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EVOLUTIONARY RELATIONSHIPS AMONG CENTRAL AMERICAN SALAMANDERS OF THE *BOLITOGLOSSA FRANKLINI* GROUP, WITH A DESCRIPTION OF A NEW SPECIES FROM GUATEMALA

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ABSTRACT: *Bolitoglossa meliana*, a new species from the mountains of central Guatemala, is described and assigned to the *B. franklini* group. Electrophoretic comparisons with the other species in the group confirm the distinctiveness of *B. meliana*, but cause us to question the validity of *B. brevipes* and *B. nigroflavescens*, which are hereby synonymized with *B. franklini*. Plio-Pleistocene tectonic and climatic events may have led to secondary contact and hybridization between previously isolated inland and Pacific coastal populations, thereby complicating patterns of genic similarity.

Key words: Amphibia; Caudata; *Bolitoglossa*; Electrophoresis; Proteins; Biogeography

NUCLEAR Central America, the tectonically and topographically complex region that lies between the Isthmus of Tehuantepec and the Nicaraguan depression (Schuchert, 1935), is the major center for species diversity in tropical plethodontid salamanders (Wake and Lynch, 1976). The present paper considers evolutionary relationships within the *Bolitoglossa franklini* species-group (Stuart, 1943) of the highlands of Guatemala and adjacent Chiapas, Mexico. This complex also has been called the "*lincolni* subgroup" of the *Bolitoglossa rostrata* species-group (Wake and Lynch, 1976), but Stuart's name has priority. We first describe a newly discovered species, and then examine electrophoretic data and selected morphological information for all species in the group. Finally, we present an evolutionary scenario that is consistent with available data.

MATERIALS AND METHODS

Electrophoretic Analysis

Samples used for electrophoretic analysis (Table 1) were obtained from three populations representing the new species of *Bolitoglossa* and six populations previously referred to *B. brevipes* (1), *B. franklini* (2), *B. nigroflavescens* (1), *B. lincolni* (1) and *B. resplendens* (1). Sample sizes per population ranged from 1 to 14 (Table 2 below). Use of small samples in interspecific comparisons has been assessed by Gorman and Renzi (1979).

All animals were brought to the laboratory alive. Freshly sacrificed specimens were dissected and samples of liver, kidney, spleen, heart and intestine were extracted, frozen, and stored at −76 C for future use. Combined tissue extracts were subjected to horizontal starch-gel electrophoresis (Selander et al., 1971).

TABLE 1.—Collecting localities for samples used in electrophoretic analysis. Numbers refer to populations in Fig. 2.

1. *Bolitoglossa meliana.* Santa Rosa Pass, 9 km NE Santa Cruz del Quiché, El Quiché, Guatemala (2520 m).
2. *B. meliana.* La Bella crest, 20 km NNW San Augustín Acasaguastlán, El Progreso, Guatemala (2725 m).
3. *B. meliana.* 4–6 km (by road) S Purulhá, Baja Verapaz, Guatemala (1650–1800 m).
4. *B. franklini nigroflavescens* (previously *B. brevipes*). Sierra Madre, 6.7 km (by road) W Motozintla, Chiapas, México (1780–1860 m).
5. *B. franklini franklini.* Above Colonia Talquian, Volcán Tacaná, Chiapas, México (2400–2510 m).
6. *B. franklini franklini.* Volcán Chicabál, 5 km SE San Martín Sacatepequez, Quezaltenango, Guatemala (2250 m).
7. *B. franklini nigroflavescens.* Cerro Ovando, Chiapas, México (1650–1680 m).
8. *B. lincolni.* Sierra de los Cuchumatanes, 3.5 km (air line) NNW Uspantán, El Quiché, Guatemala (2260–2640 m).
9. *B. resplendens.* Montañas de Cuilco, 1.5 km NE Peña Blanca, Huehuetenango, Guatemala (2800 m).

The following gel buffer systems were used: 1.—Tris Maleate EDTA, pH 7.4 (0.01 M Tris/0.1 M maleic acid/0.01 M EDTA) for malate dehydrogenase (*Mdh*; 2 loci) and malic enzyme (*Me*); 2.—Tris Citrate II, pH 8.0 (0.687 M Tris/0.15 M citric acid) for glutamate oxalate transaminases (*Got*; 2 loci), sorbitol dehydrogenase (*Sod*), mannose phosphate isomerase (*Mpi*) and 6-phosphogluconate dehydrogenase (*Pgd*), for which 1.0 cc NADP was added to 240 cc gel buffer; 3.—Tris Citrate III, pH 7.0 (0.135 M Tris/0.043 M citric acid) for glucophosphate isomerase (*Gpi*), phosphoglucomutase (*Pgm*), alpha-glycero-phosphate dehydrogenase (*α Gpd*) and isocitrate dehydrogenase (*Icd*; 2 loci); 4.—Poulik, pH 8.7 (gel: 0.076 M Tris/0.005 M citric acid; trays: 0.30 M boric acid, pH 8.2) for lactate dehydrogenase (*Ldh*; 2 loci), leucine aminopeptidase (*Lap*), and leucyl alanine peptidase (*Pep*) (Ayala et al., 1972; Selander et al., 1971). Genetic interpretations of allozymic data are based on criteria elaborated by Selander et al. (1971). Alleles are designated by letter, with "a" being the fastest migrant.

Morphological Data

Most meristic and proportional characters used in salamander systematics vary with overall size or sex, or both. With sufficiently large samples, regression methods can be used to adjust measurements for body size and sex (e.g., Lynch, 1981; Lynch and Wake, 1975, 1978), but in the present study this procedure was possible only for *B. franklini*, *B. resplendens* and the new species. For the remaining forms, only general comments on color pattern and external morphology are offered.

External measurements and tooth counts are based on examination of animals that had been fixed in dilute formaldehyde and stored in 70% ethanol. Osteological data were obtained from radiographs and from specimens that had been cleared in KOH and stained with Alizarin Red. Except as noted below, all specimens are deposited in the collection of the Museum of Vertebrate Zoology (MVZ), Berkeley, California.

DESCRIPTION OF NEW SPECIES

In 1972, we collected specimens of an unknown species of *Bolitoglossa* in a newly accessible region of Baja Verapaz, Guatemala. Subsequent collecting has revealed this large, all black species to be widespread in the Chuacús-Minas mountain system of central Guatemala. In allusion to its characteristic pigmentation, this species shall be known as:

Bolitoglossa ***meliana*** sp. nov.

Holotype.—MVZ 160736. An adult female from the vicinity of Santa Rosa Pass, 9 km NE Santa Cruz del Quiché, El Quiché, Guatemala (elevation 2520 m), collected 16 July 1978 by P. Elias, E. J. Koford, D. B. Wake, and T. A. Wake (Fig. 1).

Paratypes.—MVZ 160361–72, 160385–97, 160373–84, 160398–99, 160737–71 (74

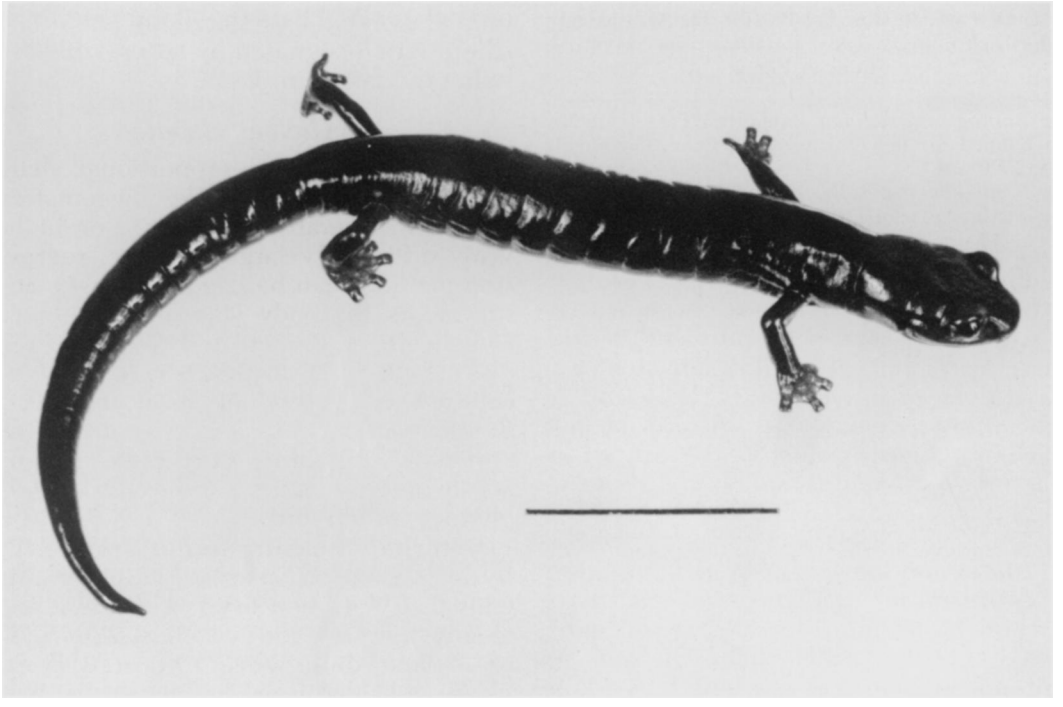

FIG. 1.—An adult specimen of *Bolitoglossa meliana* from Santa Rosa Pass, El Quiché, Guatemala. Scale is 25 mm.

specimens), all collected at the type locality; MVZ 108854, 113160–61, 150519, 169069 (5 specimens), 4–6 km (by road) S Purulhá, Baja Verapaz, Guatemala (elev. 1650–1800 m); MVZ 150804–07, 150813 (5 specimens), San Antonio, 8 km (by road) ESE Chilascó, Baja Verapaz, Guatemala (elev. 1850 m); MVZ 150789, 150812 (2 specimens), Finca Planada, 15 km NNE Río Hondo, Zacapa, Guatemala (elev. 1700 m); MVZ 150808–11, 160772 (5 specimens), Sierra de las Minas, 12 km N Santa Cruz, Zacapa, Guatemala (elev. 2200 m); MVZ 169038–39 (2 specimens), La Bella crest, 20 km NNW San Augustín Acasaguastlán, El Progreso, Guatemala (elev. 2725 m); KU 18613839 (2 specimens), El Volcancito, 3.5 km SE Purulhá, Baja Verapaz, Guatemala.

Diagnosis.—Distinguished from all other *Bolitoglossa* of México, Guatemala, Honduras and El Salvador by its combination of uniform black coloration, large adult size (males to 66 mm standard length [SL], females to 88 mm SL), and truncated tips of toes with terminal phalanges free of webbing (Fig. 1). Resembles other species of the *franklini* complex in body proportions, overall size, and in possession of slightly webbed "*Magnadigita*"-type (Taylor, 1944) hands and feet; however, other members of the *franklini* complex are marked with bright patches of white, silvery, brassy, or red-orange pigment. In addition, *B. meliana* is strongly differentiated genetically from the other species (see below).

The only other Guatemalan salamander with which *B. meliana* might possibly be confused is the sympatric *B. morio*, a much smaller, stouter species with shorter tail and limbs, and somewhat more webbing of hands and feet. As opposed to the featureless black pigmentation of *B. meliana*, *B. morio* has white flecks on the venter and irregular cream or pink spots dorsolaterally.

Variation.—In *B. meliana* and other

members of the *franklini* complex, ontogenetic and sexual differences exceed interspecific variation for most external features except coloration. The following comments are based on measurements of 17 juveniles (SL < 41 mm), ten subadults (SL 41–49 mm), 17 adult and near-adult males (SL > 49 mm) and 18 adult and near-adult females (SL > 49 mm). Linear regression methods were used to analyze bivariate trends in variation separately for juveniles, near-adult plus adult males, and near-adult plus adult females. For purposes of comparison, the value of each morphological trait was projected to a common mean value (CMV) at an SL of 30 mm for juveniles and 60 mm for near-adults and adults (Lynch and Wake, 1975, 1978; Lynch, 1981).

Males and females of *B. meliana* differ in minimum size at sexual maturity (males ca. 55 mm, females ca. 65 mm), maximum size (males 66 mm SL, females 80 mm SL), relative tail length in adults (0.84–1.10 SL [CMV = 0.94 SL] in males >49 mm SL; 0.79–0.98 SL [CMV = 0.83 SL] in females >49 mm SL), and relative limb length in adults (hind limb length 0.22–0.28 SL [CMV = 0.26 SL] in males; 0.22–0.25 SL [CMV = 0.24 SL] in females). There is no statistically significant sexual variation in relative head size or in numbers of maxillary, premaxillary, or vomerine teeth.

Relative to juveniles, adult and near-adult individuals (SL > 49 mm) have proportionately longer tails (tail length ÷ SL = 0.79–1.10 [CMV = 0.89] in adults and near-adults; 0.38–0.69 [CMV = 0.58] in juveniles), and more maxillary-premaxillary teeth (43–76 [CMV = 60] versus 11–62 [CMV = 43] in juveniles and subadults). Relative limb length and head width are similar in juveniles and adults.

Comparative osteology.—Groups within the large genus *Bolitoglossa* are not well differentiated in osteology. The only character that segregates substantial numbers of species is the complex tail base that distinguishes the "beta" group of the genus from the "alpha" group (Wake and Brame, 1969; Wake and Dresner, 1967; Wake and Lynch, 1976).

The following observations are derived from x-rays of 12 *B. meliana* (MVZ 108854, 113160, 150789, 105804–812) and one cleared and stained individual (MVZ 160740). We have examined cleared and stained specimens of *B. f. franklini*, *B. f. nigroflavescens*, *B. resplendens* and other less closely related *Bolitoglossa* but have detected no skeletal features that distinguish *B. meliana* from the other members of the *franklini* group.

The skeleton of *B. meliana* is well developed and, apart from the complex tail base, is about as generalized as is found in the genus. The single premaxillary bone bears slightly to moderately expanded frontal processes. The relatively large nasal bones are strongly protuberant and extend forward well in advance of the premaxillaries. The prefrontal bones, though discrete and well formed, are much smaller than the large nasals. Septomaxillaries are absent. Maxillaries, frontals, parietals, otic-occipitals, squamosals, quadrates, and parasphenoid bones are generalized in form. The vomers also are generalized and have long and relatively stout preorbital processes that bear teeth to their midpoint. The operculum has at most a very small stilus. The limbs are stout. The tibia bears a distinct proximal crest in larger specimens, and it is partially free (i.e., attached only at the distal end) to form a tibial spur in the two largest specimens examined. The digits are well formed, and the terminal phalanges of the longer digits are greatly expanded terminally to a "T" or "Y" shape. Phalangeal formulae are 1-2-3-2, 1-2-3-3-2. There are two caudosacral vertebrae. The first caudal vertebra has very long transverse processes which are bifid in most individuals, forming the complex tail base typical of the beta group of the genus. The long transverse processes of caudal vertebrae arise at the anterior ends of the vertebrae and extend antero-

laterally. Unregenerated tails have 27–34 trunk vertebrae in adults.

Measurements of the holotype (in mm).—Head width 10.3; snout to gular fold (head length) 14.7; head depth at posterior angle of jaw 5.4; eyelid length 3.8; anterior rim of orbit to snout 3.8; horizontal orbit diameter 2.9; interorbital distance 3.9; snout to insertion of forelimb 19.8; distance separating internal nares 2.9; distance separating external nares 3.5; projection of snout past mandible 0.7; snout to posterior angle of vent (SL) 68.7; posterior to anterior angle of vent 5.1; axilla to groin 37.1; posterior angle of vent to tip of tail (tail length) 54.1; tail width at base 4.8; tail depth at base 4.2; axilla to tip of outstretched forelimb (forelimb length) 15.5; groin to tip of outstretched hind limb (hindlimb length) 17.5; width of right hand 5.6; width of right foot 7.2. The holotype has 59 maxillary teeth, 7 premaxillary teeth, and 26 anterior vomerine teeth.

Habitat.—*B. meliana* inhabits humid forest and forest margins at moderate to high elevations (1650–2725 m). Vegetation associations at the six known localities range from extremely wet cloud forest in the east (Baja Verapaz, El Progreso, Zacapa) to less humid oak-pine forest in the west (El Quiché). A generalized map of rainfall (Instituto Geográfico Nacional de Guatemala, 1966) indicates that annual rainfall in areas where *B. meliana* occurs ranges from approximately 150–400 cm. Like *B. resplendens* and *B. lincolni* (Wake and Lynch, 1976), *B. meliana* is relatively generalized in its microhabitat requirements. Most individuals have been collected on the ground beneath logs or other cover objects, but arboreal bromeliads and the loose bark of fallen logs are also used as retreats.

Sympatric salamanders.—In the eastern portion of its range (Baja Verepaz, El Progresso, Zacapa), *B. meliana* co-occurs with two other salamanders, a medium-sized species (*B. helmrichi*) and a diminutive form (*Chiropterotriton veraepacis*). Both tend to be more arboreal than *B. meliana*, but all three species inhabit bromeliads and crevices beneath loose bark. In El Quiché, where *B. meliana* reaches its greatest local abundance (over 70 specimens collected), *Chiropterotriton* is absent but *B. morio* and an unnamed salamander possibly referable to *B. hartwegi* (Elias, 1982) are present.

Range.—Known from several localities that span approximately 160 km along the predominantly east–west axis of the Chuacús-Minas mountain systems of central Guatemala (Fig. 2). Some populations are undoubtedly isolated by intervening low mountain passes. The species should be sought in the higher mountains of Alta Verapaz, where both *B. helmrichi* and *C. verapaecis* occur (Lynch and Wake, 1978). *B. meliana* is the first salamander reported from the departamentos of El Progreso and Zacapa.

Relationships Among Populations of the *franklini* Group

Systematic background.—The first member of the *franklini* group to be described (Schmidt, 1936) was *Bolitoglossa franklini*, a common inhabitant of bromeliads in cloud forests at moderate elevations (1700–2600 m) along the Pacific escarpment of SW Guatemala.

Taylor (1941) described *B. nigroflavescens* from a large series of salamanders collected by H. Smith on Cerro Ovando, a peak in the Sierra Madre del Sur of Chiapas some 100 km NW of the nearest of Schmidt's Guatemalan localities for *B. franklini* (Fig. 2; locality 7). The two species are similar in size and habitus and in their use of bromeliads as the primary microhabitat. Both species possess a black background coloration that is marked with light-colored patches. In *B. nigroflavescens*, the iridophore patches tend to be dense and laterally concentrated, and are cream to yellow, orange-yellow, or brassy in color; in *B. franklini*, iridophores tend to be more diffuse and silver-gray to bronze in color, and they are concentrated dorsally (Schmidt, 1936; Taylor, 1941; Wake and Lynch, 1976).

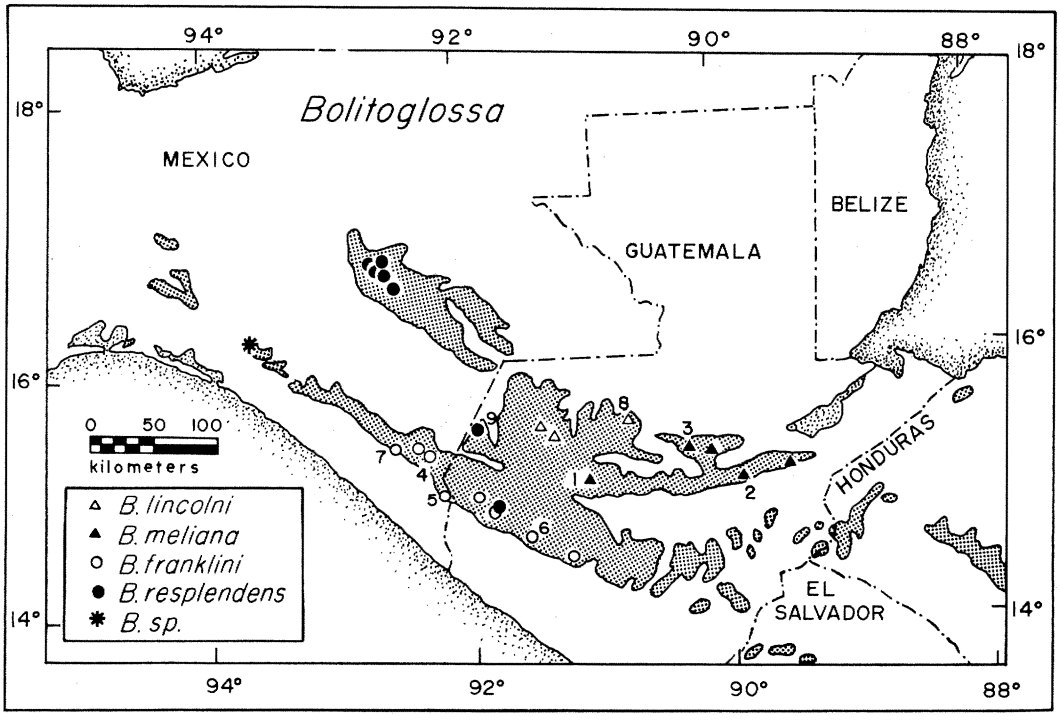

FIG. 2.—Known localities of the *B. franklini* group in Guatemala and in Chiapas, México. Numbered localities are the sites sampled for the electrophoretic analysis (see Table 1). Area above 1500 m is stippled.

Although the Sierra Madre of Chiapas is topographically continuous with the Pacific escarpment of Guatemala, Taylor (1941) did not compare *B. nigroflavescens* with *B. franklini*; instead, he suggested that relationships of the former species were with *B. engelhardti*, a much smaller and differently patterned Guatemalan species, and with *B. macrinii* of southern Oaxaca and Guerrero. In 1978, one of us (JFL) obtained the first topotypic *B. nigroflavescens* since the date of original collection by Smith in 1941. A poorly preserved series of *Bolitoglossa* in the MVZ collection, from near Mapastapec (NW of Cerro Ovando), is morphologically similar to topotypic *B. nigroflavescens*.

Bolitoglossa brevipes was described by Bumzahem and Smith (1955) from a single specimen that was present in a series of preserved amphibians and reptiles obtained from E. Matuda of Chiapas, México. The color of the badly faded holotype, a large and robust female, is brown, with yellow to yellow-green lateral blotches. The only locality data for the specimen are "Region de Soconusco, Chiapas," a local name for the eastern section of the Sierra Madre del Sur between the Guatemalan frontier and the vicinity of Cerro Ovando, where Matuda's finca was located. Today, as when the Matuda collection was assembled in the 1940's, the only road that penetrates the high elevations of this section of the Sierra Madre del Sur extends northward from the coastal plain town of Huixtla to the interior village of Motozintla. It crosses the continental divide just south of Cerro Mozotál (elevation ca. 2800 m), the highest peak in the Sierra Madre. This area is almost certainly the source of the two high elevation species of *Pseudoeu-*

rycea (*P. brunnata* and *P. goebeli*) reported by Bumzahem and Smith (1955) from the Matuda collection. Between 1972 and 1980, we collected several series of a large, dark *Bolitoglossa* marked with cream to green-yellow lateral spots at three localities near the continental divide (elevation 1900–2300 m) above Motozintla. The salamanders occurred both in bromeliads and under surface objects. On the nearby flanks of Cerro Mozotál, we collected two species of *Pseudoeurycea* (apparently the same forms reported by Bumzahem and Smith) at elevations of 2600–2800 m. Our *Bolitoglossa* specimens strongly resemble the holotype of *B. brevipes*, and we suggest that they represent the same population from which it was collected. Specimens of *Bolitoglossa* collected by Norman Hartweg a few km NW of Motozintla (UMMZ collection) also appear to be conspecific with our material from the mountains just above Motozintla.

Stuart (1943) described *B. lincolni* from a series of three red-and-black salamanders that he collected in the Sierra de los Cuchumatanes of NW Guatemala. Stuart compared the new species with *B. franklini*, but not *B. nigroflavescens*. Except for two additional specimens collected by Stuart in the 1950's, *B. lincolni* remained unknown until 1978, when Paul Elias discovered the species near the village of Uspantán in the eastern Cuchumatanes.

McCoy and Walker (1966) described *B. resplendens*, a large red-and-black salamander from the Mesa Central of Chiapas. They differentiated the new species from *B. franklini* on the basis of color differences and the supposed larger size of *B. resplendens*. McCoy and Walker further stated that *B. resplendens* differed from *B. lincolni* in body size and relative limb length. However, the largest specimen of *B. lincolni* available to McCoy and Walker measured only 57 mm SL, and showed no external signs of sexual maturity. Comparisons using recently collected, fully adult specimens have invalidated both of the diagnostic characters that previously were thought to distinguish *B. lincolni* from *B. resplendens* (Elias, 1982).

In 1970, we discovered a disjunct population of red-and-black *Bolitoglossa* at high elevations (2600–2850 m) along the Pacific escarpment near San Marcos in SW Guatemala. Typical *B. franklini* was common in cloud forest lower on the same slope (1800–2650 m), and the two forms were locally sympatric over an elevational interval of approximately 50 vertical meters. Electrophoretic analysis indicated the existence of hybridization between *B. franklini* and the red-and-black form, which we tentatively referred to *B. resplendens* (Wake and Lynch, 1976; Wake et al., 1981).

Another isolated population of red-and-black *Bolitoglossa* occurs in the Montañas de Cuilco, an isolated massif that lies between the Sierra de los Cuchumatanes and the Pacific escarpment of Guatemala (Fig. 2). This population (indicated as locality no. 9 on Fig. 2) also was tentatively assigned to *B. resplendens* (Wake and Lynch, 1976).

Finally, we note that an undescribed member of the *franklini* group occurs on Cerro Tres Picos, an isolated peak (2000 m) at the western end of the Sierra Madre, some 150 km NW Cerro Ovando. We have examined two preserved specimens of this large dark salamander, but final determination of its taxonomic status awaits the availability of living material.

Summarizing, prior to our discovery of *B. meliana*, the *franklini* complex comprised (1) two morphologically similar red-and-black species (*B. lincolni* and *B. resplendens*) found in at least four geographically isolated areas within the interior highlands of eastern Chiapas and western Guatemala, and (2) three Pacific slope species (*B. brevipes*, *B. franklini*, *B. nigroflavescens*) marked with silvery, bronze, or yellowish iridophore patches on a black background. Recently, Elias (1982) suggested that *B. resplendens* is

TABLE 2.—Genic variation in the *Bolitoglossa franklini* group. For species and localities see Table 1. Sample sizes are in parentheses.

	Population								
Locus	1 (10)	2 (2)	3 (1)	4 (13)	5 (11)	6 (14)	7 (1)	8 (7)	9 (7)
Mpi	a (0.7) b (0.3)	a (0.5) b (0.5)	a	b (0.08) c (0.92)	b (0.18) c (0.82)	c	a (0.5) b (0.5)	b (0.21) c (0.79)	c
α Gpd	a (0.2) c (0.4) d (0.4)	a (0.5) b (0.5)	a (0.5) b (0.5)	d	c (0.22) d (0.78)	d	d	d	d
Me	b	b (0.75) c (0.25)	b	a (0.31) c (0.69)	a	a	a	a	a
Pgd	a (0.06) b (0.94)	a (0.25) b (0.75)	a (0.5) b (0.5)	b (0.38) c (0.62)	b (0.82) c (0.18)	b (0.96) c (0.04)	c	b	c
Icd 1	b	b	b	b	a (0.09) b (0.91)	a	a	b	b
Icd 2	b	a (0.25) b (0.75)	b	a (0.15) b (0.85)	b (0.86) c (0.14)	b (0.88) c (0.12)	a (0.5) b (0.5)	b (0.93) c (0.07)	c
Mdh 1	a	a	a	b	a (0.09) a (0.91)	b	b	b	c
Mdh 2	a (0.7) b (0.2) d (0.1)	a	b	a (0.92) d (0.08)	a (0.9) c (0.05) e (0.05)	a	a	a (0.71) d (0.29)	a
Gpi	d	d	e	a (0.04) b (0.71) d (0.25)	c (0.09) d (0.91)	b (0.04) d (0.96)	b	b	b (0.5) d (0.5)
Pgm	a (0.1) b (0.9)	a (0.5) b (0.5)	b (0.5) c (0.5)	a (0.08) b (0.92)	b (0.09) c (0.91)	a	b	a (0.29) b (0.71)	a
Ldh 1	c	c (0.75) f (0.25)	f	a (0.27) d (0.69) e (0.04)	a (0.68) d (0.32)	a	a (0.5) d (0.5)	b (0.71) d (0.29)	a (0.5) b (0.5)
Ldh 2	b	b (0.75) c (0.25)	b	b (0.88) c (0.12)	a (0.05) b (0.95)	b	b	b	a (0.43) b (0.57)
Got 1	a (0.06) b (0.94)	b	b	b (0.92) c (0.08)	a (0.64) b (0.36)	b (0.62) c (0.38)	a (0.5) b (0.5)	b	b
Got 2	a (0.17) b (0.83)	a	a	a (0.58) b (0.42)	b	b	b	b	b
Lap	b	b	b	a	a	a	a	a	a
Sod	b	b	b	a	a	a	a	a	a
Pep	b	b	b	b	b	b	b	b	a

an invalid species, and that all of the red-and-black populations should be referred to *B. lincolni*.

Results of electrophoretic comparisons.—Table 2 summarizes the distribution of allozymes at the 17 electromorphic loci we were able to score consistently. For each between-population comparison, two genetic distances, D_N (Nei, 1972) and D_R (Rogers, 1972), were computed (Table 3). Genetic divergence within the group is relatively great, with D_N values of 0.12–1.80 ($\bar{x}$ = 0.67) and D_R values of 0.18–0.72 ($\bar{x}$ = 0.41) separating the six putative species (Table 3).

The three populations of *B. meliana* form a cluster that is well separated from the other taxa (minimum D_N = 0.54; mean D_N = 0.96). There is, however, considerable genetic differentiation among the three samples of *B. meliana*. Although two of our three samples are very small, they nevertheless contain six alleles that do not appear in the larger re-

TABLE 3.—Rogers' (above diagonal) and Nei's (below diagonal) genetic distances, based on 17 variable electromorphic loci, for populations of the *Bolitoglossa franklini* group. See Table 1 for localities.

Population	Population								
	1	2	3	4	5	6	7	8	9
1	—	0.185	0.285	0.414	0.434	0.490	0.541	0.417	0.609
2	0.117	—	0.250	0.432	0.496	0.534	0.601	0.498	0.608
3	0.314	0.256	—	0.509	0.587	0.668	0.663	0.556	0.724
4	0.545	0.615	0.796	—	0.268	0.310	0.247	0.192	0.368
5	0.604	0.788	1.064	0.233	—	0.190	0.276	0.215	0.373
6	0.880	0.871	1.381	0.319	0.162	—	0.254	0.238	0.348
7	0.743	1.156	1.338	0.204	0.299	0.275	—	0.243	0.384
8	0.595	0.796	0.979	0.130	0.197	0.251	0.239	—	0.342
9	1.100	1.189	1.797	0.448	0.444	0.425	0.493	0.404	—

maining sample. As a result, computed genetic distances within *B. meliana* are relatively large (maximum $D_N = 0.31$; maximum $D_R = 0.28$).

The greatest computed genetic distance in the entire assemblage separates the Purulhá populations of *B. meliana* from the Cuilco population assigned to *B. resplendens* ($D_N = 1.80$; $D_R = 0.72$). Populations presently assigned to *brevipes, franklini, lincolni, nigroflavescens* and *resplendens* show a complex pattern of genic relationships. Except for the samples of *meliana*, the *resplendens* sample from the Cuilco is the most divergent (mean $D_N = 0.79$; mean $D_R = 0.47$). The Uspantán sample of *lincolni*, although morphologically similar to the Cuilco sample (Elias, 1982), is closer genetically to *franklini, brevipes*, and *nigroflavescens*. Indeed, the most surprising result of the electrophoretic survey is the discovery that the Uspantán and Motozintla populations (representing *B. lincolni* and *B. brevipes*) are each other's closest genetic relatives ($D_N = 0.13$; $D_R = 0.19$).

Other results were more in line with current taxonomic assignments. Thus, the two populations presently assigned to *B. franklini* (Chicabál and Tacaná) are quite similar genetically ($D_N = 0.16$; $D_R = 0.19$). However, the mean distance of both populations to *lincolni* from Uspantán is unexpectedly small (mean $D_N = 0.22$; mean $D_R = 0.23$).

The Ovando population (topotypic *B. nigroflavescens*) is only slightly more similar to the nearby Motozintla population of *B. brevipes* ($D_N = 0.20$; $D_R = 0.25$) than it is to the relatively distant Uspantán population of *B. lincolni* ($D_N = 0.24$; $D_R = 0.24$).

Fixed differences at two loci (*Sod* and *Lap*) separate *B. meliana* from the remaining populations of the *franklini* group, and unique alleles for *Pgd* and *α Gpd* are found at relatively high frequencies in *B. meliana*. The remaining species of the *franklini* group all share two unique fixed differences (*Sod* and *Lap*). Unique alleles for *Me* and *Mpi* are widespread in the species other than *B. meliana*. Thus, *B. meliana* appears to qualify as a sister group for the remaining species of the *franklini* group.

DISCUSSION

Bolitoglossa meliana is electrophoretically and morphologically well differentiated from other members of the *franklini* group, and it clearly merits status as a full species. However, the validity of the other five nominal species in the complex must be reassessed. Based on morphological similarities and geographic relationships, we initially predicted that the four disjunct populations of red-and-black *Bolitoglossa* would prove to be close genetic relatives, possibly conspecific (this is also the view of Elias, 1982). Similarly, we suspected that the Pacific versant populations previously assigned to *B. brevipes, B. frank-*

lini, and *B. nigroflavescens* also would show very close relationships. Although the results of our study do not confirm the first prediction, the second is indeed supported. Genetic differentiation among the three supposed species-level taxa found in cloud forests along the Pacific versant of Chiapas and Guatemala is about the same as is seen within *B. meliana*. The Pacific populations are similar in ecology and morphology, and they occur along a topographically continuous mountain system. We propose that they be considered members of a single species, for which *B. franklini* (Schmidt, 1936) is the earliest available name. The Chiapan populations of *B. franklini* that occur west of the border between Guatemala and Chiapas (Volcán Tacaná) are somewhat differentiated in color pattern, and we propose that they be recognized as a distinct geographic race, *B. franklini nigroflavescens*. We consider *B. brevipes* to be a subjective junior synonym of *B. franklini nigroflavescens*. This subspecies is distinguished by the presence of dense, laterally concentrated cream, orange-yellow, or green-yellow iridophore patches. In *B. f. franklini*, the iridophore patches are more diffuse, and are often tattered or fragmented; they are usually gray, silvery, or brassy in color, and are more evenly distributed across the dorsum. Theodore Papenfuss has drawn our attention to a subtle behavioral difference between the two populations: *B. f. nigroflavescens* from the Motozintla area tends to be noticeably more sluggish than *B. f. franklini*. Populations from Volcán Tacaná to be somewhat intermediate in color pattern, but are more similar to typical *B. f. franklini*. The deeply incised, relatively xeric canyon immediately west of Volcán Tacaná may correspond to the zone of transition from *B. f. franklini* to *B. f. nigroflavescens*. While we do not advocate the unrestricted use of trinomials in salamander systematics, we believe that in this instance the subspecies designation conveys useful geographic and morphological information.

Our initial prediction that all four interior populations of red-and-black salamanders would prove to be close genetic relatives was not suppported by our electrophoretic data. *Bolitoglossa lincolni* from Uspantán is more distant genetically from the morphologically similar Cuilco population than it is from morphologically distinct populations of *B. franklini*. The Cuilco population differs from all others that we have sampled except *B. meliana* in having two unique fixed alleles (*Mdh*-1, *Pep*). We think that the relatively great genetic distance between the Cuilco population and other populations reflects a long history of isolation (see below).

Given our present ignorance of the genetic relationships of topotypic Chiapan *resplendens* to the remainder of the *franklini* group, we are hesitant to revise taxonomic status of the red-and-black populations. Future comparisons might show that Chiapan *resplendens* is genetically closer to *lincolni* from the Cuchumatanes than to *"resplendens"* from the Cuilco area. This result might justify (1) including Cuilco, San Marcos, the Chiapan and Cuchumatanes populations within *B. lincolni*, as has been suggested by Elias (1982), or (2) erecting an entirely new species to encompass the Cuilco and San Marcos populations. A practical difficulty in the latter course of action is posed by the lack of external morphological characters that reliably differentiate the various red-and-black populations (Elias, 1982). An even more serious objection to precipitous lumping of all of the red-and-black populations into a single species is that various populations of *B. franklini* (including those formerly referred to *B. brevipes* and *B. nigroflavescens*) would be closer genetically to some red-and-black populations than the latter are to supposed conspecific populations. Therefore, we tentatively continue to refer the San Marcos, Cuilco, and Chiapan populations of red-and-black *Bolitoglossa* to *B. resplendens*, and include only the Cuchumatanes population within *B.*

lincolni. This is done with the full knowledge that some future taxonomic reassessment of the red-and-black populations in the *franklini* group probably will be necessary in the future.

A basic difficulty in assessing the systematic status of populations in the *franklini* group is the strong likelihood that hybridization has occurred between previously isolated populations (Wake et al., 1981). For example, the surprisingly close genetic similarity between *B. lincolni* and some populations of *B. franklini* may reflect secondary contact, hybridization and introgression between coastal and interior populations. We think it likely that *Bolitoglossa* of the *franklini* group originally colonized the Pacific versant of Chiapas and Guatemala from the Cuchumatanes, rather than the Cuilco area. However, present-day parapatry and hybridization in the San Marcos area apparently is a result of very recent secondary contact between local *B. franklini* and *B. resplendens* from the Cuilco area (Wake et al., 1981).

The featureless black coloration of *B. meliana,* which appears to be the earliest offshoot from the ancestral stock that gave rise to the *franklini* group, may be the primitive color pattern, or may be derived from a still earlier red-and-black pattern that is preserved in the Cuilco population of *B. resplendens* (see Fig. 3 below). In either event, the red-and-black coloration of populations that inhabit the ancient interior highlands of Nuclear Central America appears ancestral to the silver-, bronze-, or yellow-and-black patterns of salamanders associated with the younger mountains along the Pacific versant (see below). Despite the close electromorphic similarity of the Uspantán population to *B. franklini,* we suggest that the close similarity in color pattern between the Uspantán and Cuilco populations reflects their retention of a primitive red-and-black pattern.

Historical development of the franklini *group.*—A brief review of the geologic history of Nuclear Central America is a necessary prelude to our attempt to reconstruct the evolutionary development of the *franklini* group.

The oldest exposed rocks in the area occur in the Sierra Madre del Sur of Chiapas and the Chuacús-Minas system of central Guatemala (Dengo, 1968; McBirney, 1963), which consist mainly of Paleozoic metamorphics and sediments. The characteristic ridge-and-valley topography in this area is controlled by fault zones that mark the boundary between the North American and Caribbean plates (Malfait and Dinkelman, 1972; Plafker, 1976).

The plateaus of the Sierra de los Cuchumatanes and the Mesa Central of Chiapas are composed mostly of somewhat younger Mesozoic sediments (Anderson et al., 1973; Dengo, 1968) that are thought to have been uplifted during the late Cretaceous or earliest Tertiary. The Paleozoic and Mesozoic sediments of the Cuilco massif are isolated from the Cuchumatanes by fault-controlled valleys (Anderson et al., 1973). Most of the area south of the Cuilco and Chuacús-Minas mountains and east of the Chiapan border is overlain by thick deposits of late Tertiary volcanics that arose from widespread fissure-type eruptions (Dengo, 1968; Williams, 1960). The spectacular strato-volcanos along the Pacific-facing slope of the Guatemalan plateau are of Pleistocene to Recent age. The focus of intense volcanic activity appears to have shifted progressively southeast from the Chiapas border to the vicinity of Guatemala City, where several presently active cones (e.g., Santa María, Fuego, Pacaya) exist.

Although the entire area inhabited by the *franklini* group has been above sea level since earliest Tertiary, the major uplift that produced the present extreme elevations in the region (the mountain systems discussed above all approach or exceed 3000 m) probably occurred after the late Miocene (Dengo, 1968; Williams, 1960). There is good evidence for the existence of small Pleistocene gla-

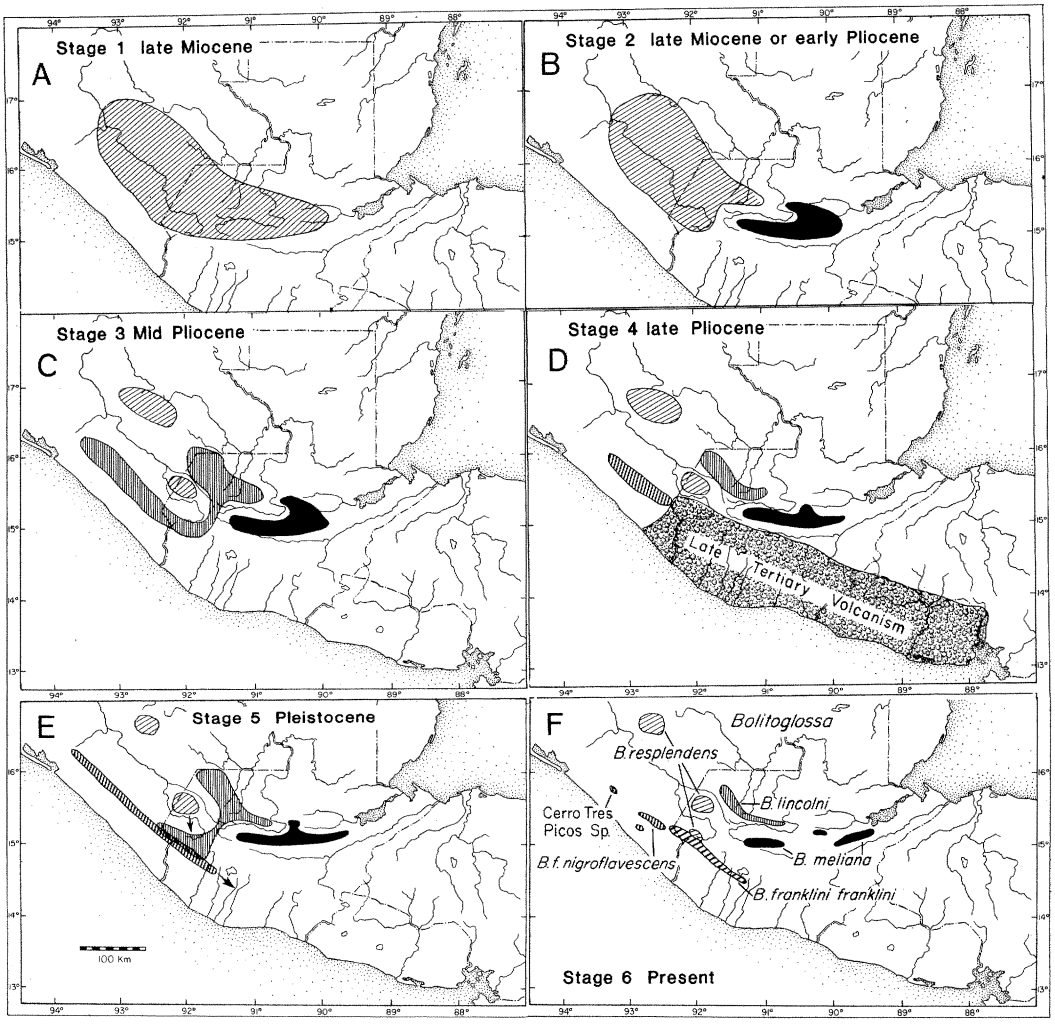

FIG. 3.—Stages in the evolutionary history of the *B. franklini* group, based on discussion in text. A common base map is used, although we recognize that geographic boundaries in this tectonically active region have changed importantly since the Miocene (cf. Plafker, 1976).

ciers throughout Middle America (Anderson et al., 1973; Weyl, 1955; White, 1960), and data from pollen profiles and plant megafossils indicate a lowering of present vegetational zones by 1000 m or more during glacial maxima in Middle America and northern South America (Graham, 1973; Martin, 1964; Simpson, 1974, 1978; Weyl, 1955). The relationships between precipitation and the stages of glacial advance and retreat are complex (Simpson, 1978), but it is reasonable to assume that some periods during the Pleistocene were substantially cooler and wetter than at present.

An evolutionary scenario for development of the franklini *group.*—With the foregoing geologic information as background, we present the following sequence of events as a plausible scenario for the evolution of the *franklini* group.

Stage 1: A mid-Tertiary forerunner of

the *franklini* group ranges throughout the ancient interior highlands of Nuclear Central America, including the major Paleozoic and Mesozoic-early Cenozoic ranges presently occupied by *B. lincolni*, *B. resplendens*, *B. meliana* and the Chiapan populations of *B. franklini* (Fig. 3A).

Stage 2: Crustal movements along the Motagua-Polochíc fault systems cause development of prominent east–west folds and fault-controlled valleys in central Guatemala. Development of the Negro-Chixoy and Cuilco river systems during the mid-Pliocene deepens these valleys by erosion, isolating the forerunners of *B. meliana* from the rest of the proto-*franklini* group (Fig. 3B).

Stage 3: Continued faulting and uplift along the Río Ocho and Paraíso-San Pedro Necta fault systems (Anderson et al., 1973) separate the Cuilco block (and perhaps the Mesa Central of Chiapas) from the Cuchumatanes in the mid-Pliocene, isolating populations of red-and-black salamanders in each area (Fig. 3C).

Stage 4: Late Pliocene fissure eruptions inundate the present-day Guatemalan Plateau. The surface of the plateau is raised to more than 3000 m near the Chiapas border, and to progressively lower levels southeastward toward El Salvador. Populations of salamanders in the Sierra Madre of Chiapas, isolated from those in the interior of Guatemala, evolve into *B. franklini* (Fig. 3D).

Stage 5: Major volcanic peaks (e.g., Tacaná and Tajamulco) arise along the Pacific escarpment near the Chiapas-Guatemala border at the close of the Pliocene; volcanic activity shifts southeastward during the Pleistocene and Recent periods. As the ash deposits that cover the volcanos and adjacent escarpment are revegetated with cloud forest, *B. franklini* disperses southeastward into Guatemala, eventually extending its range as far as Volcán Atitlán. During Pleistocene pluvial periods, secondary contact is established between *B. lincolni* and *B. franklini*. Although the two populations have evolved different color patterns and ecological associations, exchange of genetic material occurs (Fig. 3E).

Stage 6: Post-Pleistocene warming and drying trends again isolate *B. franklini* from most inland populations of the *franklini* group, but relatively recent climatic shifts (possibly higher rainfall) allow dispersal of *resplendens* from the Cuilco area to the Pacific escarpment near San Marcos (indicated by arrow in Fig. 3E). Here, hybridization with *franklini* occurs today along a limited zone of contact (Wake et al., 1981). Geographic isolation of populations generally increases as climatic-topographic barriers proliferate (Fig. 3F).

The absolute timing of these proposed events cannot be established with certainty from electrophoretic data, although there have been attempts to correlate the reasonably well-established time-immunological distance (ID) relationship with Nei distances derived from allozyme data (e.g., Maxson and Maxson, 1979; Sarich, 1977). Using data from various animal groups, Sarich (1977) concluded that a Nei distance of 1.0 is approximately equivalent to ID of 35, which in turn is thought to correspond to a divergence time of 20 million years before present. This figure has been used in several salamander studies (e.g., Wake et al., 1978; Yanev, 1980). On the other hand, in a recent study directed specifically at plethodontid salamanders, Maxson and Maxson (1979) suggested that a Nei distance (D_N) of 1.0 is equivalent to about 14 million years of divergence. Highton and Larson (1979), Larson (1980), and Larson et al. (1981) used this conversion in studies of the plethodontid genera *Plethodon* and *Aneides*. An additional problem in inferring divergence times from biochemical differences is due to scatter in the D_N-ID relationship, even within the salamander family Plethodontidae (see Fig. 2 in Maxson and Maxson, 1979). Using the 14 million year conversion,

species with D_N values of about 0.4 have ID values corresponding to estimated divergence times of 0–7 million years ago, and only about half of the statistical variation in D_N is accounted for by variation in ID ($r = 0.49$). If ID's greater than 50 units are omitted as being unreliable, r^2 increases to 0.71. However, even this degree of scatter, when combined with the considerable uncertainty concerning the proper time-ID conversion factors, indicates that all estimates of divergence times have a generous error margin.

If these caveats are kept in mind, cautious attempts to connect genetic and geologic data may be worthwhile. We suggest that the initial divergence of *B. meliana* from the remainder of the *franklini* group probably took place between late Miocene and middle Pliocene times. If molecular evolution has proceeded at equal rates in all lineages, individual D-values for *B. meliana* populations vis-à-vis other *franklini* group populations all should be equal. Instead, estimated D-values range from 0.54–1.80 (Table 3), which corresponds to a range in estimated divergence times of 7.6–25.2 million years ago. The mean D-value separating the three *B. meliana* populations from the rest of the group is 0.96, suggesting a divergence 13 million years ago (latest Miocene). However, no date between 5 and 20 million years ago (mid-Miocene to mid-Pliocene) would be inconsistent with our data. The second major phyletic event, the separation of the Cuilco and Cuchumatanes populations, may have occurred as recently as 6 million years ago. D-values separating the Cuilco population from the rest of the *franklini* complex are much less variable than was the case for *B. meliana* (Range = 0.40–0.49; mean = 0.44; $n = 5$), so we have some degree of confidence in suggesting a mid-Pliocene divergence (5–7 million years ago) for the Cuilco population. Estimated divergence times for the Uspantán population of *B. lincolni*, the four sampled Chiapan and Guatemalan populations of *B. franklini*, and the three sampled populations of *B. meliana* range from 1.6–4 million years ago (middle to late Pliocene). The maximum D-value separating *B. lincolni* from *B. franklini* ($D_N = 0.25$) is surprisingly low, given the geographic separation and phenetic distinctness of the two species, but as detailed above and elsewhere (Wake et al., 1981), this may reflect secondary contact and subsequent exchange of genetic material.

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GENETIC DIFFERENTIATION AMONG PLETHODONTID SALAMANDERS (GENUS *BOLITOGLOSSA*) IN CENTRAL AND SOUTH AMERICA: IMPLICATIONS FOR THE SOUTH AMERICAN INVASION

JAMES HANKEN AND DAVID B. WAKE

ABSTRACT: An electrophoretic survey of proteins from 14 populations representing eight species and two species groups of Central and South American *Bolitoglossa* examines patterns of intraspecific and interspecific genetic differentiation and the possible implications of these patterns for the question of the time of entry of plethodontid salamanders into South America. These species are very old, as evidenced by the great genetic differentiation among species (including presumed close cladistic relatives), the high degree of genetic subdivision within species, and the very high levels of genetic variation within individual populations. The frequency of polymorphic loci and mean heterozygosity recorded for some populations, particularly the Costa Rican species *B. subpalmata*, may be the highest levels yet recorded for vertebrates. Cluster analysis based on degree of genetic relatedness reveals only a weak distinction between South American and Central American species; furthermore, many component lineages may predate the establishment of the continuous, permanent Pliocene land connection between Panamá and Colombia. Thus, the recent South American fauna likely comprises descendants of several lineages that independently entered South America. Lastly, a specimen of *B. pandi* collected recently in Colombia indicates that this species, which previously was known from a single specimen collected near the turn of this century and feared extinct, still survives.

Key words: Amphibia; Caudata; *Bolitoglossa*; Allozymes; Biogeography; Electrophoresis; Panamanian Portal

SALAMANDERS present a classic example of a Holarctic distribution; eight of the nine extant families are found almost exclusively in the temperate regions of Europe, Asia, and North America. Only the supergenus *Bolitoglossa*, which comprises seven genera of the highly derived family Plethodontidae, has penetrated tropical latitudes to any significant degree, and only in the New World (Wake and Lynch, 1976). *Bolitoglossa* and *Oedipina*, two genera distributed mainly in Central America, have representatives in South America, and the range of *Bolitoglossa* extends as far as 17°S (Brame and Wake, 1963; Wake and Brame, 1966). However, while the South American salamander fauna is considerably richer than is generally acknowledged and occupies an enormous geographical area