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# MITOCHONDRIAL DNA SEQUENCE-BASED PHYLOGENY AND THE EVOLUTION OF VIVIPARITY IN THE *SCELOPORUS SCALARIS* GROUP (REPTILIA, SQUAMATA)

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*Abstract.*—The lizard genus *Sceloporus* contains both oviparous and viviparous species. The *scalaris* complex is the only monophyletic group within the genus that includes both reproductive modes, thus it is particularly well suited for studies of the evolution of viviparity. Approximately 874 nucleotides of mtDNA sequence data, collected from 38 specimens, comprising 25 populations of all five recognized species within the group, were used in a phylogenetic analysis of the origin of viviparity. Viviparity appears to have evolved twice in this group: once in *S. goldmani*, included in a clade formed by a northern group consisting of *S. scalaris*, *S. chaneyi*, and *S. goldmani*, and one more time in *S. bicanthalis*, included in the southern group formed by *S. bicanthalis* and *S. aeneus*. An oviparous population of *S. bicanthalis* nested within that viviparous clade, indicates that reversal from viviparity to oviparity may be possible. Degree of sequence divergence among several *S. bicanthalis* individuals pertaining to a population in which both parity modes occur, was no larger between oviparous and viviparous lizards than among viviparous lizards. This suggests that this population is a single species, and it may represent a transition from oviparity to viviparity or vice-versa.

*Key words.*—Lizard, mtDNA, phylogeny, *Sceloporus*, viviparity.

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Viviparity, defined as the retention of a developing embryo within the uterus until a relatively well-developed neonate is born (Guillette 1993), has evolved almost 100 times among squamate reptiles (Blackburn 1982, 1985; Shine 1985). The most widely accepted hypothesis for the evolution of viviparity is that it evolves in cool environments at high elevations or latitudes (Tinkle and Gibbons 1977; Shine and Berry 1978; Shine and Bull 1979; Guillette 1985, 1989, 1993; Shine 1985, 1995). Maternal thermoregulation in such environments should provide higher temperatures for the development of the embryos retained in the oviduct than for eggs laid in nests in the soil. Development rate is faster at higher temperatures (Packard et al. 1977) and therefore, egg retention, and ultimately viviparity, should be advantageous in cold environments, because early hatching may decrease offspring mortality in the nest and allow a longer growing period for the young, before the low fall and winter temperatures (Mathies and Andrews 1995).

Low partial pressure of oxygen at high elevation may also be a selective force driving egg retaining species to viviparity (Guillette et al. 1980). The problem of gas exchange between the developing embryo and the mother is solved by hypervascularity of the oviducts and extraembryonic tissues, reduction of the oviductal glandular tissues, and reductions in the shell membrane. This selective pressure may be stronger at high elevations, where many viviparous species occur (Guillette 1982).

More recently, Shine (1995) proposed that eggs incubated at maternal body temperatures produce "fitter" hatchlings than do eggs incubated at ground nest temperatures. Thus, the main selective force for prolonged intrauterine retention of eggs, and ultimately viviparity, may be related to the phenotype of the offspring and not only to increased survival to hatching or earlier hatching.

Other selective forces have been suggested to drive the evolution of viviparity (e.g., short growing season, high egg predation, xeric conditions; Packard et al. 1977; Tinkle and Gibbons 1977). However, the cold-climate hypothesis is the only one that suggests an advantage for intermediate stages of egg retention between oviparity and viviparity (Guillette 1993). Nevertheless, as Tinkle and Gibbons (1977) pointed out, viviparity may be a preadaptation to cold environments, but cold environments may not necessarily be the driving force for the evolution of viviparity.

Possible physiological mechanisms involved in the evolution of viviparity have also been studied (Guillete 1985; Shine and Guillette 1988). Guillette (1985) found that the increase in the egg retention period is correlated with a lengthening of the secretory period of corpora lutea. He hypothesized that an environmentally induced stress response would stimulate the release of adrenal steroid hormones capable of blocking prostaglandin synthesis and thus, luteolysis. Selection for this response in oviparous females living in areas with environmentally poor conditions would lead to egg retention. Under certain environmental conditions, this pathway could lead to viviparity.

The study of the selective forces and mechanisms favoring the evolution of viviparity, particularly at the transitional phases, can be clarified by understanding the phylogenetic relationships of closely related species in which both viviparity and oviparity occur. Within the genus *Sceloporus*, the *scalaris* species complex of the central Mexican highlands is particularly well suited for a study of the evolution of parity mode (Sites et al. 1992). Previous analyses of karyotype data (Cole 1978), morphology and allozyme data (Mink and Sites 1996), and morphology and DNA sequence data (Wiens and Reeder, in press) have shown this group to be monophyletic. It includes viviparous and oviparous species, with some oviparous species (*S. aeneus*) exhibiting extreme egg retention (Guillette 1981, 1982). Mink and Sites (1996) have documented a case in which both parity modes occur in a single population of *S. bicanthalis*, representing a possible transi-

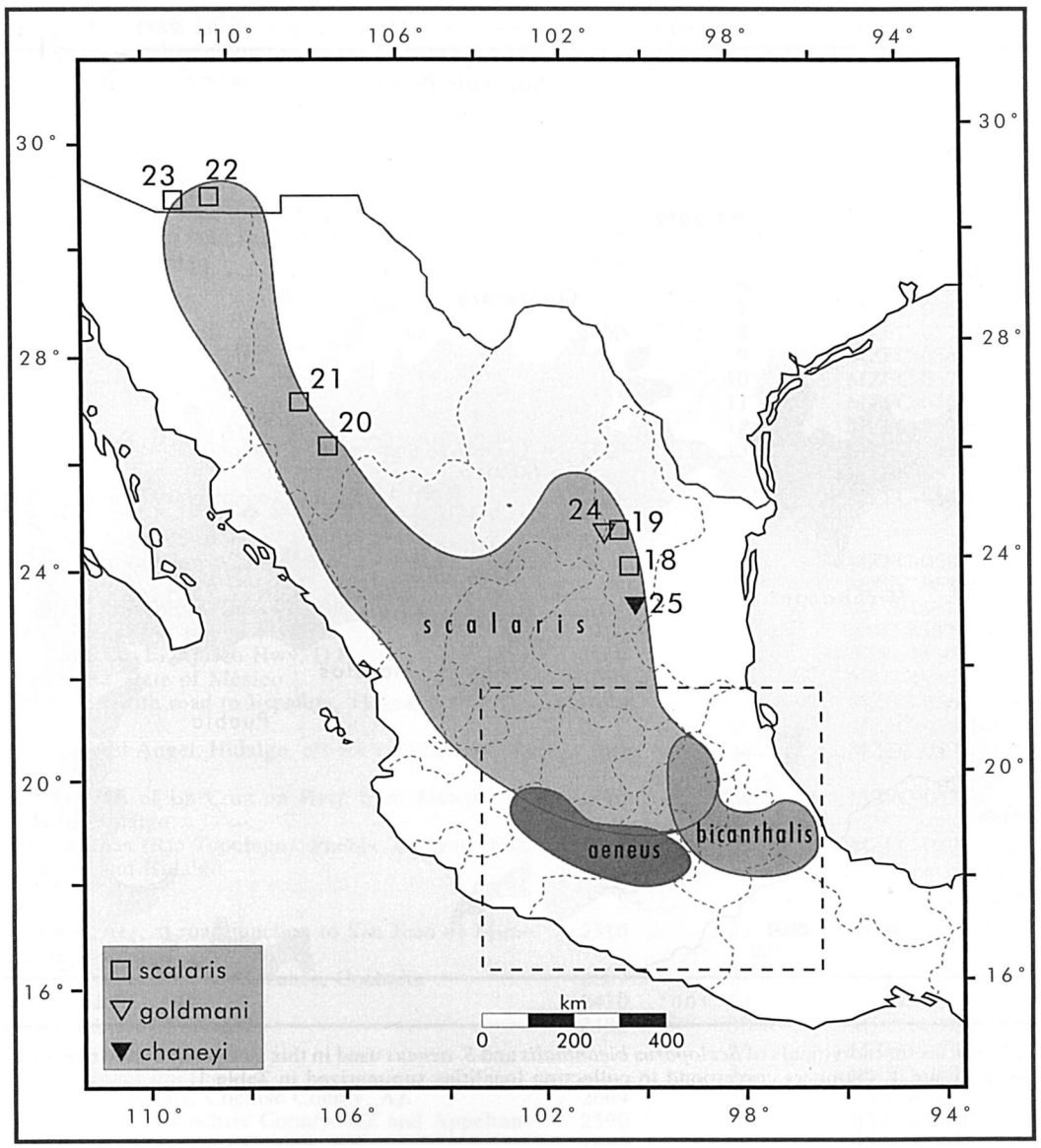


FIG. 1. Collecting localities for samples of *Sceloporus scalaris*, *S. goldmani*, and *S. chaneyi* used in this study, with numbers corresponding to collecting localities summarized in Table 1. Rectangle encloses the region depicted in Figure 2.

tional stage in the evolution of viviparity. As pointed out by Sites et al. (1992), the ecological and physiological predictions for the evolution of viviparity (Guillette 1985; Shine and Guillette 1988) provided by the study of the genus *Sceloporus*, and in particular of the *scalaris* group (Guillette 1981, 1982), make a compelling case for a thorough phylogenetic study of the entire *scalaris* group.

In this paper we present a phylogenetic analysis of mitochondrial DNA (mtDNA) sequences from 38 individuals representing 25 populations of all five recognized species within the *S. scalaris* group, and one outgroup. We incorporate data from 10 electrophoretically assayed allozymes from the same lizards, presented by Mink and Sites (1996). The purpose of this study is to obtain a well-supported phylogenetic hypothesis of this group that would provide a historical framework for the study of the evolution of viviparity in these lizards. Additionaly, we test the hypothesis that viviparity evolves at high elevations by analyzing the correlation between parity mode and altitude of the localities of the specimens included in this study.

### *Recent Phylogenetic Interpretation of the Evolution of Viviparity in the S. scalaris Group*

The *scalaris* complex has historically consisted of four or five species, depending on the author. *Sceloporus scalaris* contains four subspecies: *S. s. scalaris*, *S. s. samcolemani*, *S. s. slevini*, and *S. s. unicanthalis*, but the subspecies are currently under revision (Smith et al. 1996). *Sceloporus aeneus* includes the subspecies *S. a. aeneus* and *S. a. subniger*, although here we only refer to *S. aeneus*, because the trinomen "*subniger*" is not well defined (Sites, unpubl. data). *Sceloporus bicanthalis* has been considered a subspecies of *S. aeneus* (Thomas and Dixon 1976) or a full species due to morpho-

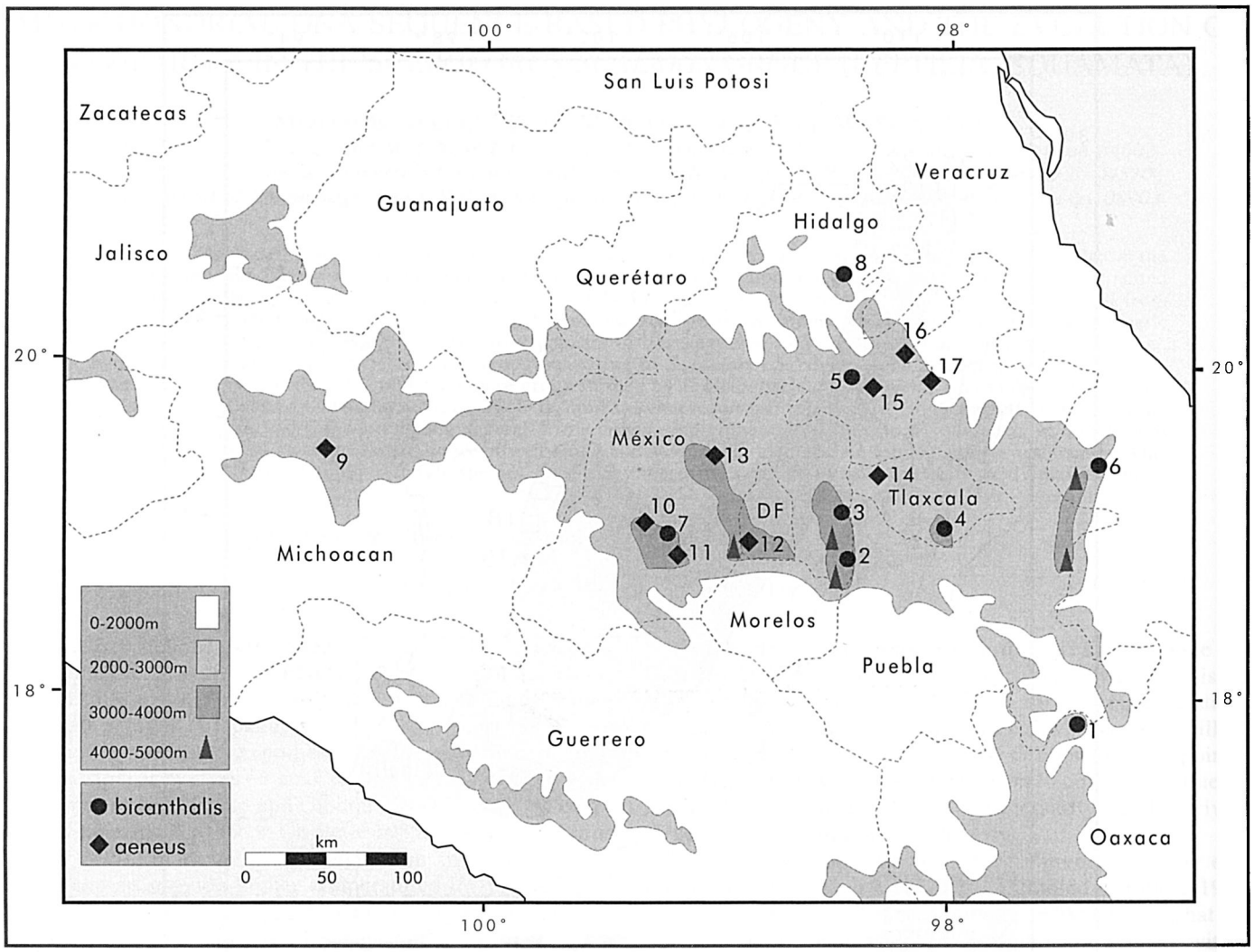


FIG. 2. Collecting localities for individuals of *Sceloporus bicanthalis* and *S. aeneus* used in this study. Area shown in map is an enlargement of dashed rectangle in Figure 1. Numbers correspond to collecting localities summarized in Table 1.

logical and allozyme differences compared to *S. aeneus* (Guillette and Smith 1985; Smith et al. 1993; Mink and Sites 1996). The species status of *S. chaneyi* and *S. goldmani* are not debated. Viviparous taxa within the group are *S. goldmani*, and most populations of *S. bicanthalis* (Guillette and Smith 1985; Mink and Sites 1996).

Mink and Sites (1996) used isozyme and morphological characters to establish phylogenetic relationships and analyze the evolution of viviparity in the *S. scalaris* group. They recovered parsimony trees supporting a "two-origin" hypothesis: once in *S. goldmani*, which grouped with *S. scalaris* and *S. chaneyi* into a "northern clade," and a second origin in *S. bicanthalis*, grouped into a "southern clade." Because of the limited number of informative characters, however, these clades were only modestly supported (bootstrap values frequently < 70%, see Mink and Sites's fig. 3), and these authors could not demonstrate that the shortest trees for the two-origin hypothesis were significantly more parsimonious than an alternative topology constraining viviparity to a single origin. Mink and Sites also recognized *S. aeneus* (oviparous) as distinct from *S. bicanthalis* (bimodal parity).

More recently, Creer et al. (in press) used mtDNA sequence data to investigate phylogenetic relationships among selected populations of the southern clade formed by *S. aeneus* and *S. bicanthalis*, as defined by Mink and Sites (1996). Creer et al. (1997) showed strong support for the separation of northern versus southern clades, and recovered *S. goldmani* and *S. bicanthalis* within each of these, respectively. However, placement of *S. goldmani* in the northern clade was based on isozyme and morphological characters only, as no specimen was available for sequencing when that study was carried out. Nevertheless, its tentative placement in the northern clade, supported one origin of viviparity within that clade. The authors suggested at least two origins of viviparity within *S. bicanthalis*, but mentioned that the weak support for an internal branch within the *bicanthalis* clade of their tree made this uncertain. The resolution of the phylogenetic relationships between *S. aeneus* and *S. bicanthalis* should clarify

TABLE 1. Representatives of the *Sceloporus scalaris* complex included in this study, by collecting localities and their elevation (m above sea level), and corresponding OTU labels, museum catalog numbers, and parity mode (oviparous = O, and viviparous = V). Locality numbers (1–26) correspond to those plotted in Figures 1 and 2. *Sceloporus jalapae* is the outgroup.

| Locality | Elevation | OTU labels | Voucher numbers | Parity |
|---|---|---|---|---|
| *Sceloporus bicanthalis* | | | | |
| 1. Junction of Peña Verde, Cuicatlán, Oaxaca | 2680 | 1 | MZFC-08461 | V |
| 2. Paso de Cortéz, state of México | 3810 | 2 | BYU-45359 | V |
| 3. km 3 of road from Llano Grande to Zoquiapan, state of México | 3000 | 3 | MZFC-05787 | V |
| 4. Volcán La Malinche, area around shelter, Tlaxcala | 3200 | 4 | BYU-45352 | V |
| 5. 2 km NE of Nopalillo, Hidalgo | 2920 | 5 | MZFC-05685 | O |
| | | 6 | MZFC-05685 | O |
| | | 7 | MZFC-05685 | O |
| 6. km 125 of Hwy 140, 2 km E of Las Vigas, Veracruz | 2460 | 8 | MZFC-05776-7 | V |
| | | 9 | MZFC-05688 | V |
| | | 10 | MZFC-05776-2 | V |
| | | 11 | MZFC-05688 | V |
| | | 12 | MZFC-05776-1 | O |
| 7. Nevado de Toluca, 4050 to 4250 m in elevation, state of México | 4150 | 13 | MZFC-05762 | V |
| | | 14 | MZFC-05762 | V |
| 8. 0.5 km NE junction to Tianguistengo, Hidalgo | 1858 | 15 | MZFC-08460 | V |
| *Sceloporus aeneus* | | | | |
| 9. 8.5 km S of Pátzcuaro on Hwy 120, Michoacán | 2250 | 16 | MZFC-05836-11 | O |
| | | 17 | MZFC-05836-15 | |
| 10. El Mapa, km 23 of Hwy Toluca-Temascaltepec, state of México | 2900 | 18 | BYU-45317 | O |
| 11. 2.8 km S-SW of Zaragoza, state of México | 2400 | 19 | BYU-45320 | O |
| 12. Between km 18 and 20, El Ajusco Hwy, D.F. | 2800 | 20 | BYU-45309 | O |
| 13. 3.5 km NE Cahuacán, state of México | 2600 | 21 | BYU-45301 | O |
| 14. Junction of Hwy 136 with road to Españita, Tlaxcala | 2760 | 22 | MZFC-05686 | O |
| | | 23 | MZFC-05686 | O |
| 15. Autódromo Bosques El Angel, Hidalgo, off Hwy 132 from D.F. to Tulancingo | 2200 | 24 | MZFC-05785 | O |
| 16. Palo Gacho, 2 km NE of La Cruz on Hwy from Metepec to Tenango de Doria, Hidalgo | 2300 | 25 | MZFC-05779 | O |
| 17. Campestre Las Truchas (Río Topolapo), Puebla, off Hwy 130, at border of Puebla and Hidalgo | 2200 | 26 | MZFC-05775 | O |
| *Sceloporus scalaris* | | | | |
| 18. 1.2 km NW of Mimbres, at road junction to San Juan de Mimbres, Nuevo León | 2310 | 27 | MZFC-05349 | O |
| 19. 14.4 km E of San Antonio de la Alazanas, Coahuila | 2607 | 28 | MZFC-05350 | O |
| 20. Sanachique, Chihuahua | 2430 | 29 | MZFC-05814-1 | O |
| 21. 11 km S of San Juanito, Chihuahua | 2430 | 30 | MZFC-05813-4 | O |
| | | 31 | MZFC-05813-1 | O |
| | | 32 | MZFC-05812 | O |
| 22. Rustler Park, Chiricahua Mts, Cochise County, AZ | 2664 | 33 | BYU-45479 | O |
| 23. Carr Peak, Huachuca Mts, Cochise County, AZ and Appelton-White Research Ranch, Sta. Cruz County, AZ | 2590<br>1463 | 34<br>35 | BYU-45474<br>BYU-45480 | O<br>O |
| *Sceloporus goldmani* | | | | |
| 24. Junction of Road from San Antonio de las Alazanas with Hwy 57, Coahuila | 2098 | 36 | MZFC-05458 | V |
| *Sceloporus chaneyi* | | | | |
| 25. 18 km NE of San Antonio Peña Nevada, Nuevo León | 2720 | 37 | MZFC-05473 | O |
| | | 38 | MZFC-05473 | O |
| *Sceloporus jalapae* | | | | |
| 26. Outgroup | — | 39 | MZFC-05958 | O |

whether viviparity has arisen two or three times independently in the *scalaris* complex.

## MATERIALS AND METHODS

### *Specimens*

Lizards were collected from 25 different localities (Figs. 1, 2) between 1991 and 1994 by D. Mink and personnel of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, as described in Mink and Sites (1996). All recognized species were represented in the sample of 29 specimens. In addition to the animals sequenced specifically for this study, the sequences of nine lizards collected by Creer et al. (in press) were included in the analysis. A single lizard from most localities was included, but two individuals from five localities, and three individuals from two localities, were used to assess within-versus between-population sequence variation. Five *S. bicanthalis* from Las

TABLE 2. Pairwise comparisons of nucleotide differences among haplotypes. OTU numbers correspond to those in Table 1.

| OTUs | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | | | | | | |
| 2 | 23.0 | | | | | | | | | | | | | | | | | | | |
| 3 | 21.0 | 19.0 | | | | | | | | | | | | | | | | | | |
| 4 | 19.0 | 19.0 | 16.0 | | | | | | | | | | | | | | | | | |
| 5 | 19.0 | 23.0 | 18.0 | 18.0 | | | | | | | | | | | | | | | | |
| 6 | 24.0 | 23.0 | 19.0 | 19.0 | 8.0 | | | | | | | | | | | | | | | |
| 7 | 20.0 | 20.0 | 15.0 | 15.0 | 5.0 | 6.0 | | | | | | | | | | | | | | |
| 8 | 18.0 | 31.0 | 24.0 | 26.0 | 25.0 | 29.0 | 25.0 | | | | | | | | | | | | | |
| 9 | 18.0 | 30.0 | 23.0 | 25.0 | 25.0 | 28.0 | 24.0 | 3.0 | | | | | | | | | | | | |
| 10 | 19.0 | 31.0 | 24.0 | 26.0 | 26.0 | 29.0 | 25.0 | 4.0 | 1.0 | | | | | | | | | | | |
| 11 | 20.0 | 32.0 | 25.0 | 25.0 | 27.0 | 30.0 | 26.0 | 4.0 | 2.0 | 3.0 | | | | | | | | | | |
| 12 | 17.0 | 29.0 | 22.0 | 24.0 | 24.0 | 27.0 | 23.0 | 2.0 | 1.0 | 2.0 | 3.0 | | | | | | | | | |
| 13 | 23.0 | 22.0 | 20.0 | 16.0 | 16.0 | 21.0 | 17.0 | 23.0 | 21.0 | 24.0 | 25.0 | 19.0 | | | | | | | | |
| 14 | 19.0 | 19.0 | 16.0 | 12.0 | 17.0 | 19.0 | 15.0 | 20.0 | 19.0 | 20.0 | 21.0 | 17.0 | 2.0 | | | | | | | |
| 15 | 20.0 | 18.0 | 17.0 | 13.0 | 19.0 | 20.0 | 16.0 | 24.0 | 23.0 | 24.0 | 25.0 | 22.0 | 14.0 | 10.0 | | | | | | |
| 16 | 80.0 | 81.0 | 81.0 | 81.0 | 82.0 | 82.0 | 79.0 | 76.0 | 75.0 | 76.0 | 75.0 | 70.0 | 78.0 | 75.0 | 75.0 | | | | | |
| 17 | 78.0 | 81.0 | 78.0 | 78.0 | 79.0 | 79.0 | 76.0 | 73.0 | 72.0 | 73.0 | 72.0 | 67.0 | 76.0 | 72.0 | 72.0 | 11.0 | | | | |
| 18 | 73.0 | 78.0 | 73.0 | 72.0 | 66.0 | 70.0 | 66.0 | 69.0 | 67.0 | 74.0 | 73.0 | 62.0 | 65.0 | 63.0 | 67.0 | 69.0 | 64.0 | | | |
| 19 | 69.0 | 72.0 | 66.0 | 65.0 | 64.0 | 68.0 | 65.0 | 67.0 | 66.0 | 67.0 | 65.0 | 60.0 | 64.0 | 62.0 | 60.0 | 62.0 | 57.0 | 5.0 | | |
| 20 | 73.0 | 72.0 | 66.0 | 69.0 | 69.0 | 73.0 | 69.0 | 71.0 | 70.0 | 71.0 | 70.0 | 65.0 | 70.0 | 66.0 | 64.0 | 62.0 | 57.0 | 17.0 | 12.0 | |
| 21 | 69.0 | 73.0 | 67.0 | 66.0 | 66.0 | 70.0 | 66.0 | 67.0 | 67.0 | 68.0 | 67.0 | 62.0 | 64.0 | 62.0 | 61.0 | 65.0 | 60.0 | 7.0 | 8.0 | 12.0 |
| 22 | 65.0 | 69.0 | 64.0 | 62.0 | 61.0 | 64.0 | 60.0 | 58.0 | 59.0 | 60.0 | 58.0 | 54.0 | 60.0 | 58.0 | 61.0 | 55.0 | 54.0 | 43.0 | 43.0 | 47.0 |
| 23 | 61.0 | 65.0 | 60.0 | 58.0 | 57.0 | 60.0 | 56.0 | 54.0 | 55.0 | 56.0 | 54.0 | 51.0 | 58.0 | 54.0 | 58.0 | 56.0 | 55.0 | 49.0 | 42.0 | 49.0 |
| 24 | 63.0 | 67.0 | 62.0 | 60.0 | 58.0 | 62.0 | 58.0 | 57.0 | 57.0 | 58.0 | 57.0 | 52.0 | 57.0 | 56.0 | 59.0 | 54.0 | 54.0 | 42.0 | 42.0 | 49.0 |
| 25 | 66.0 | 74.0 | 67.0 | 63.0 | 60.0 | 68.0 | 64.0 | 63.0 | 62.0 | 59.0 | 58.0 | 56.0 | 63.0 | 62.0 | 65.0 | 61.0 | 60.0 | 45.0 | 47.0 | 54.0 |
| 26 | 64.0 | 68.0 | 63.0 | 61.0 | 60.0 | 63.0 | 59.0 | 57.0 | 58.0 | 59.0 | 57.0 | 53.0 | 59.0 | 57.0 | 60.0 | 56.0 | 55.0 | 44.0 | 44.0 | 50.0 |
| 27 | 118.0 | 112.0 | 114.0 | 110.0 | 110.0 | 112.0 | 112.0 | 112.0 | 111.0 | 112.0 | 110.0 | 103.0 | 109.0 | 107.0 | 109.0 | 108.0 | 107.0 | 103.0 | 100.0 | 102.0 |
| 28 | 119.0 | 113.0 | 115.0 | 111.0 | 111.0 | 113.0 | 113.0 | 113.0 | 112.0 | 113.0 | 111.0 | 104.0 | 111.0 | 108.0 | 110.0 | 110.0 | 111.0 | 113.0 | 107.0 | 107.0 |
| 29 | 111.0 | 108.0 | 107.0 | 106.0 | 100.0 | 107.0 | 104.0 | 103.0 | 104.0 | 105.0 | 103.0 | 98.0 | 106.0 | 102.0 | 102.0 | 99.0 | 100.0 | 110.0 | 104.0 | 107.0 |
| 30 | 106.0 | 107.0 | 104.0 | 103.0 | 99.0 | 106.0 | 103.0 | 98.0 | 99.0 | 100.0 | 97.0 | 92.0 | 101.0 | 99.0 | 98.0 | 97.0 | 97.0 | 102.0 | 101.0 | 104.0 |
| 31 | 104.0 | 106.0 | 103.0 | 102.0 | 99.0 | 106.0 | 102.0 | 97.0 | 98.0 | 99.0 | 97.0 | 92.0 | 100.0 | 98.0 | 98.0 | 94.0 | 94.0 | 100.0 | 99.0 | 102.0 |
| 32 | 104.0 | 103.0 | 101.0 | 100.0 | 95.0 | 101.0 | 98.0 | 94.0 | 93.0 | 96.0 | 95.0 | 86.0 | 97.0 | 93.0 | 95.0 | 101.0 | 101.0 | 104.0 | 97.0 | 102.0 |
| 33 | 114.0 | 112.0 | 109.0 | 110.0 | 105.0 | 110.0 | 108.0 | 103.0 | 104.0 | 105.0 | 103.0 | 99.0 | 107.0 | 105.0 | 105.0 | 108.0 | 106.0 | 107.0 | 104.0 | 105.0 |
| 34 | 115.0 | 113.0 | 111.0 | 112.0 | 107.0 | 111.0 | 108.0 | 105.0 | 104.0 | 107.0 | 107.0 | 100.0 | 109.0 | 105.0 | 108.0 | 110.0 | 108.0 | 110.0 | 103.0 | 106.0 |
| 35 | 116.0 | 113.0 | 111.0 | 112.0 | 106.0 | 111.0 | 109.0 | 104.0 | 104.0 | 107.0 | 105.0 | 99.0 | 110.0 | 105.0 | 107.0 | 108.0 | 106.0 | 108.0 | 102.0 | 105.0 |
| 36 | 122.0 | 115.0 | 118.0 | 114.0 | 111.0 | 115.0 | 115.0 | 114.0 | 113.0 | 115.0 | 113.0 | 105.0 | 112.0 | 110.0 | 113.0 | 112.0 | 113.0 | 117.0 | 109.0 | 110.0 |
| 37 | 107.0 | 101.0 | 101.0 | 101.0 | 98.0 | 99.0 | 100.0 | 102.0 | 101.0 | 102.0 | 100.0 | 92.0 | 101.0 | 98.0 | 97.0 | 106.0 | 103.0 | 93.0 | 89.0 | 91.0 |
| 38 | 107.0 | 101.0 | 101.0 | 101.0 | 97.0 | 98.0 | 98.0 | 102.0 | 101.0 | 102.0 | 101.0 | 93.0 | 100.0 | 98.0 | 98.0 | 106.0 | 103.0 | 92.0 | 90.0 | 92.0 |
| 39 | 159.0 | 157.0 | 158.0 | 151.0 | 151.0 | 154.0 | 153.0 | 153.0 | 152.0 | 153.0 | 153.0 | 145.0 | 150.0 | 148.0 | 147.0 | 145.0 | 145.0 | 144.0 | 136.0 | 139.0 |

TABLE 2. Extended.

| OTUs | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | |
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| 19 | | | | | | | | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | | | | | | | |
| 22 | 41.0 | | | | | | | | | | | | | | | | | | |
| 23 | 43.0 | 6.0 | | | | | | | | | | | | | | | | | |
| 24 | 44.0 | 5.0 | 5.0 | | | | | | | | | | | | | | | | |
| 25 | 45.0 | 13.0 | 9.0 | 7.0 | | | | | | | | | | | | | | | |
| 26 | 44.0 | 5.0 | 5.0 | 4.0 | 12.0 | | | | | | | | | | | | | | |
| 27 | 100.0 | 103.0 | 97.0 | 99.0 | 105.0 | 102.0 | | | | | | | | | | | | | |
| 28 | 107.0 | 104.0 | 102.0 | 102.0 | 108.0 | 105.0 | 11.0 | | | | | | | | | | | | |
| 29 | 101.0 | 99.0 | 99.0 | 99.0 | 106.0 | 99.0 | 83.0 | 86.0 | | | | | | | | | | | |
| 30 | 98.0 | 95.0 | 94.0 | 94.0 | 101.0 | 95.0 | 83.0 | 88.0 | 23.0 | | | | | | | | | | |
| 31 | 96.0 | 92.0 | 92.0 | 92.0 | 99.0 | 92.0 | 81.0 | 86.0 | 21.0 | 3.0 | | | | | | | | | |
| 32 | 96.0 | 93.0 | 92.0 | 90.0 | 92.0 | 91.0 | 86.0 | 90.0 | 33.0 | 16.0 | 12.0 | | | | | | | | |
| 33 | 103.0 | 96.0 | 94.0 | 96.0 | 101.0 | 96.0 | 84.0 | 85.0 | 48.0 | 46.0 | 41.0 | 51.0 | | | | | | | |
| 34 | 103.0 | 95.0 | 96.0 | 94.0 | 93.0 | 95.0 | 82.0 | 86.0 | 48.0 | 46.0 | 41.0 | 51.0 | 5.0 | | | | | | |
| 35 | 101.0 | 92.0 | 92.0 | 92.0 | 97.0 | 92.0 | 87.0 | 91.0 | 49.0 | 48.0 | 43.0 | 53.0 | 11.0 | 10.0 | | | | | |
| 36 | 109.0 | 105.0 | 104.0 | 102.0 | 109.0 | 106.0 | 14.0 | 3.0 | 89.0 | 90.0 | 88.0 | 91.0 | 86.0 | 87.0 | 92.0 | | | | |
| 37 | 88.0 | 91.0 | 87.0 | 87.0 | 92.0 | 90.0 | 40.0 | 41.0 | 72.0 | 74.0 | 72.0 | 76.0 | 75.0 | 75.0 | 78.0 | 43.0 | | | |
| 38 | 89.0 | 89.0 | 86.0 | 86.0 | 92.0 | 88.0 | 41.0 | 42.0 | 71.0 | 73.0 | 71.0 | 74.0 | 74.0 | 73.0 | 76.0 | 44.0 | 1.0 | | |
| 39 | 137.0 | 140.0 | 139.0 | 139.0 | 144.0 | 140.0 | 165.0 | 170.0 | 166.0 | 170.0 | 167.0 | 166.0 | 168.0 | 167.0 | 163.0 | 168.0 | 154.0 | 154.0 | |

Vigas, Veracruz (locality 6 in Fig. 2), the locality where both parity modes were found (Mink and Sites 1996), were included: two viviparous females, one oviparous female, and two males. Three lizards (13, 14, and 15) came from populations that were presumably within the range of *S. a. subniger* (localities 7 and 8), based on Smith et al. (1993), but opposite to what those authors mention, these samples were all viviparous. We consider all of these to be *S. bicanthalis*. A list of specimens used, museum catalog numbers (BYU = Brigham Young University, and MZFC = Museo de Zoología, Facultad de Ciencias, UNAM), and localities, is presented in Table 1.

### *Sequencing*

Total genomic DNA was extracted from heart and liver tissue samples following the procedures described by Hillis and Davis (1986). *Taq* DNA polymerase (GibcoBRL) mediated PCR amplifications of mtDNA were performed as described in Sambrook et al. (1989) using ND4 and Leu primers designed by Arévalo et al. (1994). DNA was denatured initially at 94°C for 3 min, followed by 35 cycles of amplification under the following conditions: denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min.

Amplified products were purified either by a glass bead system (Gene-Clean; BIO 101, Inc.) or through magnetic strand separation (Streptavidin Dynabeads M-280; Dynal A.S). Purified DNA was then sequenced with Sequenase enzyme, version 2.0 (United States Biochemical Corp.) using the amplification primers ND4 (reference positions: 11165–11196), and Leu (reference positions: 12111–12036), and three additional internal primers to the ND4-Leu sequence that were later developed. Internal primer sequences in a 3′ to 5′ direction, and annealing positions of first and last nucleotides, were: 3′-TGA GGC ATT GTT ATA ACA AG-5′, annealing between nucleotides 11309 and 11328; 3′-ACA GGA CTA GGW ACA CTA AT-5′, annealing between nucleotides 11696 and 11715; and 3′-ATTAGGCAGTGGCCCTAAAA-CAG-5′, annealing between nucleotides 11911 and 11934. Confirmation of DNA sequence was obtained either by sequencing both complementary strands within the target DNA, or by overlapping DNA sequence obtained with different primers. DNA sequences were aligned to the *S. poinsetti* sequence presented by Arévalo et al. (1994). The vertebrate tRNA secondary structure models described by Kumazawa and Nishida (1993) were followed in the alignment of those sequences.

### *Analysis*

DNA sequences of closely related populations or of individuals from the same population often differ by very few characters. Traditional methods for phylogeny reconstruction using parsimony analysis may not be suitable for analyses of recent divergence events in which the ancestral haplotype may still be extant. The algorithm of Templeton et al. (1992) estimates gene trees under the assumption of low levels of divergence, that is, where few mutational changes separate operational taxonomic units (OTUs), and therefore multiple substitutions at the same site are unlikely. This algorithm has recently been shown to accurately reconstruct relationships, with greater statistical power, than does maximum parsimony when few nucleotide substitutions are available (Crandall 1994). Therefore, we used this algorithm to assess the relationships of the most closely related OTUs (individual haplotypes in this case) as explained below.

The statistical power of this method is obtained by conditioning the probability of a parsimonious connection between OTUs by the number of nucleotides shared between them (Crandall and Fitzpatrick 1996). The probability of a parsimonious connection between a pair of haplotypes is given by the number of differing nucleotides relative to the number of shared nucleotides, as described by Templeton et al. (1992). After generating a matrix of the number of nucleotide differences between each pair of haplotypes with MEGA (Kumar et al. 1993; Table 2), we used the algorithms of Templeton et al. (1992) available in *Mathematica* (Wolfram 1991) to calculate the probability of parsimonious connections between all possible haplotype pairs. Relationships of haplotypes estimated to be related at a probability $\geq 0.95$ were depicted in networks (see Crandall 1994), and these primary relationships were used as constraints, retaining only one or two haplotypes from each network to estimate the remaining tree structure with parsimony analyses using the "branch and bound" option of PAUP (vers. 3.1.1.; Swofford 1993). Additionally, a heuristic search using the tree bisection-reconnection option, and including 100 random additions with all 38 taxa, was run in PAUP for comparison with the branch-and-bound tree.

To examine the stability of our hypothesis under weighted parsimony, we added transversion and amino acid characters to our matrix. The original data matrix was copied twice. The nucleotides in the first copy were converted into purines and pyrimidines, and the codons in the second copy were converted into amino acids, and these new characters were added to the original matrix. Thus, the new three-tiered data matrix contained 1995 characters. A similar approach was defended in Agosti et al. (in press). In all analyses, gaps in the tRNA sequences were treated as additional characters.

Phylogenetic signal in the data set was evaluated using the $g_1$-statistic of the distribution of 10,000 randomly generated trees (the "stopping algorithm" of Hillis 1991). Support for the relationships recovered by the parsimony analyses was estimated by bootstrap analyses (Felsenstein 1985) with 1000 replicates. Since there was no evidence of multiple topology islands in the random addition searches, a single replicate was considered sufficient in each of the 1000 bootstrap searches. Additionaly, the Bremer (1988) support index was determined for each interior node.

According to the high elevation hypothesis for the evolution of viviparity, the proportion of viviparous lizards inhabiting high elevations should be higher than at low elevations, where oviparous lizards should be more common. Thus, there should be a positive correlation between elevation and viviparity. This prediction was tested by an independent comparison method (Harvey and Pagel 1991) using the statistical package CAIC (Comparative Analysis by Independent Contrasts; Purvis and Rambaut 1995). Parity mode was coded as a categorical variable, and elevation of each collecting locality was used as a continuous variable. The analysis was

run twice: once using the phylogeny generated using all 38 specimens, and the second time using only those presented in Figure 5 to avoid biases due to the inclusion of more than one specimen per locality. The only exception was the inclusion of the oviparous and one of the viviparous lizards from Las Vigas (locality 6), to account for the occurrence of both parity modes in that population.

## Results

### *Variation in the ND4 Sequences*

We obtained mitochondrial DNA sequence data of approximately 700 nucleotides from the ND4 gene, and 175 nucleotides from the histidine, serine, and leucine tRNAs (total of 874 nucleotides), from 38 individuals belonging to the ingroup, and one individual for the outgroup (GENBANK accession numbers: U88255–U88293).

The total number of variable sites was 325, of which 205 were parsimony informative. Sequence divergence between pairs of individuals ranged from 1 to 165 nucleotides. Variable nucleotides in the ND4 sequence summed to 40.20%, and 26.04% of the sites in the ingroup were parsimony informative. The level of variation in the tRNA sequences was much lower: 16.0% of nucleotides were variable across all individuals, and 8.36% of these sites in the ingroup were parsimony informative. Patterns of variation within each codon position were typical of mtDNA ND4 sequences collected for other squamates (Arévalo et al. 1994; Forstner et al. 1995), being highest at third positions and lowest at second positions. Nucleotide positions featuring only transitions were most common, followed by positions exhibiting both transitions and transversions. Positions presenting only transversions were the least common. In the tRNA sequences, transitions were the most common substitutions followed by transversions, and then positions where both had occurred.

Levels of saturation of transitions (ts) and transversions (tv) at first, second, and third positions in codons in the ND4 gene were examined by plotting the uncorrected p-distance versus the number of each type of base-pair change for each pair of haplotypes (Fig. 3). Transversional changes remained linear at all codon positions across all levels of divergence, as did transitional changes in first and second positions. Transitions at third positions reached an asymptote at distances over about 0.17. The ratio of transitions to transversions (ts/tv) ranged from 0.0 to 12.0.

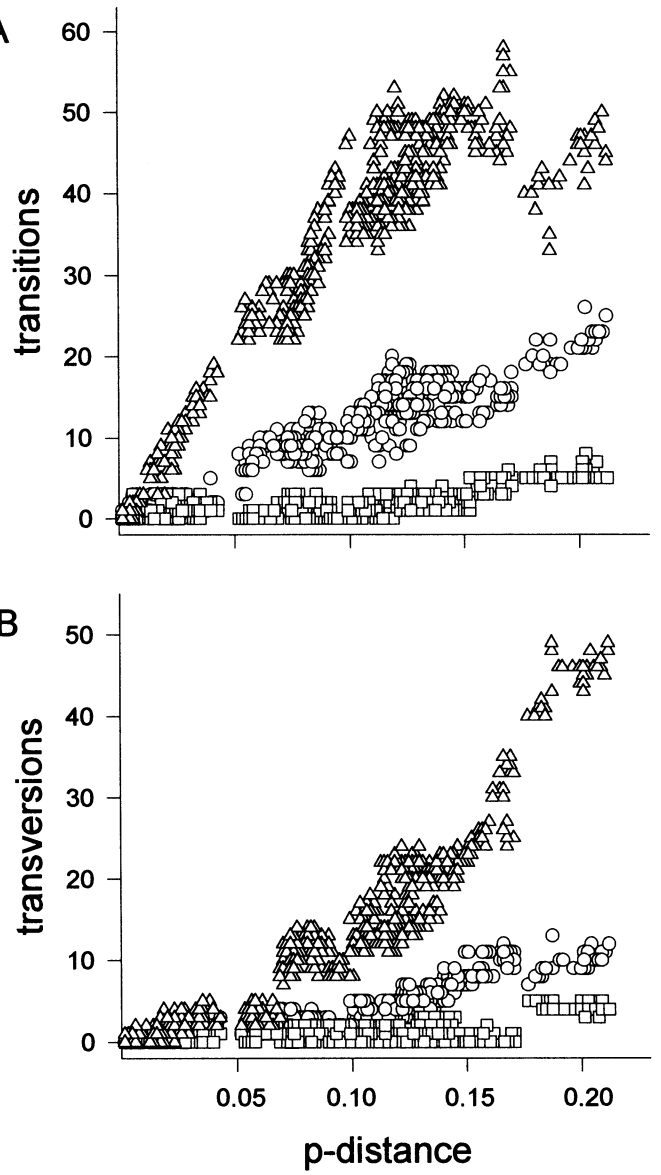


FIG. 3. Relationships between the number of transitions (A) and transversions (B) at each codon position and the uncorrected p-distance between each pair of haplotypes for the ND4 gene sequence (□ first, ○ second, △ third positions).

### *Phylogenetic Relationships*

Three different species in the genus *Sceloporus* that previous studies (Smith 1939; Larsen and Tanner 1975; Wiens and Reeder, in press) suggested to be closely related to the *scalaris* group were originally chosen as outgroups: *S. jalapae*, *S. ochoterenae*, and *S. pyrocephalus*. A jackknife deletion analysis showed that the use of any combination of these outgroups did not affect the monophyly of the ingroup, nor the topology of the tree. Therefore, we used *S. jalapae* as an outgroup for the remaining analyses, because we had the most complete data for that taxon. Sequential calculation of the $g_1$-statistic by iterative deletion of best supported branches in the tree (ranges of bootstrap proportions used in the sequential deletions were 100, 99, 94–95, 72–82, and 61) revealed significant phylogenetic signal in all pruned trees (using *P*-values in Hillis and Huelsenbeck 1992).

The probability of a most parsimonious relationship between each haplotype pair, as calculated by the algorithm of Templeton et al. (1992), was statistically significant at $P < 0.05$ for pairs separated by 12 or fewer mutational steps. Distance values between haplotype pairs ranged from 1 to 170 (Table 2). Ten networks were obtained that clustered individuals from the same taxon (species or subspecies) from geographically close localities, or individuals belonging to the same population (Fig. 4).

The strict consensus of five trees (Fig. 5) that resulted from the "branch and bound" analysis with 20 taxa (retaining one

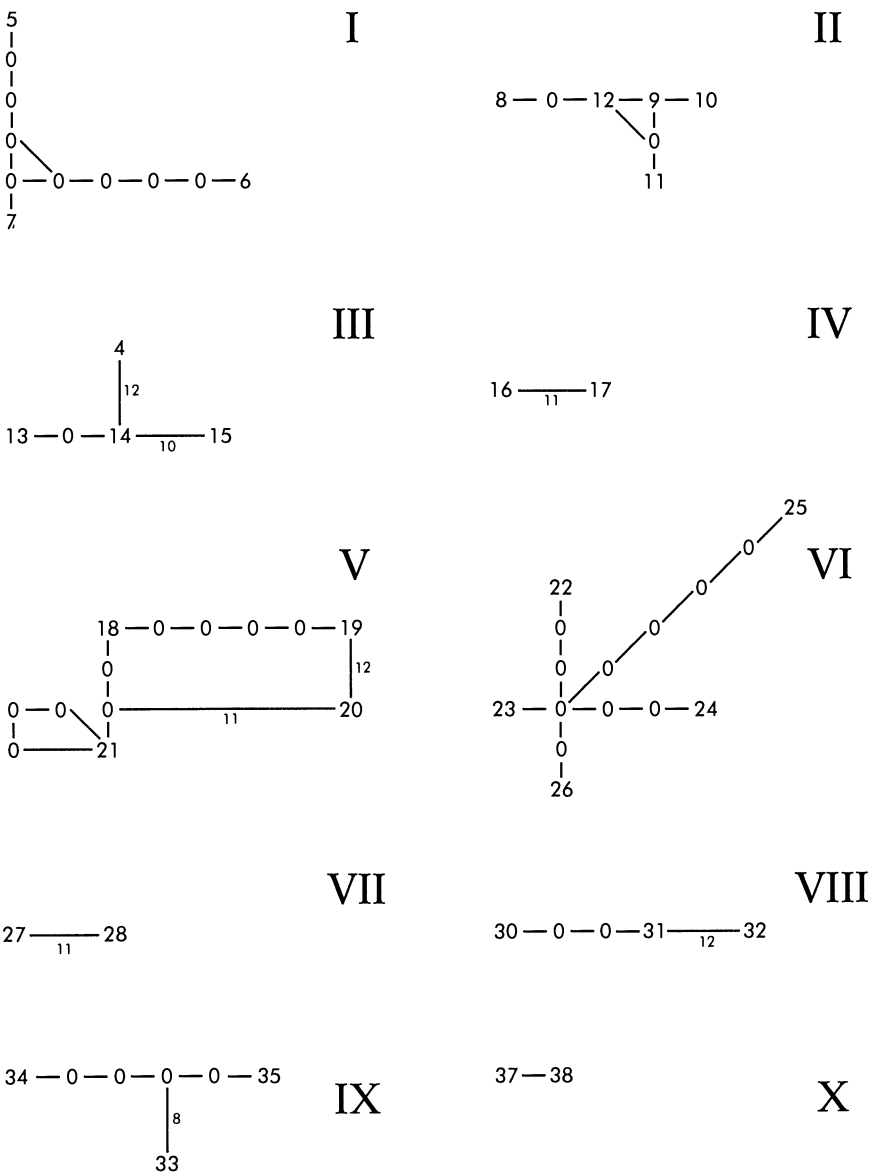


FIG. 4. Unrooted networks depicting the parsimony relationships of haplotypes differing by up to 12 nucleotide changes, as estimated by samples selected by the algorithm of Templeton et al. (1992). Each line connecting 0 represents one mutational event, and each 0 represents a missing intermediate haplotype that connect the OTUs in the networks. Small-sized numbers close to long lines indicate abbreviated number of missing intermediate haplotypes. OTU labels correspond to those in Table 1.

or two taxa from each unrooted network) gave basically the same results as the strict consensus of 75 trees obtained from the heuristic search (100 random addition searches) that included all taxa. The internal structure of both trees was the same, except that the three branches of *S. aeneus* had unresolved relationships in the heuristic search tree, and an unresolved relationship of one terminal branch within the *S. bicanthalis* clade was resolved in the branch and bound analysis. The consensus tree resulting from weighting transversions and nonsilent mutations was identical to the trees derived from the "equally weighted" matrix.

All *S. bicanthalis* form a strongly supported monophyletic group (bootstrap proportion = 100%), apart from a nonmonophyletic *S. aeneus* that appear as three separate branches with weakly resolved relationships (Fig. 5). Another strongly supported monophyletic group is formed by *S. scalaris*, *S. goldmani*, and *S. chaneyi* (again with bootstrap proportions of 100%). All of the remaining samples of *S. scalaris*, from Chihuahua and Arizona (OTUs 29, 31–34), are recovered as another strongly supported monophyletic group, and these two are recovered together as a monophyletic "northern clade" (localities 18–25; bootstrap proportion = 100% in both; Fig. 5).

### *Evolution of Viviparity*

According to the topology of the tree shown in Figure 5, viviparity evolved once in *S. goldmani*, and a second time in the branch leading to *S. bicanthalis*. Within *S. bicanthalis*, the lizards from Nopalillo, Hidalgo (OTUs 5–7, locality 5),

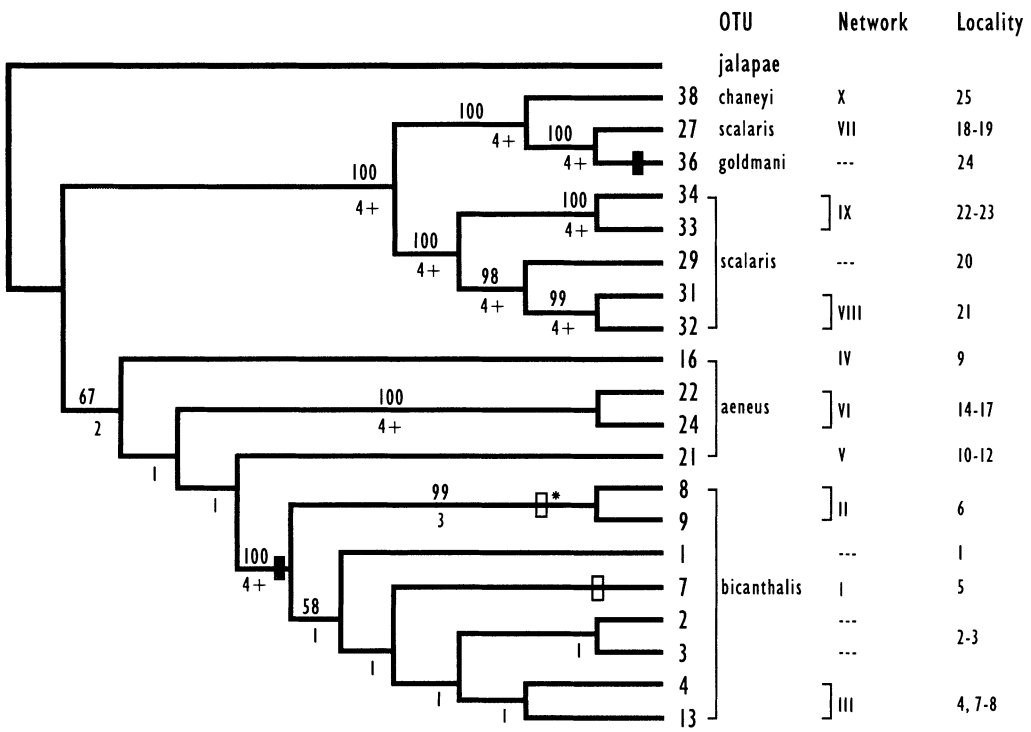


FIG. 5. Strict consensus of five trees obtained by the "branch and bound" option in PAUP, including 20 taxa (one or two members of each of the networks depicted in Fig. 4). OTU labels and locality numbers correspond to those in Table 1. Tree length = 505; consistency index = 0.6851; retention index = 0.7488; rescaled consistency index = 0.5130. Support for the internal nodes is indicated as follows: numbers above the branches are bootstrap proportions (if > 50%), and numbers below are Bremer (1988) support indexes. Solid rectangles indicate most parsimonious interpretations of the appearance of viviparity, while open rectangles indicate either reproductively bimodal (*) or oviparous populations in *Sceloporus bicanthalis*.

appear to have reversed from viviparity to oviparity. Among the lizards from Las Vigas, Veracruz, where both parity modes occur, there is no difference in sequence divergence between the single oviparous specimen (OTU 12) and of the viviparous specimens from that location (OTUs 8–11, Table 2).

The significance of the independent origins of viviparity in the *S. scalaris* group was tested by modifying the topology of the tree in two ways. First, we constrained *S. goldmani* to the base of all *S. bicanthalis*, therefore forming a monophyletic viviparous clade (except for the oviparous lizards from Nopalillo, Hidalgo, that would represent the reversal noted above). The most parsimonious tree was compared with the constrained tree using the large-sample approximation for the sign test (Hollander and Wolfe 1973). The constrained tree (693 steps, including uninformative characters) was significantly longer ($n$ = 85, $B^*$ = 8.785, $P < 0.0001$) than the original tree (611 steps). Another sign test was performed to check if the support for the hypothesis of the reversal of viviparity to oviparity in the *S. bicanthalis* lizards from Nopalillo was significant, versus the alternative hypothesis that the lizards from that locality are basal to the rest of *S. bicanthalis*, and viviparity has never evolved (and then reversed) in the Nopalillo clade. The constrained tree (616 steps) was not significantly longer ($n$ = 17, $B^*$ = 10, $P$ = 0.3145) than the original tree. The hypothesis that all viviparous taxa form one clade was tested by constraining the position of *S. goldmani* to the base of the *S. bicanthalis* clade, and the position of the oviparous *S. bicanthalis* from Nopalillo external to *S. goldmani*. The resulting tree (721 steps) was significantly longer ($n$= 121, $B^*$ = 9.364, $P < 0.0001$) than the original tree.

The comparative analysis by independent contrasts showed no correlation between the contrasts on parity mode and the contrasts on elevation, using all OTUs except for only one of those from the same locality ($r^2$ = 0.01; $P$ = 0.97) nor in the analysis using the OTUs shown in Figure 5 ($r^2$ = 0.08; $P$ = 0.91). *Sceloporus goldmani*, the viviparous species within the northern clade, was collected at the lowest elevation locality. The oviparous *S. aeneus* were collected in the low to intermediate elevation ranges. Among *S. bicanthalis*, the population from Las Vigas, where both parity modes were found, inhabits the lowest elevation, although Nopalillo, the locality where *S. bicanthalis* is oviparous, has an intermediate elevation. Only four lizards used in this study came from elevations of 3000 m above sea level or higher (Table 1).

## DISCUSSION

### *Phylogenetic Relationships*

Our results confirm and complement those of Mink and Sites (1996), showing two main clades within the *S. scalaris* complex: one clade inhabiting northern Mexico and Arizona,

comprised of all populations of *S. scalaris*, *S. goldmani*, and *S. chaneyi* (OTUs 27, 29, 31–34, 36, and 38), and a second clade from central Mexico including all populations of *S. aeneus* and *S. bicanthalis* (OTUs 1–4, 7–9, 13, 16, 21, 22, and 24). Because more populations were included here, some differences between the present study and that of Mink and Sites (1996) emerged, particularly the paraphyly of *S.* scalaris.

Paraphyly of the *S. scalaris* mtDNA tree with respect to *S. chaneyi* and *S. goldmani* may be due to any of four causes. First, interspecific hybridization and subsequent introgression of *S. scalaris* mtDNA into *S. chaneyi* and *S. goldmani* could produce a mtDNA gene tree discordant with the species tree (Smith 1992). Second, a recent origin of *S. goldmani* or *S. chaneyi* from within part of *S. scalaris* could produce a morphologically distinct species nested well within its ancestral taxon (Theriot 1992; Patton and Smith 1994). However, this expectation is inconsistent with the origin of *S. goldmani*, which is unambiguously differentiated from *S. scalaris* in allozyme and morphological characters (Mink and Sites 1996). Third, incomplete sampling of *S. scalaris* taxa may result in an inaccurate estimate of phylogeny (Wheeler 1992), and can only be corrected by inclusion of the other named races of *S. scalaris*. Fourth, given the disjunct distribution of taxa now under the name *S. scalaris*, the presence of cryptic species and inaccurate taxonomy is the most likely explanation. The very high bootstrap proportions for all internal nodes of the "northern clade" (OTUs 27–38 in Fig. 5) supports this notion, and a detailed study of "*S. scalaris*" throughout its range is now needed. For a consideration of the evolution of viviparity in the *S. scalaris* group, however, placement of *S. goldmani* within the *S. scalaris* clade is irrelevant. It represents one unambiguous origin of viviparity, regardless of its affinities within this group.

Recognition of *S. aeneus* and *S. bicanthalis* as separate species has been proposed by Guillette and Smith (1985), Smith et al. (1993), and Mink and Sites (1996). However, the mtDNA data presented by Creer et al. (in press) are equivocal in the resolution of these relationships. The inclusion of mtDNA sequence data from members of more populations of these taxa in this study shows *S. bicanthalis* to be monophyletic, supporting the division of two species. Mink and Sites (1996) found strong support (bootstrap proportions = 85%) for monophyly of *S. aeneus*, but the mtDNA sequences did not corroborate the monophyly of this species. As in the case with *S. scalaris*, such results may stem from a number of possible causes that cannot be determined at this time. Ultimate resolution of the *S. aeneus* populations, however, will have little impact on the issue of viviparity and its origin in this clade. The key point is that *S. bicanthalis* is strongly corroborated as a monophyletic taxon, on the basis of allozyme, morphological, and mtDNA evidence, and it includes both oviparous and viviparous populations.

### *Evolution of Viviparity*

Our analyses using *S. jalapae* as an outgroup assumed that oviparity was ancestral for the *S. scalaris* group. A very recent study (Wiens and Reeder, in press) recovered *S. graciosus* as the sister group to a clade formed by *S. scalaris* plus the large-scaled, large-bodied *Sceloporus* species. Even though *S. jalapae* does not appear now to be the closest outgroup to the *S. scalaris* group, our basic assumption of oviparity as the ancestral parity mode does not change.

Mink and Sites (1996) suggested at least two independent origins of viviparity within the *S. scalaris* group, one in *S. goldmani*, and at least one in *S. bicanthalis*. However, due to a limited number of parsimony informative characters, Mink and Sites (1996) could not reject the single origin hypothesis with any statistical confidence. The subsequent study of Creer et al. (in press), adding mtDNA data, suggested two origins of viviparity within *S. bicanthalis*. That study also suggested an independent origin of viviparity in *S. goldmani*, although it did not include sequence data for that species.

Our analysis includes a larger number of populations within each clade and suggests that viviparity evolved once in *S. goldmani*, due to its placement well within the oviparous *S. scalaris* clade from northern Mexico and Arizona, and again in *S. bicanthalis*. The possibility of *S. goldmani* belonging to the *bicanthalis* clade is statistically unlikely, as shown by the sign test. Our placement of *S. goldmani* within the *scalaris* clade thus confirms the results reported by Mink and Sites (1996) on the basis of isozyme characters, but with a high level of statistical confidence ($P < 0.0001$).

Evolution of viviparity in the *bicanthalis* clade seems more complex. The most interesting observations are: (1) a fully oviparous population is nested within a viviparous clade; and (2) the most basal population (Las Vigas) is reproductively bimodal.

### *Reversal of Parity Mode*

Placement of the oviparous *S. bicanthalis* from Nopalillo at the base of all viviparous *S. bicanthalis* does not significantly change the length of the tree. Therefore, although the population from Nopalillo may be basal and therefore viviparity may have appeared once in the node leading to the rest of the *bicanthalis* clade, the most parsimonious topology indicates a reversal from viviparity to oviparity at this locality (Fig. 5). This possibility is supported by the consistent nesting of the Nopalillo population within the *bicanthalis* clade in all of the analyses, including weighted parsimony. Rigorous confirmation of this hypothesis has interesting implications for hypothesis of the evolution of viviparity in squamate reptiles.

In an analysis of reproductive mode in relation to several ecological factors, Guillette et al. (1980) suggested that viviparity evolved primarily in cold environments of high elevation, and proposed that the presence of several viviparous sceloporines in the lowlands may indicate that viviparity evolves as an irreversible trait. However, other studies (de Fraipont et al. 1996) based on phylogenetic analysis suggest that reversal from viviparity to oviparity is possible among squamates. Studies of the morphological and physiological mechanisms involved in the evolution of viviparity in reptiles suggest that the main changes necessary for its appearance are related to the evolution of a placenta. This involves a decrease in the thickness of the eggshell and lengthening of the gestation period, or egg retention with embryonic development, coupled with the maintenance of the corpus lu-

teum. Thinning of the eggshell allows maternal recognition of pregnancy to occur, because the uterus can be exposed to chemical signals secreted by the embryo (Guillette 1989). Thus, the transition of oviparity to viviparity seems to involve a fine-tuned balance among these factors, and as long as the shelling glands of the oviduct are not lost (as in the lizard *Mabuya*; Vitt and Blackburn 1983; Blackburn et al. 1984), reversal from viviparity back to oviparity should be possible (Guillette, pers. comm.).

Reproductive bimodality occurs in other lizards (e.g., *Lacerta vivipara*) that appear to have recently evolved viviparity (Guillette 1993). The transition from oviparity to viviparity must at some point involve polymorphism for the length of egg retention, and eventually for parity mode, and that stage is what may be evident in the population from Las Vigas, Veracruz (locality 6 in Fig. 2). There is genetic evidence that oviparous and viviparous lizards from this location belong to a single random-mating population, as no differences in allozymes between lizards showing different parity modes were found by Mink and Sites (1996). That conclusion is supported by our analysis of mtDNA showing that within this population there is no larger degree of sequence divergence between oviparous and viviparous lizards than among viviparous lizards only. Fertile $F_2$ hybrids have been obtained in laboratory crosses between oviparous and viviparous populations of *L. vivipara* (Heulin et al. 1989), suggesting that shifts in parity mode do not affect reproductive compatibility. These observations are strong evidence that oviparous and viviparous individuals in this population are conspecific, and the basal position of this sample within the *S. bicanthalis* clade could represent either a retained ancestral polymorphism, or a transition in either direction.

### *Relationship of Parity Mode to Elevation*

The complexity of this issue is further underscored by the absence of significant correlation between parity mode and elevation in our sample, in opposition to the expectations of the high elevation hypothesis of the evolution of viviparity in reptiles. This lack of correlation may be an artifact of either: (1) sampling only the two extremes (oviparity vs. viviparity) of a reproductive continuum; or (2) restricting the use of "elevation" to single data points on an elevational gradient, when in fact the real issue may be the range of elevation occupied by these populations. It is possible that the lower limit of *S. bicanthalis* elevational range overlaps with the upper extreme for *S. aeneus*, but in fact, the total ranges occupied by both taxa may really show very little overlap. If data for complete elevation ranges were available, significant correlations might be found. Other variables expected to covary along an elevational gradient, like egg retention and eggshell thickness of the oviparous populations, were not available to us. As a result, our single category of oviparity may be too simplistic, and may hide significant correlations.

Even if the general analysis of the correlation of elevation and parity mode failed to support the predictions, elevation could correlate with parity mode within the southern (*aeneus-bicanthalis*) clade, or within the *bicanthalis* clade only. For example, within *S. bicanthalis*, the Las Vigas (where both parity modes occur) and Nopalillo (oviparous *bicanthalis*) populations could fit the predicted pattern (lower elevations than the fully viviparous *bicanthalis* populations). The Las Vigas population could represent an example of a nearly complete transition to viviparity, with oviparity existing at a very low frequency, while Nopalillo, if it is a lower-elevation site, may represent a reversal selected for in the low-elevation habitat. However, Nopalillo is at a higher elevation (2920 m) than Las Vigas (2460 m), and there is one viviparous population (locality 8) that is the lowest of all (1858 m). The analysis including *S. aeneus* does not support the predictions either.

Mathies and Andrews (1995) showed a correlation between length of egg retention period and elevation, and between eggshell thickness and elevation in an oviparous lizard (*S. scalaris*), claiming that this results support the idea that viviparity evolves at high elevations. They assumed, then, that oviparous lizards at high elevations tend to evolve toward viviparity. The evidence within *S. bicanthalis* that at least one population is bimodal for parity mode, and another may have reversed from viviparity back to oviparity, makes the assumption of Mathies and Andrews (1995) invalid for at least some populations.

Little is known about the comparative ecology of the populations included in this study, and thus, a more detailed analysis regarding the possible selective forces involved in the evolution of viviparity (including the high altitude hypothesis of Guillette et al. 1980) in the *S. scalaris* complex should be insightful. The selective forces driving the transition from oviparity to viviparity may best be investigated by looking for consistent differences between closely related oviparous and viviparous species (Shine 1985). The *S. scalaris* complex appears to be a good model for such studies. Comparisons of ecological, endocrinological, and morphological studies of *S. bicanthalis* from Las Vigas (locality 6) where both parity modes occur, and from the oviparous *S. bicanthalis* from Nopalillo (locality 5), relative to viviparous conspecific populations, will be of great importance in the understanding of the evolution of viviparity in these lizards.

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