a previously described fossil species) from San Luis Potosi, the first locality for a member of this group from the Sierra Madre Oriental. A second species of this group from the Sierra Madre Oriental was described

by Zweifel (1955)—*Rana pueblae* from northwestern Puebla. The most recently-named member of the *R. tarahumarae* group from western Mexico is *Rana sinaloae*, described by Zweifel (1954) from southern Sinaloa near the Durango border. Zweifel (1954) distinguished *R. sinaloae* from what he considered to be *R. pustulosa* to the south by several pattern characteristics, and stated that the relationships of *R. sinaloae* were within the *R. palmipes* group.

Boulenger (1920) considered *R. tarahumarae* to be "... very closely allied to *R. boyllyi* ...," and *R. pustulosa* to be "... nearly allied to *R. palmipes*." Although Zweifel (1954) placed *R. sinaloae* in the *R. palmipes* group, he considered *R. tarahumarae*, *R. pustulosa*, *R. johni* and *R. pueblae* as part of the *R. boyllyi* group (Zweifel, 1955). However, in a biochemical study of several species of the genus *Rana* in North America, Case (1978) concluded that *R. tarahumarae* is allied to members of the *R. palmipes* group much more closely than to the *R. boyllyi* group. On the basis of Case's (1978) study and several morphological considerations, Webb (1978) defined the *R. tarahumarae* group to include: *R. tarahumarae*, *R. pustulosa*, *R. sinaloae*, *R. pueblae* and *R. johni*. However, recent examination of the holotypes of *R. pustulosa*, *R. trilobata* and *R. sinaloae* has indicated that all three names apply to the same species (Hillis et al., 1983a; Webb, 1984). Because *R. pustulosa* was the first of these species to be described that name has priority. Therefore, the species that has traditionally been called *R. pustulosa* (e.g., Blair, 1947; Hillis et al., 1983a; McDiarmid et al., 1976; Oliver, 1937; Smith and Taylor, 1948; Taylor, 1942; Webb and Korky, 1977; Webb, 1978) is presently unnamed. In recognition of the numerous important contributions of Richard G. Zweifel to the systematics of New World *Rana*, and because he adequately distinguished what he (incor-



Fig. 1. *Rana zweifeli* from Copuyo, Michoacan, Mexico, collected and photographed by William E. Duellman.

rectly) called *R. pustulosa* from the species he named *R. sinaloae* (a synonym of *R. pustulosa*), we name the new species in his honor:

Rana zweifeli, n. sp.
(Fig. 1)

Rana pustulosa: Oliver, 1937; Taylor, 1942; Blair, 1947 (in part); Smith and Taylor, 1948 (in part); Zweifel, 1954, 1955 (in part); Duellman, 1958, 1961; McDiarmid et al., 1976; Webb, 1978; Hillis et al., 1983a.

Holotype.—Adult female, University of Kansas 192466, from 12 km E (by road) of Teloloapan, Guerrero, Mexico, collected on 18 May 1982 by David M. Hillis and John S. Frost.

Diagnosis.—A frog of the *R. tarahumarae* group distinguished by the following combination of characters (Table 1): well-developed dorsolateral folds that are not sharply distinct from body color, no ventrolateral stripe (see Webb, 1978), labial stripe indistinct or absent in adults (especially anteriorly), head relatively short and wide (see morphometric analysis, below), tibiofibula relatively short compared to body size (see morphometric analysis) and a distinct tympanum. The crossbars on the thighs of *R. zweifeli* are usually similar in width to the spaces between them, whereas the crossbars on the thighs of *R. pustulosa* are usually narrower than the spaces between them, although this character is variable in both species. Tadpoles of *R. zweifeli* are similar to those of *R. pustulosa* (Webb and Korky, 1977), but differ in less commonly developing a sixth upper tooth row in the later stages, in having a relatively blunt (instead of a tapering) tail tip, and in having a relatively longer tail.

Description of holotype.—Adult female with the following body measurements (in millimeters): snout–urostyle length: 100.1; head width: 38.2; head length: 33.8; tibiofibula length: 47.5; foot length: 67.0; tympanum diameter: 6.0; length of first finger: 20.8; length of second finger: 18.3; distance from eye to naris: 7.1; internarial distance: 7.5; distance from tympanum to eye: 5.5. Distinct dorsolateral folds that are slightly lighter than body color (light brown on a dark olive-brown background). Numerous diffuse black spots over body. No labial stripe. Posterior surface of thighs with light reticulations.

TABLE 1. DIFFERENTIATION OF QUALITATIVE CHARACTERS AMONG MEMBERS OF THE *Rana tarahumarae* GROUP.

Character	Species				
	<i>R. zweifeli</i>	<i>R. pustulosa</i>	<i>R. tarahumarae</i>	<i>R. johni</i>	<i>R. pueblae</i>
1. Dorsolateral folds	Distinct	Distinct, white	Absent or very faint	Absent	Absent
2. Ventrolateral stripe	Absent	Present	Absent	Absent	Absent
3. Labial stripe	Indistinct or absent	Distinct	Absent	Distinct posteriorly	Absent
4. Head shape	Short, wide	Long, narrow	Short, wide	Long, narrow	Short, wide
5. Tibia length	Short	Long	Short	Very short	Very short
6. Tympanum	Distinct	Distinct	Indistinct, pustulose	Distinct	Indistinct

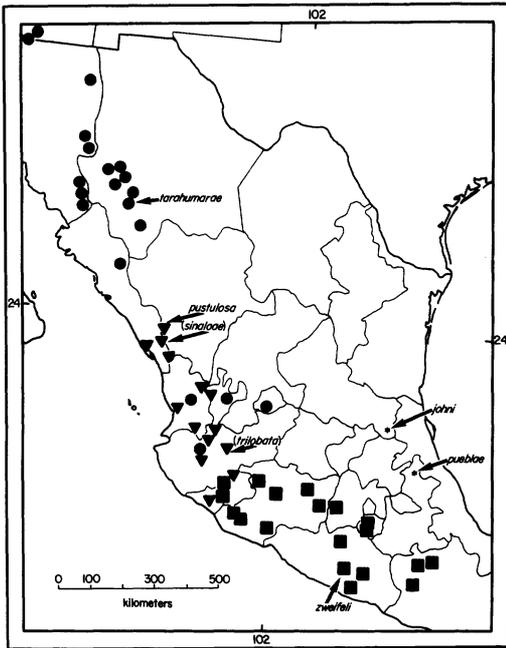


Fig. 2. Known distributions of members of the *Rana tarahumarae* group in western Mexico, as determined from literature records and examined specimens. Circles designate localities for *R. tarahumarae*, triangles for *R. pustulosa* and squares for *R. zweifeli*. The asterisks designate the type localities of *R. johni* and *R. pueblae*. Type localities are indicated by arrows; junior synonyms are enclosed in parentheses.

Four (right) and five (left) dark crossbars across thighs, approximately same width as space between. Dark crossbars across tibia and foot. Webbing and underside of feet marked with black and light reticulations. Venter cream with some melanism on legs and around chin. Second finger distinctly shorter than first or third. Tympanum distinct and smooth, widely separated from eye. Surface of body covered with small pustules, becoming more distinct and numerous laterally. Section of liver and section of femoral muscle removed for electrophoretic analysis; section of intestine removed for chromosomal analysis.

Distribution.—*R. zweifeli* occurs from southern Jalisco and Colima southeast along the escarpment of the Mexican Plateau in Michoacan, Mexico (state) and Morelos, throughout low and moderate elevations of Guerrero, and into northwestern Oaxaca (Fig. 2). It has not been

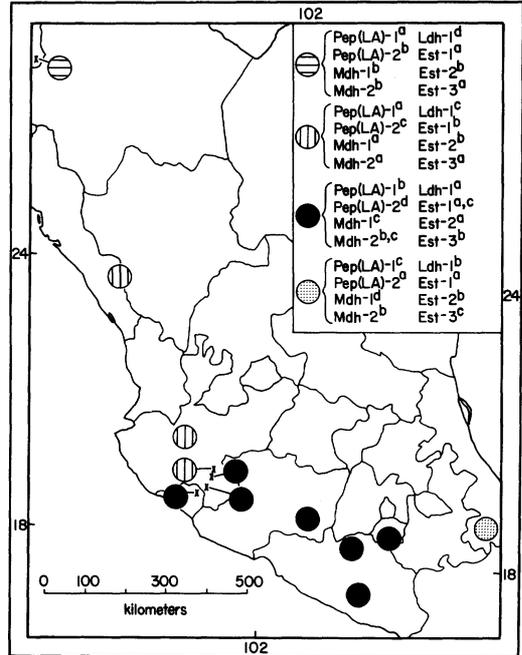


Fig. 3. Observed distribution of electromorphs among three species of the *Rana tarahumarae* group (*R. tarahumarae*, horizontally-striped circles; *R. pustulosa*, vertically-striped circles; and *R. zweifeli*, solid circles) and *R. palmipes* (shaded circle).

collected sympatrically with any other member of the *R. tarahumarae* group, although specimens of *R. pustulosa* have been collected within 15 km of *R. zweifeli* in Jalisco (the former specimens came from higher elevation and considerably different habitat). *R. zweifeli* is sympatric with *R. sierramadrensis* in the Sierra Madre del Sur of Guerrero (Webb, 1978). A record of *R. palmipes* from Cuernavaca, Morelos (Kellogg, 1932) is based on a specimen of *R. zweifeli*.

Electrophoretic analysis.—Sections of liver and femoral muscle were removed from specimens of *R. tarahumarae*, *R. pustulosa*, *R. zweifeli* and *R. palmipes* in the field and stored in liquid nitrogen for transport back to the laboratory. Tissues were kept frozen in the laboratory at -80°C for up to six months. Specimens were collected at the following localities (Fig. 3): *R. palmipes*: Rio Blanco, 27 km E of Cordoba, Veracruz (N = 2). *R. zweifeli*: Santa Fe, Morelos (N = 5); near Chichihualco, Guerrero (N = 6); 12 km E Teloloapan, Guerrero (N = 4; type locality); 22 km SW Zitacuaro, Michoacan (N = 3); Con-

tlá, Jalisco (N = 6); Atenquique, Jalisco (N = 6); and 18 km SW Atenquique (Jalisco), in Colima (N = 2). *R. pustulosa*: 18 km NW Contla, Jalisco (N = 2); 32 km NW Magdalena, Jalisco (N = 2); 10 km (by road) SW El Batel, Sinaloa (N = 13; this is near the type locality of *R. sinaloae* and about 30 km straight line distance from the type locality of *R. pustulosa*). *R. tarahumarae*: 15 km E Yecora, Sonora (N = 2). All specimens were preserved after tissues were removed and have been deposited at the Museum of Natural History, The University of Kansas.

Tissues were separately homogenized in a solution of 0.01 M tris-HCl, 0.001 M EDTA, and 0.001 M β -mercaptoethanol adjusted to pH 7.5 (muscle homogenates diluted 1:1, liver homogenates diluted 1:3). Homogenates were refrozen at -80°C , and then thawed and centrifuged at 15,000 rpm for 15 min before use. Gels were made with Connaught starch (12%); buffer systems employed were the tris-citrate pH 7.0 and tris-versene-borate pH 8.0 systems of Siciliano and Shaw (1976). Two drops of β -mercaptoethanol were added to the gel buffer mixture after boiling and degassing. Gels were electrophoresed for seven hours at approximately 350 V. Staining procedures employed are described by Siciliano and Shaw (1976) and Harris and Hopkinson (1976).

The samples of *R. palmipes* were examined for outgroup comparison to determine primitive and derived electromorphs. The observed electrophoretic patterns of eight variable loci are shown in Fig. 3. The observed isozyme phenotypes substantiate the specific designations of the various species and correspond to the morphotypes presented in Table 1. Based on our sample, the adjacent populations of *R. pustulosa* and *R. zweifeli* in Jalisco show no indication of gene exchange.

In a previous study (Hillis et al., 1983b) 50 genetic loci were examined electrophoretically in single individuals of *R. tarahumarae*, *R. pustulosa*, and *R. zweifeli*. Nei's genetic distance between *R. pustulosa* and *R. tarahumarae* was found to be $D = 0.264$, whereas between *R. tarahumarae* and *R. zweifeli* $D = 0.455$, and between *R. pustulosa* and *R. zweifeli* $D = 0.478$. These data suggest that *R. tarahumarae* and *R. pustulosa* are more closely related to one another than either is to *R. zweifeli*. The electrophoretic data presented in Fig. 3 (of this report) also support this hypothesis. Among the eight loci, the only alleles shared among the three members of the *R. tarahumarae* group that are not

symplesiomorphic (i.e., also present in *R. palmipes*) occur in *R. tarahumarae* and *R. pustulosa* (Pep(LA)-1^a and Est-3^a).

Morphometric analyses.—Eleven morphometric variables were measured on the preserved specimens used in the electrophoretic analysis, as well as on other specimens of *R. pustulosa*, *R. tarahumarae* and *R. zweifeli* in the collections of the Museum of Natural History, The University of Kansas and specimens from the type localities of *R. johnei* and *R. pueblae* in the collections of Strecker Museum, Baylor University. Individuals of *R. pustulosa*, *R. tarahumarae* and *R. zweifeli* ranged from recently transformed juveniles to adults; only adults were available for *R. johnei* and *R. pueblae*. The variables measured were: 1) head width between tympana, 2) head length from tip of snout to corner of mouth, 3) tibiofibula length from middle of joint at each end, 4) foot length from middle of joint between astragalus-calcaneum and tibiofibula to tip of longest toe, 5) vertical tympanum diameter, 6) distance between the tympanum and the eye, 7) length of first finger from base of palmer tubercle to tip of finger, 8) length of second finger measured as in 7, 9) distance between the anterior edge of the eye and the posterior edge of the naris, 10) distance between the nares and 11) snout-urostyle length. All measurements were rounded to the nearest 0.1 mm.

The above morphometric data were used in two stepwise discriminant function analyses (BMDP7M; Dixon et al., 1981). The species were grouped based on the morphological characters listed in Table 1 and the electrophoretic characters listed in Fig. 3. The program was stopped when the F's-to-remove of all variables in the model were above the $\alpha = 0.05$ level of significance ($F_{2,60} = 3.15$) and none of the F's-to-enter of the variables not in the equation were above this level.

The first discriminant function analysis was of the three western members of the *R. tarahumarae* group: *R. pustulosa*, *R. tarahumarae*, and *R. zweifeli*. Six variables were selected by the program as having significant F's-to-enter: head width (x_1), tibiofibula length (x_2), tympanum-eye distance (x_3), eye-naris distance (x_4), internarial distance (x_5), and snout-urostyle length (x_6). The canonical analysis produced two new variables that are plotted along canonical axis I and canonical axis II in Fig. 4. These canonical variables are defined by the following equations:

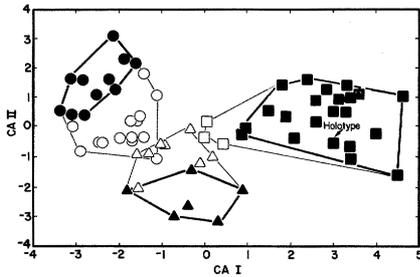


Fig. 4. Plot of scores of individuals of *R. tarahumarae* (triangles; $N = 14$), *R. pustulosa* (circles; $N = 25$), and *R. zweifeli* (squares; $N = 25$) on canonical axes I and II. Open symbols represent juveniles (less than 60 mm), solid symbols represent individual 60 mm and larger. See text for definition of canonical variables.

$$\begin{aligned} \text{CV I} &= 0.0872x_1 - 0.2351x_2 + 0.9241x_3 \\ &\quad - 0.03210x_4 - 1.7887x_5 \\ &\quad + 0.2083x_6 + 0.56779; \\ \text{CV II} &= -0.8934X_1 + 0.1695X_2 - 0.6162X_3 \\ &\quad + 1.5429X_4 - 1.4340X_5 \\ &\quad + 0.2825X_6 + 0.47242. \end{aligned}$$

The plot of the specimens measured in this analysis along the two canonical axes (Fig. 4) reveals three distinct groups corresponding to the three species in the analysis. There is very little overlap of the groups; only one recently transformed specimen of *R. pustulosa* occurs within the polygon that incorporates all the individuals of *R. tarahumarae*. This specimen, as well as three recently transformed *R. zweifeli*, are classified as *R. tarahumarae* by the jackknifed classification functions. Juveniles of all three species generally occupy central positions in the multivariate space defined by the two canonical axes, although all adults are well separated (Fig. 4). Apparently, juveniles of all three species are very similar in shape at metamorphosis (although *R. zweifeli* transform at a slightly larger size), but the three species mature along different developmental trajectories.

The greatest separation of species is between *R. pustulosa* and *R. zweifeli*. These two species are separated primarily along the first canonical axis. Therefore, *R. zweifeli* differs from *R. pustulosa* in having a wider head with nares that are relatively closer to each other and to the eyes, shorter legs relative to body size, and tympana that are more distantly separated from the eyes. *Rana tarahumarae* is intermediate between *R. pustulosa* and *R. zweifeli* along this first axis of variation and is primarily separated from them along the second canonical axis. Along the sec-

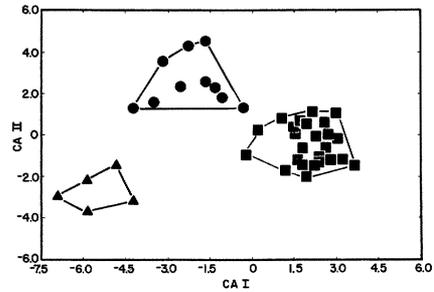


Fig. 5. Plot of scores of individuals of *R. johnei* (circles; $N = 10$), *R. pueblae* (triangles; $N = 5$), and *R. zweifeli* (squares; $N = 25$) on canonical axes I and II. See text for definition of canonical variables.

ond axis, *R. tarahumarae* is characterized by relative increase of head width, internarial distance, and tympanum-eye distance compared to tibiofibula length, eye-naris distance, and snout-urostyle length.

The second discriminant function analysis was performed to compare *R. zweifeli* with the two eastern members of the *R. tarahumarae* group, *R. johnei* and *R. pueblae*. The following six variables had significant F 's-to-enter: head width (y_1), tibiofibula length (y_2), foot length (y_3), length of first finger (y_4), internarial distance (y_5), and snout-urostyle length (y_6). The two new variables that were produced in the canonical analysis are plotted along canonical axes I and II in Fig. 5. The canonical variables are defined by the following equations:

$$\begin{aligned} \text{CV I} &= -0.05057y_1 + 0.2891y_2 \\ &\quad - 0.2361y_3 + 0.6441y_4 \\ &\quad - 1.9901y_5 + 0.1949y_6 \\ &\quad + 4.97723; \\ \text{CV II} &= -0.8220y_1 + 0.7321y_2 \\ &\quad - 0.2731y_3 + 1.0888y_4 \\ &\quad + 2.6490y_5 - 0.2500y_6 \\ &\quad - 2.55459. \end{aligned}$$

The plot of specimens of *R. johnei*, *R. pueblae* and *R. zweifeli* along canonical axes I and II (Fig. 5) reveals no overlap between the species. All of the specimens were correctly identified by the jackknifed classification functions, except for one specimen of *R. johnei* that had a higher posterior probability for *R. zweifeli* (0.731) than for *R. johnei* (0.269). *R. pueblae* differs from *R. johnei* and *R. zweifeli* in having a relatively wider head, shorter tibiofibula length, longer foot length, and shorter first finger length. *R. johnei* differs from *R. zweifeli* primarily in the relation-

ship of internarial distance to body size; *R. johnei* has a relatively wider internarial distance compared to *R. zweifeli*.

Remarks.—The status of frogs assigned herein to *R. tarahumarae* from Aguascalientes, Jalisco and Nayarit requires confirmation (Zweifel, 1968). It is possible that these individuals are not conspecific with any described species. The relationships of the frogs of the *R. tarahumarae* group to frogs of the *R. palmipes* group also needs investigation.

No mating call has been described for any member of the *R. tarahumarae* group. Zweifel (1955) noted that these frogs lack vocal sacs and openings; they may have no mating call. Observations of breeding behavior in these frogs would be of considerable interest.

ACKNOWLEDGMENTS

We thank William E. Duellman, Linda Trueb and Richard G. Zweifel for reading the manuscript and providing suggestions. Parts of this study have been supported by NSF grants DEB81-07625 and DEB81-08266 (to JSF), by the Center for Biomedical Research—The University of Kansas, and by an NSF graduate fellowship (to DMH).

LITERATURE CITED

- BLAIR, A. P. 1947. A new *Rana* from San Luis Potosi, Mexico. *Amer. Mus. Nov.* 1353.
- BOULENGER, G. A. 1883. Description of new species of lizards and frogs collected by Herr A. Forrer in Mexico. *Ann. Mag. Nat. Hist.* 5:342-344.
- . 1917. Description of new frogs of the genus *Rana*. *Ann. Mag. Nat. Hist.* 8:413-418.
- . 1920. A monograph of the American frogs of the genus *Rana*. *Proc. Amer. Acad. Arts Sci.* 55: 413-480.
- CASE, S. M. 1978. Biochemical systematics of members of the genus *Rana* native to western North America. *Syst. Zool.* 27:299-311.
- DIXON, W. J., M. B. BROWN, L. ENGELMAN, J. W. FRANE, M. A. HILL, R. I. JENNRICH AND J. D. TOPOREK. 1981. BMDP statistical software 1981. Univ. California Press, Berkeley.
- DUELLMAN, W. E. 1958. A Preliminary analysis of the herpetofauna of Colima, Mexico. *Occ. Pap. Mus. Zool. Univ. Mich.* 594.
- . 1961. The amphibians and reptiles of Michoacan, Mexico. *Univ. Kansas Publ. Mus. Nat. Hist.* 15.
- HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publ. Co., Amsterdam.
- HILLIS, D. M., D. R. FROST AND J. S. FROST. 1983a. Allocation and distribution of *Rana trilobata* Mocquard. *J. Herpetol.* 17:73-75.
- , J. S. FROST AND D. A. WRIGHT. 1983b. Phylogeny and biogeography of the *Rana pipiens* complex: A biochemical evaluation. *Syst. Zool.* 32:132-143.
- KELLOGG, R. 1932. Mexican tailless amphibians in the United States National Museum. *Bull. U.S. Natl. Mus.* 160.
- MCDIARMID, R. W., J. F. COPP AND D. E. BREEDLOVE. 1976. Notes on the herpetofauna of western Mexico: New records from Sinaloa and the Tres Marias Islands. *Cont. Sci. Nat. Hist. Mus. LA Co.* 275.
- MOCQUARD, M. F. 1899. Reptiles et batraciens recueillis au Mexique par M. Léon Digué en 1896 et 1897. *Bull. Soc. Phil. Paris* 9:154-169.
- OLIVER, J. A. 1937. Notes on a collection of amphibians and reptiles from the state of Colima, Mexico. *Occ. Pap. Mus. Zool. Univ. Mich.* 360.
- SICILIANO, M. J., AND C. R. SHAW. 1976. Separation and localization of enzymes of gels, p. 184-209. *In: Chromatographic and electrophoretic techniques*, 2, 4th edition. I. Smith (ed.). William Heinemann Medical Books Ltd., London.
- SMITH, H. M., AND E. H. TAYLOR. 1948. An annotated checklist and key to the Amphibia of Mexico. *Bull. U.S. Nat. Mus.* 194:1-118.
- TAYLOR, E. H. 1942. Tadpoles of Mexican anura. *Kansas Univ. Sci. Bull.* 28:37-55.
- WEBB, R. G. 1966. The lizard *Sceloporus bulleri* in western Mexico. *Yearbook Amer. Phil. Soc.* 1965: 356-357.
- . 1978. A systematic review of the Mexican frog *Rana sierramadrensis* Taylor. *Cont. Sci. Mus. Nat. Hist. Los Angeles Co.* 300:1-13.
- . 1984. Herpetogeography in the Mazatlan-Durango region of the Sierra Madre Occidental, Mexico. *In: Vertebrate ecology and systematics: A tribute to Henry S. Fitch*. R. A. Seigel et al. (eds.). *Mus. Nat. Hist. Univ. Kansas Spec. Publ.* 10.
- , AND J. K. KORKY. 1977. Variation in tadpoles of frogs of the *Rana tarahumarae* group in western Mexico (Anura: Ranidae). *Herpetologica* 33:73-82.
- ZWEIFEL, R. G. 1954. A new frog of the genus *Rana* from western Mexico with a key to the Mexican species of the genus. *Bull. So. Calif. Acad. Sci.* 53: 131-141.
- . 1955. Ecology, distribution and systematics of frogs of the *Rana boylei* group. *Univ. Calif. Pubs. Zool.* 54:207-292.
- . 1968. *Rana tarahumarae*. *Cat. Amer. Amph. Rep.* 66.1-66.2.
- MUSEUM OF NATURAL HISTORY AND DEPARTMENT OF SYSTEMATICS AND ECOLOGY, THE UNIVERSITY OF KANSAS, LAWRENCE, KANSAS 66045 AND DEPARTMENT OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF TEXAS AT EL PASO, EL PASO, TEXAS 79968. Accepted 21 April 1983.