

Reproductive biology of the bushmaster *Lachesis muta* (Serpentes: Viperidae) in the Brazilian Atlantic Forest

Fátima Q. Alves¹, Antônio J. S. Argôlo², and Gilson C. Carvalho³

¹ CEPEC/CEPLAC, Rod. Ilhéus/Itabuna, Km 22, Caixa Postal 7, 45600-000, Itabuna, BA, Brazil.

E-mail: queirozalves@gmail.com.

² UESC - Departamento de Ciências Biológicas. Rodovia Jorge Amado, km 16, Bairro Salobrinho, Ilhéus, BA, Brazil. 45662-900. E-mail: ajargolo@gmail.com.

³ Departamento de Biointeração, ICS. Universidade Federal da Bahia - UFBA, BA, Brazil. E-mail: biogilson@gmail.com.

Abstract

Reproductive biology of the bushmaster *Lachesis muta* (Serpentes: Viperidae) in the Brazilian Atlantic Forest. The Neotropical genus *Lachesis* Daudin, 1803 includes snakes restricted to the humid forests of Central and South America. The species *L. muta* is the most widely dispersed, occurring throughout the Amazon rainforest, and with an isolated population in the Atlantic Forest, from the north of the state of Ceará to the south of the state of Rio de Janeiro, Brazil. We present information about the reproductive biology of *L. muta* from a large series of samples gathered in southern Bahia. Mature males *L. muta* are larger than females. Degree of sexual size dimorphism (SSD) is 0.16. Vitellogenetic follicles and eggs are not distributed equally throughout the year. Clutch size was 3–14 oviductal eggs and was positively correlated with female SVL. Testes volume and ductus deferens diameter in mature individuals did not vary significantly throughout the year. The reproductive pattern of *L. muta* differs from that of the phylogenetically related genera because *L. muta* is oviparous, and has a discontinuous cycle and reproductive synchrony between the sexes.

Keywords: fecundity, oviparity, reproductive cycle, South America.

Resumo

Biologia reprodutiva da surucucu *Lachesis muta* (Serpentes: Viperidae) da Floresta Atlântica brasileira. O gênero Neotropical *Lachesis* Daudin, 1803 inclui serpentes restritas às florestas úmidas da América do Sul e Central. A espécie *Lachesis muta* é mais amplamente distribuída, ocorrendo em toda a Floresta Amazônica e com uma população isolada na Mata Atlântica, desde o norte do estado

Received 10 June 2014.

Accepted 24 November 2014.

Distributed December 2014.

do Ceará, ao sul do estado do Rio de Janeiro, Brasil. Apresentamos informações sobre a biologia reprodutiva de *L. muta* a partir da análise de uma grande série de amostras recolhidas no sul da Bahia. Os machos maduros de *L. muta* foram maiores que as fêmeas. O grau de dimorfismo sexual (SSD) foi 0,16. Folículos vitelogênicos e ovos não foram distribuídos homogeneamente ao longo do ano. O tamanho da ninhada foi 3–14 ovos e positivamente correlacionado com o CRC das fêmeas. O volume testicular e o diâmetro dos ductos deferentes nos indivíduos maduros não apresentaram variação significativa ao longo do ano. O padrão reprodutivo de *L. muta* difere daquele de gêneros filogeneticamente relacionados, pois essa espécie é ovípara, tem ciclo descontínuo e sincronia reprodutiva entre os sexos.

Palavras-chave: América do Sul, ciclo reprodutivo, fecundidade, oviparidade.

Introduction

The Neotropical genus *Lachesis* Daudin, 1803 includes snakes restricted to the humid forests of Central and South America (Vial and Porras-Jímenez 1967, Campbell and Lamar 2004). The four recognized species (Campbell and Lamar 2004, Fernandes *et al.* 2004) occur in relatively restricted regions. *Lachesis stenophrys* occurs on the Atlantic slope of Costa Rica, Panama, and possibly southern Nicaragua. *Lachesis melanocephala* occupies southwestern Costa Rica and possibly occurs in the western part of Panama on the Pacific slopes and *Lachesis achrochorda* is distributed on both the Atlantic and Pacific slopes of eastern Panama, northwestern Colombia (Atlantic coast) to northwestern Ecuador. The fourth species, *Lachesis muta*, is the most widely distributed, occurring in the equatorial forests of Brazil, Venezuela, Trinidad, Guyana, Suriname, French Guiana, Ecuador, Peru, and Colombia, in addition to an isolated population located in the Atlantic Forest, from the north of the state of Ceará to the south of the state of Rio de Janeiro (Campbell and Lamar 2004).

Lachesis, which can exceed 3 m in length, is the largest known viperid (Campbell and Lamar 2004). Except for *Bothrocophias colombianus*, they also are the only egg-laying viperids in the Neotropics (Greene and Santana 1983, Campbell and Lamar 2004). Observations made on captive,

as well as wild *Lachesis*, suggest that these vipers protect their clutches (Ripa 1994, Melgarejo *et al.* 1999, Campbell and Lamar 2004, Souza 2007). Despite the peculiar biological attributes of *Lachesis*, the little information that exists about reproduction in the genus mainly is based on captive Central American species (Campbell and Lamar 2004 and related work). In Brazil, publications on the ecology of *L. muta* focus on the Amazonian populations (Beebe 1946, Cunha and Nascimento 1978, 1993), and the scarce information available on the breeding populations in the Atlantic Forest references records of oviparity in species (Amaral 1926) and births in captivity (Campbell and Lamar 2004, Souza 2007).

It is important to document the reproductive biology of the population of *Lachesis muta* in the Atlantic Forest because this population was listed as vulnerable by the IUCN (2012) owing to habitat loss caused by the massive destruction of this ecosystem; currently, only 78% of the original area remains intact. Recently, experts asserted that this species is endangered in Bahia as a result of habitat restrictions (A. J. S. Argôlo 2014, pers. comm.). Thus, populations of *L. muta* inhabiting these forest fragments are at risk for survival because of their isolation. Reproductive studies of these oviparous viperids are critical to formulate successful strategies for captive management and species conservation *in situ*.

Nearly all Neotropical snake reproductive studies are based primarily on the morphology of the gonads (Zug *et al.* 1979, Pizzatto and Marques 2002, Pinto and Fernandes 2004, Alves *et al.* 2005, Marques and Muriel 2007). In males, gonadal variation can only be assessed by histological examination of the testis and deferens duct (Mathies 2011). Herein we present the first information about the reproductive biology of *L. muta* from regions of south of Bahia, based on morphological and histological techniques.

Materials and Methods

We examined 139 specimens (Appendix I) of *Lachesis muta* that were killed between 1989 and 2007 in farming areas in a strip of the Atlantic Forest in southern Bahia and subsequently sent to the Coleção Zoológica Gregório Bondar (CZGB) of the Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC) in Ilhéus, Bahia, Brazil. The climate in the study region is Type Af (Koeppen 1948) —i.e., hot and humid, without a well-marked dry season (Roeder 1975). Winter (June–September) temperatures average 18°C and summer (December–March) temperatures average 26°C. The average annual temperature is 26°C. The humidity reaches 78% and exceeds 80% near the coast. Rain falls throughout most of the year, but it is concentrated from March–August. The annual average rainfall is 1500 mm, but can be as much as 2000 mm near the coast owing to high temperature, humidity, and condensation (Sá *et al.* 1987).

The following variables were measured in each specimen: (1) head diameter (HD); (2) head length (HL, measured with calipers); (3) snout-vent length (SVL); (4) tail length (TL) (measured with measuring tape in mm); (5) largest and smallest testicle diameter (e.g., Saint Girons 1982); (6) ductus deferens diameter in three regions (proximal, middle and distal); (7) diameter of the largest follicle and egg (measured with calipers); (8) number of follicles and eggs

in the oviducts; and (9) reproductive condition (recorded as mature or immature). Testicular volume was calculated using the ellipsoid volume formula $TV = 4\pi/3ab^2$, where a = largest radius, and b = smallest radius (see James and Shine 1985). We used “*t*-tests” to compare the SVL and TL of males and females.

We considered mature males those with enlarged testes, and opaque and convoluted ductus deferens, indicating the presence of sperm (Shine 1977a,b, 1980, 1982) and mature females those with vitellogenic follicles or mature oviducts (Shine 1987). The developmental stages of follicles were based on those of Dessauer and Fox (1959), Aldridge (1979) and Zug *et al.* (1979). Because the oviducts vary during the breeding season (e.g., Dessauer and Fox 1959, Fox 1956, Blackburn 1998, Janeiro and Cinquini 2004), their condition could be used to infer maturity.

The analysis of the female reproductive cycle was based on the number of mature females collected each month, and the number of females with vitellogenic follicles or eggs in the same period, using the Kruskal-Wallis test. The male reproductive cycle was inferred through an ANOVA that evaluated the diameter of the ductus deferens and the residuals resulting from the regression of the SVL and the testicular volume each month.

Reproductive organs were prepared histologically and examined under a light microscope to complete our morphometric data. Three females with vitellogenic follicles, and one or two mature males were examined each month. We removed the distal region of the ductus deferens and the distal lobe of the right testicle, and the posterior oviduct and vagina in females. The oviduct was divided into the vagina, posterior and anterior uterus, and infundibulum (e.g., Blackburn 1998). The preparation of tissues for a histological analysis of testicles, deferens duct, and oviducts followed routine histological techniques (Kiernan 1990). Sections of 7 µm were made and stained with Harris hematoxylin and eosin. Light microscopy was

used to detect the presence of sperm in the oviducts and vagina of females. The spermatogenetic stages were classified according to Goldberg and Parker (1975). The type of reproductive cycle was characterized according to Saint-Girons (1982) and Mathies (2011).

Egg laying, hatching periods, clutch size, and SVL of hatchlings were obtained from preserved specimens, and from eggs and newborns observed in nature. We used correlation analyses to relate female SVL with clutch size and female SVL with the diameter of the follicles and egg length. We include numeric size instead of clutch mass because we were unable to remove the oviductal eggs of museum specimens.

Degree of sexual size dimorphism (SSD) was calculated by dividing the mean adult SVL of the larger sex by the mean adult SVL of the smaller sex minus 1 (Shine 1994). All tests were performed using Statistica software (Ver. 8.0), assuming $p < 0.05$ for significance.

Results

Sexual Maturity and Body Size

The smallest mature female has a SVL of 1118 mm, whereas the SVL of the smallest mature male is 1292 mm. Mature female SVL ranges from 1118–2200 mm (mean = 1604.61 ± 318.97 , $N = 65$), and TL from 55–180 mm (mean = 136.87 ± 20.48 , $N = 65$). Mature male SVL ranges from 1292–2375 mm (mean = 1731.20 ± 389.66 , $N = 74$), and TL from 55–180 mm (mean = 150.37 ± 22.18 , $N = 74$). Mature female SVL ($t = 3.95$; $p < 0.0492$) and TL ($t = 2.4$ $p < 0.0006$) is significantly shorter male SVL. The degree of sexual size dimorphism (SSD) is 0.16.

Female Reproductive Cycle

Vitellogenic follicles and eggs do not vary significantly during the year (Kruskal-Wallis test = 5.6404; $p = 0.8962$; Figure 1). We observed females with vitellogenic follicles from April–

August (fall/winter) and in December (late spring and early summer); follicles in primary vitellogenesis were observed from January–April (between summer and fall) and October–November (spring), and follicles in secondary vitellogenesis from June–July (late fall and early winter), and in December at the beginning of summer. We observed as many as 48.22 ± 5.67 mm in March ($N = 1$), August ($N = 2$), September ($N = 3$) and December ($N = 1$), summer, winter, and spring respectively and shelled oviductal eggs in January ($N = 3$), November ($N = 1$) and December ($N = 1$).

We collected a female (CZGB 3123) with 14 developing eggs in her oviducts in August 1994. A second female (CZGB 6669) was captured in October 1995 with another individual (possibly a male); these snakes were held in captivity, and the female laid nine eggs on the 8th and 9th of January 1996. Following incubation, the eggs produced seven hatchlings. We captured another female (CZGB 8041) in October 1998 and held her captive; she laid six eggs on 30 November 1998. One egg was stuck near the cloaca and removed on 4 December 1998; three other eggs remained in the oviduct.

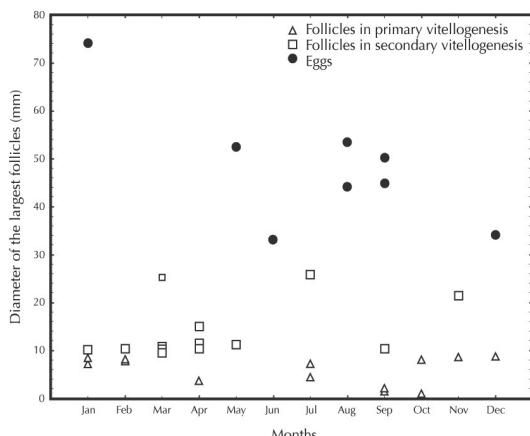


Figure 1. Seasonal variation in the diameter of the largest ovarian follicles and eggs in adult females (SVL ≥ 1118 mm) of *Lachesis muta*.

We collected eggs and hatchlings in the wild from January–April and young (SVL < 500 mm; average size of a hatchling) from March–June. These dates indicate that oviposition occurs in the summer and early fall.

Light microscopy of a female (CZGB 7354) captured in early winter (June) with follicles in secondary vitellogenesis revealed no sperm in the posterior region of the uterus. Females (CZGB 299 and 2965) killed in late summer (March) and early fall (April) with follicles in primary vitellogenesis also lacked sperm in the vagina or in the posterior end of the uterus (Figure 2).

Fecundity

Lachesis muta clutch sizes are 3–14 eggs (5.83 ± 1.48 ; $N = 12$) and not positively correlated with female SVL ($r^2 = 0.0172$; $p = 0.5146$). Egg length is 64.07 ± 21.2 mm (44.2–92.5 mm, $N = 54$ eggs from 12 clutches, from dissected specimens). The largest follicle measured was 34.3 mm and the smallest egg was 44.2 mm. Female SVL is significantly correlated

with the diameter of the follicles and with egg length ($r^2 = 0.207$, $p = 0.0047$, $N = 55$). The hatchlings are 505 ± 14.4 mm (492–614, $N = 11$) long, approximately 31.5% of maternal SVL.

Male Reproductive Cycle

The testicular volume ($F = 0.84887$, $p = 0.58017$, $N = 60$; Figure 3) and deferens duct diameter in mature individuals ($F = 0.68370$, $p = 0.71692$, $N = 60$) shows no significant variation during the year.

The light microscopic examinations revealed that in the summer, the germinal epithelium of seminiferous tubules is large (Figure 4A) and in spermiogenesis. At the end of summer, starts the recrudescence phase begins (Figure 4B) and continues throughout the fall. During this period two or three layers of cells are present (mainly spermatogonia) and the tubule lumen becomes empty. In the winter (Figure 4C, D), the epithelium of the seminiferous tubules undergoes spermiogenesis and in late spring, recrudescence occurs. Deferens ducts contain spermatozoa in all seasons (Figure 4E).

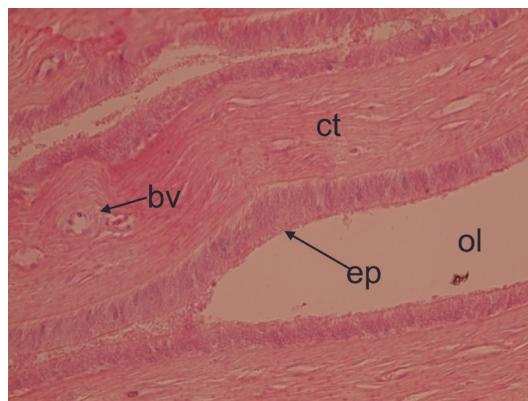


Figure 2. Transverse paraffin sections of the vagina of *Lachesis muta* in the early winter. ep: epithelium; ct: connective tissue; ol: oviductal lumen; bv: blood vessels.

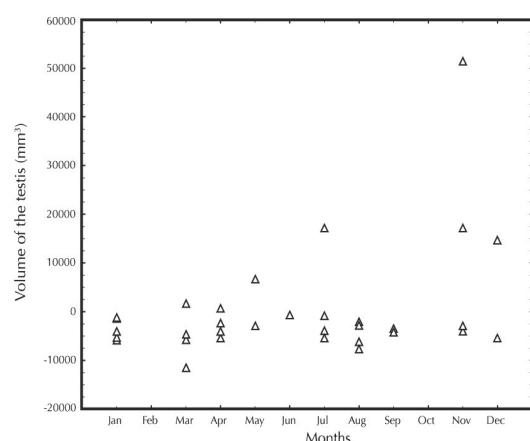


Figure 3. Relative volume of the testes in mature males of *Lachesis muta* throughout the year.

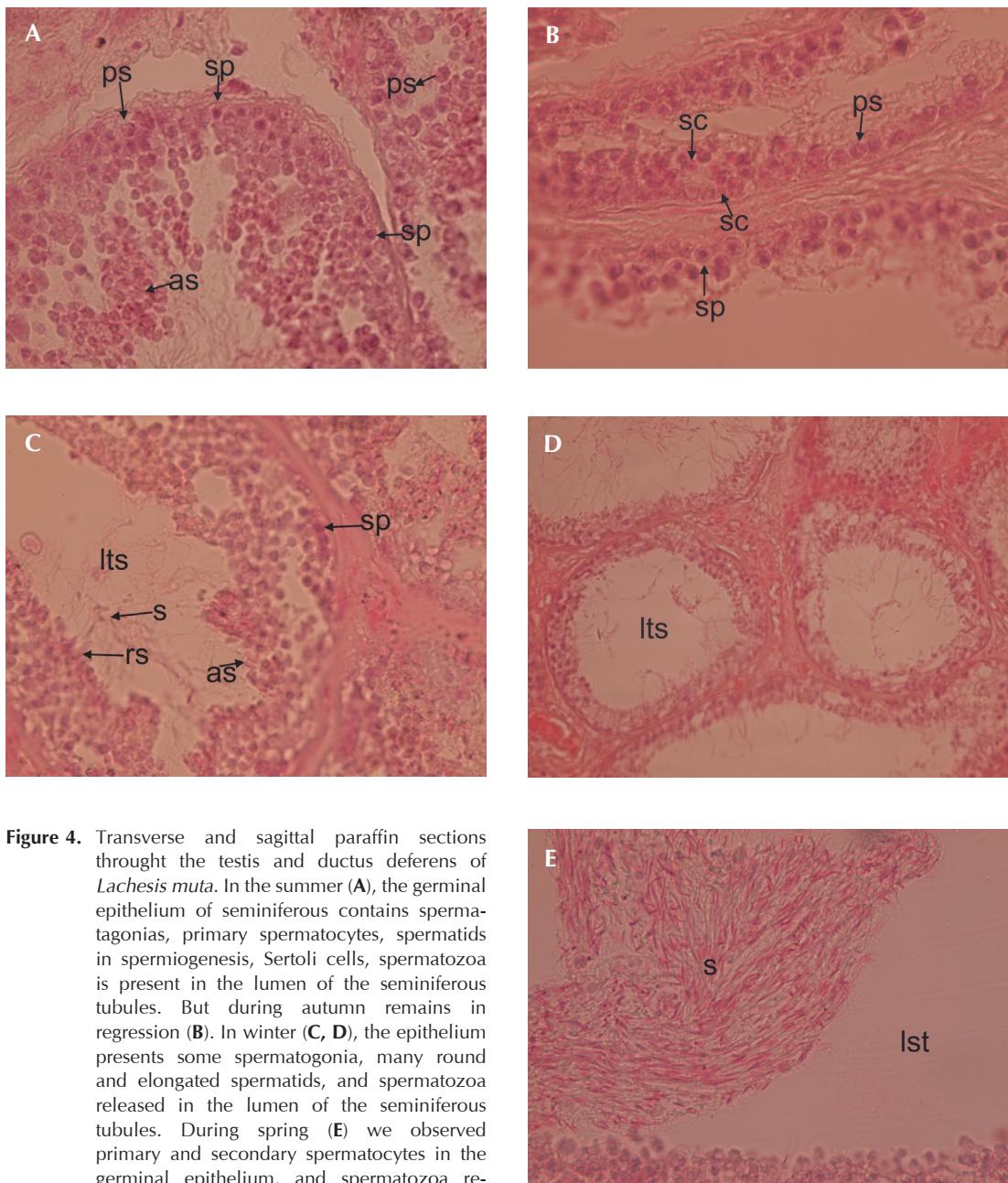


Figure 4. Transverse and sagittal paraffin sections through the testis and ductus deferens of *Lachesis muta*. In the summer (**A**), the germinal epithelium of seminiferous contains spermatagonias, primary spermatocytes, spermatids in spermiogenesis, Sertoli cells, spermatozoa is present in the lumen of the seminiferous tubules. But during autumn remains in regression (**B**). In winter (**C, D**), the epithelium presents some spermatogonia, many round and elongated spermatids, and spermatozoa released in the lumen of the seminiferous tubules. During spring (**E**) we observed primary and secondary spermatocytes in the germinal epithelium, and spermatozoa released in the lumen of the seminiferous tubules. sp: spermatogonia; ps: primary spermatocytes; ss: secondary spermatocytes; as: elongated spermatids; rs: round spermatid; s: spermatozoa; sc: Sertoli cells; lst: lumen of seminiferous tubules.

Discussion

In many ophidian species, females are larger than males (Shine 1994, 2003, Hartmann *et al.* 2004, Alves *et al.* 2005). However, unlike other viperids (Solorzano and Cerdas 1989, Hartmann *et al.* 2004), male *Lachesis muta* are longer than females. Larger body size is advantageous for males in species that perform ritual combat during courtship to determine dominant relationships (Shine 1978, 1994, 2003). Possible ritual combat has been observed among males of *L. melanocephala* (Ripa 1994). According to the author, combat does not seem to be necessary to encourage sexual activity in the male. The degree of sexual dimorphism observed in the present study is less than 0.25, and snakes with this pattern demonstrate combat between males (Shine 1994).

With regard to clutch size, Melgarejo *et al.* (1999) recorded 15 and 13 eggs in two clutches from the same captive female of *Lachesis muta* from the state of Alagoas. Boyer *et al.* (1989) reported 10 eggs in a captive female of *L. muta* from Trinidad. Ripa (1994, 2004) studied litters of 13 and 16 eggs in *L. melanocephala* and *L. stenophrys*, respectively, held in captivity, and Chacon and Valverde (2004) reported *L. stenophrys* to have laid 11 eggs within 3 mo of having been captured in the wild. According to Campbell and Lamar (2004), the average litter size in *Lachesis* probably is 6–11 eggs. Based on a larger sample, the present study reported clutches from 3–14 eggs in the *L. muta* population of southern Bahia, with an average of 5.83 eggs per clutch; this average is not correlated to the female SVL. This pattern may be related to the fact that female *L. muta* mature with a smaller SVL than that of the male, and according to Shine (2003), female body size determines clutch mass only if the snake delays reproduction until it reaches a larger body size.

A contrasting pattern has been observed in species in which mature females are longer than males (e.g., Shine 1994, Pizzatto 2002, Hartmann *et al.* 2004, Alves *et al.* 2005, Pleguezuelos *et al.*

2007, Pinto *et al.* 2010) because larger body size confers a selective advantage for the female by increasing her ability to contain eggs or neonates (Shine 1994, 2003). Although the fecundity of females could be determined by the stored energy (body fat), we did not analyze the correlation between corporeal mass and litter size. However, we found (unpubl. data) the five levels of abdominal fat (classification proposed by Pleguezuelos and Feriche 2006) in the females at all stages of vitellogenesis and containing oviductal eggs.

With respect to egg size, Carrillo (1970) reported that the eggs of Peruvian *Lachesis muta* are as large as 10 cm long. The maximum size of 92.5 mm that we recorded exceed that of *L. melanocephala* (88 mm) (Ripa 1994, 2004), *L. stenophrys* (76 and 79.9 mm) (Ripa 1994, 2004, Chacon and Valverde 2004), and *L. muta* from the Atlantic Forest (69.9 mm) (Melgarejo *et al.* 1999). The egg length in *L. muta* is not positively correlated with female SVL.

The reproductive strategy of *Lachesis muta* in the Atlantic Forest of southern Bahia encompasses a seasonal reproductive cycle. Mating can occur in winter and summer, once we find follicles in secondary vitellogenesis in fall, winter and summer, and eggs in the summer, winter and spring. Light microscopy of the posterior oviduct revealed no sperm in the vagina of CZGB 7354, which was in secondary vitellogenesis in early winter, and CZGB 299 and 2965, which were in primary vitellogenesis in late summer. However, our data are insufficient to infer that female *L. muta* store sperm.

Oviposition occurs mostly from November–February, the warmest time of the year and the most suitable for embryonic development, whereas births occur from January–April, between the hottest period and the beginning of the season with the greatest concentration of rain. The only two published records of copulation and oviposition in this species are consistent with our data. From one female (not 2, as reported in Campbell and Lamar 2004) captured in the state of Alagoas, Melgarejo *et al.*

(1999) recorded mating on 22 August and egg-laying on 06 December 1995. In Itacaré, Souza (2007) studied a pair in copulation in September and subsequent oviposition in December. Although the annual temperature variation in the Atlantic Forest of southern Bahia falls within a range of 7°C and rainfall is distributed throughout most of the year (e.g., Sa *et al.* 1987), oviposition and births of *Lachesis muta* are concentrated in the warmer months, whereas secondary vitellogenesis and spermatogenesis occur in the colder months, demonstrating determined seasonality in the reproductive cycle of the species. These data agree with those postulated by Mathies (2011), and contradict those assumed by Souza (2007) for *L. muta*.

With respect to congeners of *Lachesis muta*, nothing is known about *L. acrochorda* reproduction; however, mating in captivity by *L. stenophrys* and *L. melanocephala* was recorded in January, February, and March, with oviposition in May and births in July (Ripa 1994, 2004). In Costa Rica, Chacon and Valverde (2004) recorded *L. stenophrys* laying eggs in August and births occurring in November. Although these records are insufficient for us to ascertain the reproductive cycle of these species, the month for births falls during the rainy season in Central America where the species occur.

The length of the mating season and the timing of egg laying and births, as well as others features, may be related to the local environment of *Lachesis muta* in the same way as it is in other tropical snakes (e.g., Vitt and Vangilder 1983). In *Bothrops pubescens*, a viviparous viperid in southeast Brazil, the reproductive cycle also is related to warmer periods of the year (Hartmann *et al.* 2004), although the seasons in this region are more distinct. In tropical Colubridae (Pizzatto and Marques 2002, Pinto *et al.* 2010) and Elapidae (Marques 1996b), which have seasonal reproductive cycles, the breeding season is concentrated in the hot, rainy months.

Testicular volume is lower between January and April—i.e., the time for births, when there is

no courtship activity. In contrast, testicular volume increases in winter and summer during the peak of vitellogenesis in females. Spermatogenesis corresponds to an increase in testicular volume, with a peak in the coldest period of the year (June) and another in the summer (January), demonstrating synchrony between secondary vitellogenesis in females and spermatogenesis in males. The timing of vitellogenesis and spermatogenesis relative to the mating period between winter and early summer suggests that the reproductive cycle in *Lachesis muta* should be classified as pre-nuptial (Saint-Girons 1982). However, according to the recent classification of the reproductive cycles of tropical snakes (Mathies 2011), the cycle in *L. muta* is discontinuous. Owing to the synchrony of the mating season and spermatogenesis, perhaps male *L. muta* have no need to store sperm.

According to the most recent phylogeny of Viperidae (e.g., Gutberlet and Harvey 2004, Castoe and Parkinson 2006), *Lachesis* is closely related to *Bothrocophias* and *Bothrops* (*sensu* Carrasco *et al.* 2012). The reproductive cycles of several species of *Bothrops* shows marked seasonality, with variations conditioned by climate (e.g., Schuett 1992; Solorzano and Cerdas 1986, Alves *et al.* 1998, Alves *et al.* 2000, Hartmann *et al.* 2004, Almeida-Santos and Solomão 2002). The reproduction pattern of *L. muta* differs from those related taxa in having discontinuous cycles, reproductive synchrony between the sexes, and being oviparous.

Acknowledgments

We would like to thank Guisla Boehs for access to the Laboratório de Histologia Animal of the Universidade Estadual de Santa Cruz, Mariane Aguiar for her help in preparing the histological slides, Linda Trueb for valuable comments on the manuscript and English review, Marcos F. Vila Nova for his help in the selection of specimens and José Montival Alencar Jr. and Alvaro Coelho B. de Alencar for assisting with the figures.

References

- Aldridge, R. D. 1979. Female reproductive cycles of the snakes *Arizona elegans* and *Crotalus viridis*. *Herpetologica* 35: 256–261.
- Almeida-Santos, S. M. and M. G. Salomão. 2002. Reproduction in Neotropical pitvipers, with emphasis on species of the genus *Bothrops*. Pp. 307–328 in G. Schuett, M. Höggren, and H. W. Greene (eds.), *Biology of the Vipers*. Utah. Eagle Mountain Publishing.
- Almeida-Santos, S. M., I. L. Laporta-Ferreira, M. M. Antoniazzi, and C. Jared. 2004. Sperm storage in males of the snake *Crotalus durissus terrificus* (Crotalinae: Viperidae) in southeastern Brazil. *Comparative Biochemistry, Physiology. Part A: Molecular and Integrative Physiology* 139: 169–174.
- Alves, M. L. M., M. L. Araujo, and E. Caberlon. 1998. Atividade reprodutiva de *Bothrops neuwiedi* em cativeiro (Serpentes, Viperidae). *Iheringia, Série Zoologia* 84: 185–191.
- Alves, M. L. M., M. L. Araujo, and A. A. Witt. 2000. Aspectos da biologia reprodutiva de *Bothrops jararaca* em cativeiro (Serpentes, Viperidae). *Iheringia, Série Zoologia* 89: 187–192.
- Alves, F. Q., A. J. S. Argolo, and J. Jim. 2005. Biologia reprodutiva de *Dipsas neivai* Amaral e *D. catesbyi* (Sentzen) (Serpentes, Colubridae) no sudeste da Bahia, Brasil. *Revista Brasileira de Zoologia* 22: 573–579.
- Amaral, A. 1926. On the oviparity of *Lachesis muta* Daudin, 1803. *Copeia* 149: 93–94.
- Beebe, W. 1946. Field notes on the snakes of Kartabo, British Guiana, and Caripito, Venezuela. *Zoologica* 31: 11–52.
- Blackburn, D. G. 1998. Structure, function and evolution of the oviducts of squamate reptiles, with special reference to viviparity and placentation. *Journal of Experimental Zoology* 282: 560–617.
- Boyer, D. M., L. A. Mitchell, and J. B. Murphy. 1989. Reproduction and husbandry of the Bushmaster *Lachesis muta muta* at the Dallas Zoo. *International Zoo Yearbook* 28: 190–194.
- Campbell, J. A. and W. W. Lamar. 2004. *The Venomous Reptiles of the Western Hemisphere*. Vol. I. Ithaca. Comstock Publishing Associates. 976 pp.
- Carrasco, P. A., C. I. Mattoni, G. C. Leynaud, and G. J. Scrocchi. 2012. Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). *Zoologica Scripta* 41: 109–124.
- Carrillo, D. E. 1970. Contribución al conocimiento de los reptiles del Perú. *Publicaciones del Museo de Historia Natural Javier Prado. Serie A. Zoología* 22: 1–64.
- Castoe, T. A. and C. L. Parkinson. 2006. Bayesian mixed models and the phylogeny of pitvipers (Viperidae, Serpentes). *Molecular Phylogenetics and Evolution* 39: 91–110.
- Chacon, D. and D. Valverde. 2004. *Lachesis stenophrys* (Bushmaster). Reproduction. *Herpetological Review* 35: 68.
- Cunha, O. R. and F. P. Nascimento. 1978. Ofídios da Amazônia X. As cobras da região leste do Pará. *Boletim do Museu Paraense Emílio Goeldi* 31: 1–218.
- Cunha, O. R. and F. P. Nascimento. 1993. Ofídios da Amazônia. As cobras da região leste do Pará. *Boletim do Museu Paraense Emílio Goeldi* 9: 1–191.
- Dessauer, H. C. and W. Fox. 1959. Changes in ovarian follicle composition with plasma levels of snakes during estrus. *American Journal of Physiology* 197: 360–366.
- Fernandes, D. S., F. L. Franco, and R. Fernandes. 2004. Systematic revision of the genus *Lachesis* Daudin, 1803 (Serpentes, Viperidae). *Herpetologica* 60: 245–260.
- Fitch, H. S. 1982. Reproductive cycles in tropical reptiles. *Occasional Papers of the Museum of Natural History, University of Kansas* 96: 1–53.
- Fox, W. 1956. Seminal receptacles of snakes. *Anatomical Record* 124: 519–540.
- Gutberlet, R. L. and M. B. Harvey. 2004. Phylogenetic relationships of new world pitvipers as inferred from anatomical evidence. Pp. 51–68 in G. Schuett, M. Höggren, and H. W. Greene (eds.), *Biology of the Vipers*. Utah. Eagle Mountain Publishing.
- Greene, H. W. and M. A. Santana. 1983. Field studies of hunting behavior by bushmasters. *American Zoologist* 23: 897.
- Goldberg, S. R. and W. S. Parker. 1975. Seasonal testicular histology of the colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. *Herpetologica* 31: 317–322.
- Hartmann, M. T., O. A. V. Marques, and S. M. Almeida-Santos. 2004. Reproductive biology of the southern Brazilian pitviper *Bothrops pubescens* (Serpentes, Viperidae). *Amphibia-Reptilia* 25: 77–85.
- IUCN. 2014. IUCN Red List of Threatened Species. Version 2012.2. Electronic Database accessible at <http://www.iucnredlist.org>. Captured on 29 January 2014.

- James, C. and R. Shine. 1985. The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia* 67: 464–474.
- Janeiro-Ciniquini, T. 2004. Variação anual do sistema reprodutor de fêmeas de *Bothrops jararaca* (Serpentes, Viperidae). *Iheringia, Série Zoologia* 94: 325–328.
- Jones, R. E., R. R. Tokarz, F. T. LaGreek, and K. T. Fitzgerald. 1976. Endocrine control of clutch size in reptiles: VI. Patterns of FSH-induced ovarian stimulation in adult *Anolis carolinensis*. *General and Comparative Endocrinology* 30: 101–116.
- Koeppen, W. 1948. *Climatología: con un estudio de los climas de la Tierra*. México. Fondo de Cultura Económica. 478 pp.
- Kiernan, J. A. 1990. *Histological and Histochemical Methods: theory and practice*. New York. Pergamon Press. 513 pp.
- Marques, O. A. V. 1996. Reproduction, seasonal activity and growth of the coral snake, *Micrurus corallinus* (Elapidae), in the southeastern Atlantic Forest in Brazil. *Amphibia-Reptilia* 17: 277–285.
- Marques, O. A. V. and A. P. Muriel. 2007. Reproductive biology and food habitats of the swamp racer snake *Mastigodryas bifossatus* (Colubridae) from southeastern South America. *Journal of Herpetology* 17: 104–109.
- Mathies, T. 2011. Reproductive cycles of tropical snakes. Pp. 511–550 in R. D. Aldridge and D. M. Sever (eds.), *Reproductive Biology and Phylogeny of Snakes*. Boca Raton. CRC Press.
- Melgarejo, A. R., A. C. M. Cunha, and Aguiar A. S. 1999. Reprodução de *Lachesis muta rhombata* Wied, 1824 (Serpentes: Viperidae). V Congreso Latinoamericano de Herpetología. Montevideo, Uruguay. Publicación extra, Museo Nacional de Historia Natural.
- Pleguezuelos, J. M., X. Santos, J. C. Brito, X. Parellada, G. A. Llorente, and S. Fahde. 2007. Reproductive ecology of *Vipera latastei*, in the Iberian Peninsula: Implications for the conservation of a Mediterranean viper. *Zoology* 110: 9–19.
- Pinto, R. R. and R. Fernandes. 2004. Reproductive biology and diet of *Liophis poecilogyrus poecilogyrus* (Serpentes: Colubridae) from southeastern Brazil. *Phyllomedusa* 3: 9–14.
- Pinto R. R., O. A. V. Marques, and R. Fernandes. 2010. Reproductive biology of two sympatric colubrid snakes, *Chironius flavolineatus* and *Chironius quadricarinatus*, from the Brazilian Cerrado domain. *Amphibia-Reptilia* 31: 463–473.
- Pizzatto, L. and O. A. V. Marques. 2002. Reproductive biology of the false coral snake *Oxyrhopus guibei* (Colubridae) from southeastern Brazil. *Amphibia-Reptilia* 23: 495–504.
- Ripa, D. 1994. The reproduction of the Central American Bushmasters (*Lachesis muta melanoleuca* and *Lachesis muta stenophrys*) for the first time in captivity. *Vivarium* 5: 36–37.
- Ripa, D. 2004. The Bushmasters (Genus *Lachesis* Daudin, 1803): Morphology in Evolution and Behaviour. CD-ROM. Wilmington, Ripa Ecologica.
- Roeder, M. 1975. Diagnóstico sócio-econômico da região caqueira. Vol. 4. Reconhecimento climatológico. Ilhéus. Convênio IICA/CEPLAC. 89 pp.
- Sá, D. F., H. A. Almeida, I. F. Silva, and A. C. Leão. 1987. Fatores edafo-climáticos seletivos ao zoneamento da cacaueira. *Revista Theobroma* 12: 169–186.
- Saint-Girons, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica* 38: 5–16.
- Schuett, G. W. 1992. Is long-term sperm storage an important component of the reproductive biology of temperate pitvipers? Pp. 169–184 in J. A. Campbell and E. D. Brodie Jr. (eds.), *Biology of the Pitvipers*. Austin. Selva.
- Shine, R. 1977a. Reproduction in Australian elapid snakes II. Female reproductive cycles. *Australian Journal of Zoology* 25: 655–666.
- Shine, R. 1977b. Reproduction in Australian elapid snakes I. Testicular cycles and mating seasons. *Australian Journal of Zoology* 25: 647–653.
- Shine, R. 1978. Sexual size dimorphism and male combat in snakes. *Oecologia* 33: 269–277.
- Shine, R. 1980. Comparative ecology of three Australian snake species of the genus *Cacophis*. *Copeia* 1980: 831–838.
- Shine, R. 1982. Ecology of the Australian elapid snake *Echiopsis curta*. *Journal of Herpetology* 16: 388–393.
- Shine, R. 1987. Food habits and reproductive biology of Australian snakes of the genus *Hemiaspis* (Elapidae). *Journal of Herpetology* 21: 71–74.
- Shine, R. 1994. Sexual size dimorphism in snakes revisited. *Copeia* 1994: 326–345.
- Shine, R. 2003. Reproductive strategies in snakes. *Proceedings of the Royal Society of London B* 270: 995–1004.

- Souza, R. C. G. 2007. Reproduction of the Atlantic Bushmaster (*Lachesis muta rhombeata*) for the first time in captivity. *Bulletin of the Chicago Herpetological Society* 47: 41–43.
- Solórzano, A. and L. Cerdas. 1986. A new subspecies of the bushmaster, *Lachesis muta*, from southeastern Costa Rica. *Journal of Herpetology* 20: 463–466.
- Solórzano, A. and L. Cerdas. 1989. Reproductive biology and distribution of the terciopelo, *Bothrops asper* Garman (Serpentes: Viperidae) in Costa Rica. *Herpetologica* 45: 444–450.
- Vial, J. L. and J. M. Jimenez-Porras. 1967. The ecogeography of the bushmaster, *Lachesis muta*, Central America. *American Midland Naturalist* 78: 182–187.
- Vitt, L. J. and L. D. Vangilder. 1983. Ecology of a snake community in Northeastern Brazil. *Amphibia-Reptilia* 4: 273–296.
- Zug, G. R., B. S. Hedges, and S. Sunkel. 1979. Variation in reproductive parameters of three Neotropical snakes, *Coniophanes fissidens*, *Dipsas catesbyi* and *Imantodes cenchoa*. *Smithsonian Contributions in Zoology* 300: 1–20.

Appendix I. Specimens examined.

Arataca (MZUESC 2162–63, 4263), Camacá (CZGB 5656, MZUESC 4395), Camamu (CZGB 1030, 1276, 2965, 4524, 5442–43, 6669, 7349, 7802, 8040, 8658–59, 9318, MZUESC 314, 325, 327, 619, 622, 625, 1081, 1402, 1404–05, 1407, 1533–34, 3011–12, 3724, 4859), Ibicarai (CZGB 5359, MZUESC 299, 1053), Ibirataia (CZGB 8538), Igrapiúna (MZUESC 3941), Ilhéus (CZGB 262, 276, 402, 1157, 1741, 2069, 2084, 2085, 2319, 2669, 2684, 2845, 2913, 2916, 3026, 3068, 3031, 3123, 3971, 3955, 3991, 4071, 4096, 4550, 4553, 4648, 5029, 5174, 5599, 6198, 6603, 7344, 7585, 9313, 9355, MZUESC 110, 618, 710–11, 1210, 1402, 2001, 2575, 2683–85, 3717, 3794, 3797, 4055, 4094, 7344), Itacaré (CZGB 288, 550, 1603, 3316, 4321, 6149, MZUESC 3917–18, 3936), Itapebi (MZUESC 778), Itamaraju (CZGB 9107), Ituberá (CZGB 7354, 7429–30, 7431, 7803, 8400, 8041, 9356, MZUESC 1079–80, 1429, 3013–15), Medicilândia (CZGB 3068), Mutuípe (MZUESC 1472, 2968–69), Porto Seguro (CZGB 3689), Una (CZGB 1528, 4617, MZUESC 536), Uruçuca (CZGB 1128), Valença (CZGB 1138, 1203, 1714, 3850).

Editor: Kate Jackson