Herpetologica, 58(4), 2002, 415–421

CONTINUOUS SPERMATOGENESIS IN THE LIZARD *SCELOPORUS BICANTHALIS* (SAURIA: PHRYNOSOMATIDAE) FROM HIGH ELEVATION HABITAT OF CENTRAL MEXICO

OSWALDO HERNÁNDEZ-GALLEGOS[1], FAUSTO ROBERTO MÉNDEZ-DE LA CRUZ[1], MARICELA VILLAGRÁN-SANTA CRUZ[2], AND ROBIN M. ANDREWS[3,4]

[1]*Laboratorio de Herpetología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70-515, C. P. 04510, México, D. F. México*
[2]*Laboratorio de Biología de la Reproducción Animal, Facultad de Ciencias, Universidad Nacional Autónoma de México, A. P. 70-515, C. P. 04510, México, D. F. México*
[3]*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

ABSTRACT: *Sceloporus bicanthalis* is a viviparous lizard that inhabits high altitude temperate zone habitats in México. Our histological observations indicate that adult males exhibit spermatogenesis and spermiogenesis throughout the year; no seasonal differences were found in testes mass, height of epididymal epithelial cells, and number of layers of spermatogonia, primary and secondary spermatocytes, and spermatids. Seminiferous tubules exhibited slight, but statistically significant, seasonal variation in diameter. Continuous spermatogenesis and spermiogenesis of *S. bicanthalis* differ from the cyclical pattern exhibited by most species of lizards and from lizard species sympatric with *S. bicanthalis.* Continuous reproductive activity of males of *S. bicanthalis,* and maturation at a relatively small size, is associated with a female reproductive activity in which vitellogenic or pregnant females are present in the population during all months of the year. As a consequence, males can encounter potential mates as soon as they mature.

Key words: Reproductive cycle; *Sceloporus bicanthalis*; Spermatogenesis; Viviparity

REPRODUCTIVE activity of reptiles is usually seasonal. In the temperate zone, seasonal reproduction is associated with the alternation of warm and cold seasons (Duvall et al., 1982; Fitch, 1970; Sherbrooke, 1975). In the lowland tropics, most reptiles also exhibit seasonal reproductive cycles, but, in this case, they are associated with the alternation of wet and dry seasons (Howland et al., 1990; Vitt and Morato de Carvalho, 1992). Similarly, in high elevation subtropical areas of México and Central America, most lizards also exhibit seasonal reproductive cycles (Guillette and Méndez-de la Cruz, 1993; Méndez-de la Cruz et al., 1998; Ramírez-Bautista et al., 1998). Aseasonal reproduction is, thus, an uncommon reproductive pattern for reptiles, regardless of habitat. At high elevations in Costa Rica, however, males of *Barisia monticola* exhibit continual spermatogenesis and spermiogenesis and appear to be reproductive throughout the year (Vial and Stewart, 1985).

Our study examines the testicular activity of *Sceloporus bicanthalis,* a phrynosomatid lizard of high elevations of the central mountains of México. This species is viviparous as are most other lizards that live at comparable elevations. Based on variation in the volume of testes, Guillette (1981*a*, 1982) reported that males of this species exhibit a seasonal reproductive cycle. In contrast, our histological analysis shows that males are actually spermatogenic year round.

MATERIALS AND METHODS

We collected *Sceloporus bicanthalis* at Parque Nacional Zoquiapan, State of México (98° 37′ 39″ W, 19° 13′ 10″ N), at 3200 m. The study area has a relatively cool climate with low temperature variation during the year, and most rain falls during summer (Fig. 1). Vegetation is dominated by pines, and the understory consists of various species of bunch-grasses, including *Festuca* and *Muhlenbergia* (Rzedowsky, 1981). *Sceloporus bicanthalis* is terrestrial and is associated with the bunch-grass microhabitat; individuals use the grasses for perches and for shelter. Collections were

[4] CORRESPONDENCE: e-mail, randrews@vt.edu

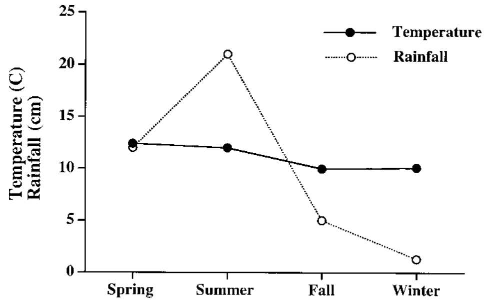

FIG. 1.—Mean temperature (solid circles) and rainfall (open circles) averaged over 3-mo intervals at the weather station (Río Frío) located closest (about 15 km) to the Zoquiapan study site (García, 1973).

TABLE 1.—Monthly sample sizes for males of *Sceloporus bicanthalis* and individuals selected for histological analysis. J = juvenile and Ad = adult males.

	Sample size		Males selected for histological analysis	
Month	Ad	J	Ad	J
April	4	0	3	0
May	4	2	3	0
June	4	0	3	0
July	4	6	2	0
August	5	0	4	0
September	4	3	3	0
October	4	1	3	0
November	3	0	3	0
December	2	6	2	1
January	5	0	3	0
February	5	0	2	0
March	4	1	2	1

made monthly (Table 1) but were analyzed seasonally: spring 1993 (April–June, n = 14), summer 1993 (July–September, n = 22), fall 1993 (October–December, n = 16), and winter 1994 (January–March, n = 15). Morphological measurements taken from each animal were as follows: snout–vent length (SVL), body mass, and testicular mass.

We performed conventional histological analyses (Estrada-Flores et al., 1990) of testes from males exhibiting secondary sexual characteristics (Table 1). An ocular micrometer was used to make the following measurements on the left testis: mean diameter of 25 representative seminiferous tubules and height of 25 epididymal epithelial cells. Germinal cells (spermatogonia, primary and secondary spermatocytes, and spermatids) were classified according to size and position in the seminiferous tubules. Relative abundance of germinal cells was estimated by counting the number of layers in 25 tubules. The presence of spermatozoa in the seminiferous tubules and epididymis ducts was used to define the minimum size of sexual maturity. We averaged the measurements for each individual; individuals were, thus, represented only once in statistical analyses.

All statistical analyses were conducted with SAS software (SAS Institute, Inc., Version 6.12, 1997). We used ANCOVA's to compare seasonal means of morphological measurements. In these analyses, we used body mass as the covariate for testis mass and SVL as the covariate for other measurements. In no case was the interaction term between the class variable and the covariate significant at $P < 0.05$.

RESULTS

The smallest sexually mature male was 35 mm SVL as judged by both gross morphology and histological analysis. Externally, all individuals 35 mm in length or more exhibited bright blue ventral coloration and active femoral pores. Moreover, all males 35 mm or more (n = 33) had sperm in their seminiferous tubules and epididymal ducts. Adult males ranged in size from 35–51 mm SVL ($\bar{x}$ = 42.6 mm, SE = 0.61). Two males with SVL of 34 mm had traces of blue coloration and were selected for histological analysis. They did not, however, have sperm in the seminiferous tubules and epididymis duct and, therefore, were considered immature.

Testis mass of adult males did not vary seasonally ($F_{3,43}$ = 0.8, P = 0.51, ANCOVA), although testis mass was related to body mass ($F_{1,43}$ = 65.6, $P < 0.0001$). Analyses of histological data also indicated that adult males of *S. bicanthalis* exhibit continuous testicular activity throughout the year; no testicular recrudescence or regression was observed. Mature sperm was found in the seminiferous tubules (Fig. 2) and epididymal ducts in all specimens in all seasons. The height of epididymal epithelial cells and the number of layers of

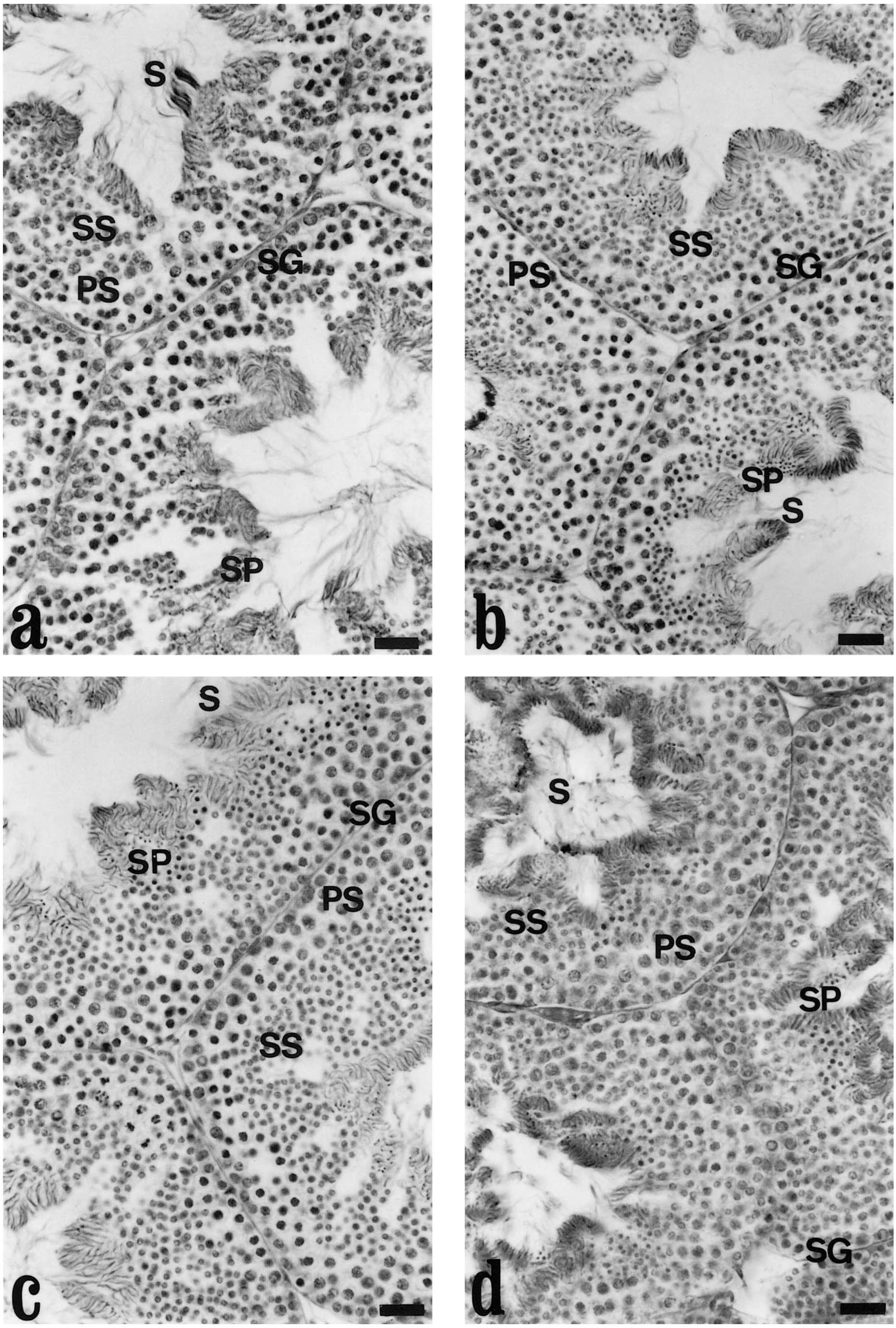

FIG. 2.—Seminiferous tubules of *Sceloporus bicanthalis* from Zoquiapan, State of México: (a) spring, (b) summer, (c) fall, and (d) winter. Note the presence of spermatogonia (SG), primary spermatocytes (PS), secondary spermatocytes (SS), spermatids (SP), and spermatozoa (S) in all seasons. Scale bar = 30 μm.

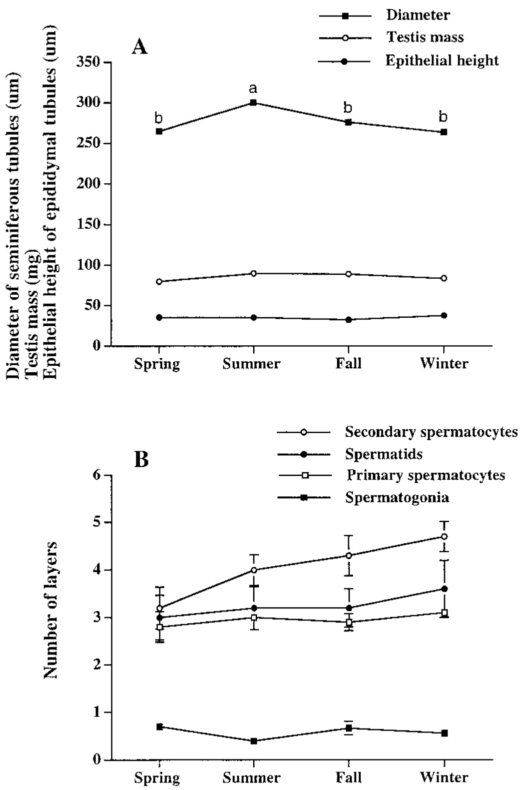

FIG. 3.—Morphological measurements of testis mass and histological structures expressed as means and their standard errors. When standard error bars are not shown, they were too small to be distinguished from the means. Values with different superscripts are significantly different ($P < 0.05$).

spermatogonia, primary and secondary spermatocytes, and spermatids did not vary seasonally or as a function of SVL (P values 0.11–0.95 for overall models). The only feature that varied seasonally was the diameter of seminiferous tubules ($F_{3,28} = 4.0$, $P = 0.018$, ANCOVA) (Fig. 3A,B).

DISCUSSION

Reproductive Seasonality

Our observations indicate that adult males of *S. bicanthalis* exhibit continuous spermatogenesis and spermiogenesis throughout the year. The relative amount of proliferation from one germinal cell layer to another within the testes did not vary seasonally nor did the height of epithelial cells in the epididymis duct. The epithelial lining of the epididymis duct, as well as the sexual segment of the kidney, are known to increase in height and secretory activity in association with reproduction (Wilhoft, 1963). While we did not study the sexual segment of the kidney, the lack of seasonal variation in the size of the cells in the epithelial lining of the epididymis duct indicates that reproduction by males was possible year round. The bright blue ventral coloration and active femoral pores throughout the year further suggest continual reproductive activity; these features presumably reflect high androgen levels throughout the year (Cooper, 1995; Hews and Moore, 1995).

Our conclusion about the reproductive cycle of males of *S. bicanthalis* differs from that of Guillette (1981*a*, 1982). A possible reason for this difference is that observations by Guillette and by us were made on different populations and for different years. Geographic variation in reproductive cycles is known for several species (Méndez-de la Cruz et al., 1994; Villagrán-Santa Cruz et al., 1994). More likely explanations, however, relate to methodology. Guillette's study is based on estimates of testis volume whereas our conclusions are based on histological analysis. The problem with the use of testis size to assess reproductive status is that variation in testis size may reflect the degree of hydration (Vitt, 1986) or variation in intensity of sperm production (García-Collazo et al., 1993). Moreover, Guillette did not correct estimates of testis size for the size of the lizard, and testis size is strongly correlated with body size in our investigation.

Our results call into question male reproductive cycles assessed only by macroscopic measures, particularly when females show noticeably asynchronous reproduction. For example, *Sceloporus formosus* (Guillette and Sullivan, 1985) and *Eumeces copei* (Ramírez-Bautista et al., 1996) are classified as lizards with fall reproductive activity, but vitellogenic females are found during most of the year, which suggests that male reproductive activity could be similarly protracted. Only histological studies of testes will clarify the reproductive cycles for males of these species, because the variation in reproductive activity through histological examination provides confirmation of the degree of correspondence between macroscopic changes in gonadal cycles and gametogenic activity (Estrada-Flores et al., 1990).

The only morphological measurement that varied seasonally was the diameter of the seminiferous tubules, which was greatest in summer. Seasonal change in tubule diameter is inexplicable because the change in diameter was not associated with variation in the number of cells (the number of germinal cells did not vary seasonally). Moreover, because testis mass did not vary seasonally, the increase in the diameter of the seminiferous tubules was probably not associated with greater hydration of the tubules. Given these observations, and the relatively small variation in the diameter of the tubules, we consider that the seasonal change in tubule diameter is not likely to be significant biologically.

Females of *S. bicanthalis* at Zoquiapan also exhibit continuous reproduction; vitellogenic and pregnant females are present in the population year round (Manríquez-Morán, 1995; Méndez-de la Cruz et al., 1998). The presence of vitellogenic females during most months is associated with the continuous reproductive activity in males; potential mates are always available. Males and females of *S. bicanthalis* at other sites, Nopalillo, Hidalgo (2900 m), and Nevado de Toluca (4100 m), State of México, also exhibit continuous reproduction (F. R. Méndez-de la Cruz, unpublished data).

For *S. bicanthalis,* continuous testicular activity could be associated with low seasonal variation in temperature (Duvall et al., 1982; Vial and Stewart, 1985). This explanation is unlikely, as males of the sympatric *Sceloporus mucronatus* (Guillette, 1981b) and *Sceloporus grammicus* (Guillette and Casas-Andreu, 1980) exhibit seasonal reproduction. Moreover, two species from different genera that share the bunch grass microhabitat with *S. bicanthalis* (*E. copei:* Guillette, 1983; Ramírez-Bautista et al., 1996; and *B. imbricata:* Guillette and Casas-Andreu, 1987) appear to exhibit seasonal reproduction as well. Continuous reproductive activity of *S. bicanthalis,* thus, is not necessarily related to climate or microhabitat features.

Continuous spermatogenesis by males of *S. bicanthalis* is associated with two unusual features of the reproductive biology of this species. The SVL of males of *S. bicanthalis* at sexual maturity is the smallest among species of *Sceloporus* (Fitch, 1978; Tinkle et al., 1970). Small size at maturity may be advantageous for males because receptive females are available throughout the year and mating could be possible whenever males mature. Moreover, the ratio of male SVL to female SVL (0.87) is the lowest in the genus (Fitch, 1978). In the bunch-grass microhabitat of *S. bicanthalis,* larger males may not have an advantage over smaller males. Low visibility precludes the effective territorial monitoring and long-range territorial displays of species that occupy open microhabitats. In this latter situation, growth to relatively large body sizes is beneficial to males (Fitch, 1978). In contrast, males of *S. bicanthalis* may employ strategies of courtship that are effective at small as well as large body sizes.

Evolution of the Reproductive Cycle

Sceloporus bicanthalis is the only member of the genus *Sceloporus* known to exhibit continuous spermatogenesis and spermiogenesis. We postulate that the largely aseasonal reproduction by both males and females of *S. bicanthalis* is related to the recent origin of viviparity of this species. *Sceloporus bicanthalis* is the sister-species of the oviparous *Sceloporus aeneus,* and these species are genetically very similar (Benabib et al., 1997; Guillette, 1982; Mink and Sites, 1996). In *Sceloporus,* the evolution of viviparity is associated with a change in reproductive phenology from spring mating and fertilization in oviparous species to spring and summer mating and fall fertilization in viviparous species (asynchronous reproduction). This change in phenology is associated with the invasion of cool climates at high elevations (Méndez-de la Cruz et al., 1998). The reproductive cycle of *S. bicanthalis* suggests that an initial prolongation of the reproductive season may represent a transition between the reproductive cycles of oviparous and viviparous species (Hernández-Gallegos, 1995; Méndez-de la Cruz et al., 1998).

Resumen

Sceloporus bicanthalis es una lagartija vivípara que habita en elevaciones altas de ambientes templados en México. De acuerdo al análisis histológico, los machos adultos presentan espermatogénesis y espermiogénesis continuas; no se encontraron diferencias estacionales en el peso testicular, altura del epitelio de los conductos del epidídimo, número de capas de espermatogonias, espermatocitos primarios y secundarios y espermátidas. En contraste, se encontró variación estacional en el diámetro de los túbulos seminíferos. El patrón reproductor continuo de *S. bicanthalis* es atípico cuando se compara con los ciclos reproductores de otras lagartijas, incluyendo especies simpátricas. Aparentemente, la actividad reproductora continua de los machos de *S. bicanthalis* y su madurez sexual temprana, están asociadas con la actividad reproductora de las hembras, en el cual la vitelogénesis o preñez se presentan durante todo el año. En consecuencia, los machos pueden aparearse una vez que alcanzan la madurez sexual.

Acknowledgments.—We thank Felipe Rodríguez-Romero for field assistance and Robert Jaeger, Steve Tilley, and two anonymous reviewers for their constructive and insightful comments on the manuscript.

Literature Cited

Benabib, M., K. M. Kjer, and J. W. Sites, Jr. 1997. Mitochondrial DNA sequence-based phylogeny and the evolution of viviparity in the *Sceloporus scalaris* group (Reptilia, Squamata). Evolution 51: 1262–1275.

Cooper, W. E., Jr. 1995. Effects of estrogen and male head coloration on chemosensory investigation of female cloacal pheromones by male broad-headed skinks (*Eumeces laticeps*). Physiology and Behavior 58:1221–1225.

Duvall, D., L. J. Guillette, Jr., and R. E. Jones. 1982. Environmental control of reptilian reproductive cycles. Pp. 201–231. *In* C. Gans and F. H. Pough (Eds.), Biology of the Reptilia, Vol. 13D. Academic Press, New York, New York, U.S.A.

Estrada-Flores, E. M., M. Villagrán-Santa Cruz, F. R. Méndez-de la Cruz, and G. Casas-Andreu. 1990. Gonadal changes throughout the reproductive cycle of the viviparous lizard *Sceloporus mucronatus* (Sauria: Iguanidae). Herpetologica 46:43–50.

Fitch, H. S. 1970. Reproductive cycles in lizards and snakes. University of Kansas Museum of Natural History, Miscellaneous Publication 52:1–247.

———. 1978. Sexual size differences in the genus *Sceloporus.* University of Kansas Science Bulletin 51:441–461.

García, E. 1973. Modificaciones al sistema de clasificación climática Köppen. Instituto de Geografía, Universidad Nacional Autónoma de México, México.

García-Collazo, R., T. Altamirano-Álvarez, and M. Gómez-Soto. 1993. Reproducción continua en *Sceloporus variabilis variabilis* (Sauria: Phrynosomatidae) en Alvarado, Veracruz, México. Boletin de la Sociedad Herpetológica de México 5:51–59.

Guillette, L. J., Jr. 1981*a*. Reproductive Strategies and Evolution of Viviparity in Two Allopatric Populations of the Mexican Lizard, *Sceloporus aeneus.* Ph.D. Dissertation, University of Colorado, Boulder, Colorado, U.S.A.

———. 1981*b*. Fall reproductive activity in high elevation reptiles of the Parque Nacional de Zoquiapan, México. Journal of the Colorado-Wyoming Academy of Science 13:152.

———. 1982. The evolution of viviparity and placentation in the high elevation, Mexican lizard *Sceloporus aeneus.* Herpetologica 38:94–103.

———. 1983. Notes concerning reproduction of the montane skink, *Eumeces copei.* Journal of Herpetology 17:144–148.

Guillette, L. J., Jr., and G. Casas-Andreu. 1980. Fall reproductive activity in the high altitude Mexican lizard *Sceloporus grammicus microlepidotus.* Journal of Herpetology 14:143–147.

———. 1987. The reproductive biology of the high elevation Mexican lizard, *Barisia imbricata.* Herpetologica 43:29–38.

Guillette, L. J., Jr., and F. R. Méndez-de la Cruz. 1993. The reproductive cycle of the viviparous Mexican lizard *Sceloporus torquatus.* Journal of Herpetology 27:168–174.

Guillette, L. J., Jr., and W. P. Sullivan. 1985. The reproductive and fat body cycles of the lizard, *Sceloporus formosus.* Journal of Herpetology 19:474–480.

Hernández-Gallegos, O. 1995. Estudio comparativo del patrón reproductor de los machos de dos especies de lagartijas emparentadas con distinto modo reproductor: *Sceloporus aeneus* y *S. bicanthalis.* Tesis de Licenciatura en Biología, Facultad de Ciencias, Universidad Nacional Autónoma de México, México.

Hews, D. K., and M. C. Moore. 1995. Influence of androgens on differentiation of secondary sex characters in tree lizards, *Urosaurus ornatus.* General and Comparative Endocrinology 97:86–102.

Howland, J. M., L. J. Vitt, and P. T. Lopez. 1990. Life on the edge: the ecology and life history of the tropidurine iguanid lizard *Uranoscodon superciliosum.* Canadian Journal of Zoology 68:1366–1373.

Manríquez-Morán, N. L. 1995. Estrategias reproductoras en las hembras de dos especies hermanas de lacertilios: *Sceloporus aeneus* y *S. bicanthalis.* Tesis de Licenciatura en Biología, Facultad de Ciencias, Universidad Nacional Autónoma de México, México.

Méndez-de la Cruz, F. R., M. Villagrán-Santa Cruz, and R. M. Andrews. 1998. Evolution of viviparity in the lizard genus *Sceloporus.* Herpetologica 54:521–532.

Méndez-de la Cruz, F. R., M. Villagrán-Santa Cruz, and O. Cuellar. 1994. Geographic variation of spermatogenesis in the Mexican viviparous lizard *Sceloporus mucronatus.* Biogeographica 70: 59–67.

Mink, D. G., and J. W. Sites, Jr. 1996. Species limits, phylogenetic relationships, and origins of viviparity in the scalaris complex of the lizard genus *Sceloporus* (Phrynosomatidae: Sauria). Herpetologica 52:551–571.

Ramírez-Bautista, A., J. Barba-Torres, and L. J. Vitt. 1998. Reproductive cycle and brood size of *Eumeces lynxe* from Pinal de Amoles, Queretaro, México. Journal of Herpetology 32:18–24.

Ramírez-Bautista, A., L. J. Guillette, Jr., G. Gutiérrez-Mayén, and Z. Uribe-Peña. 1996. Reproductive biology of the lizard *Eumeces copei* (Lacertilia: Scincidae) from the Eje Neovolcánico, México. Southwestern Naturalist 41:103–110.

Rzedowsky, J. 1981. Vegetación de México. Limusa, México.

SAS Institute, Inc. 1997. SAS/STAT User's Guide. Statistical Analysis Systems Institute, Inc., Cary, North Carolina, U.S.A.

Sherbrooke, W. C. 1975. Reproductive cycle of a tropical teiid lizard, *Neusticurus ecpleopus* Cope, in Peru. Biotropica 7:194–207.

Tinkle, D. W., H. M. Wilbur, and S. G. Tilley. 1970. Evolutionary strategies in lizard reproduction. Evolution 24:55–74.

Vial, J. L., and J. R. Stewart. 1985. The reproductive cycle of *Barisia monticola*: a unique variation among viviparous lizards. Herpetologica 41:51–57.

Villagrán-Santa Cruz, M., F. R. Méndez-de la Cruz, and L. Parra-Gámez. 1994. Ciclo espermatogénico del lacertilio *Sceloporus mucronatus* (Reptilia: Phrynosomatidae). Revista de Biología Tropical 42:289–296.

Vitt, L. J. 1986. Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. Copeia 1986:773–786.

Vitt, L.J., and C. Morato de Carvalho. 1992. Life in the trees: the ecology and life history of *Kentropyx striatus* (Teiidae) in the lavrado area of Roraima, Brazil, with comments of the life histories of tropical teiid lizards. Canadian Journal of Zoology 70:1995–2006.

Wilhoft, D. C. 1963. Gonadal histology and seasonal changes in the tropical Australian lizard *Leiolopisma rhomboidalis.* Journal of Morphology 113: 185–204.

Accepted: 23 January 2002
Associate Editor: Stephen Tilley