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Contribution to the Reproductive Biology of *Bothrops erythromelas* (Squamata: Viperidae) in the Semiarid Region of Brazil

Although oviparity is the prevalent reproductive mode among reptiles, squamates exhibit significant reproductive plasticity, with oviparous and viviparous species in numerous clades (viperids in particular, see Fenwick et al. 2012) and with different structural adaptations in both reproductive modes. In viviparous species, embryonic development occurs entirely in the oviduct, and the embryos can be fed entirely by the vitellus (lecithotrophic viviparity) or by nutrient transfer from the mother via the placenta (placentotrophic viviparity) (Yaron 1985; Stewart and Thompson 2000).

Reproductive seasonality is a widely discussed phenomenon in squamate reptiles from temperate zones, where there are marked variations in temperature and photoperiod (Marion 1982). On the other hand, a more frequent occurrence in tropical regions is an adjustment between the reproductive period and rainfall seasonality. This tendency seems to be related to food availability, which can significantly decrease during the dry season (Janzen and Schoener 1968; Ribeiro and Freire 2011). In this sense, most data on snake reproduction refer to temperate and subtropical regions. Neotropical snakes were until recently relatively little studied with regard to reproduction (Amaral 1977; Vanzolini et al. 1980; Seigel and Ford 1987). However, this scenario has changed considerably and several studies have produced a substantial amount of information, thereby significantly increasing our knowledge about the reproduction of neotropical snakes (David and Lewis 2011; Mesquita et al. 2011; Gomes and Marques 2012; Bellini et al. 2013; Figueroa et al. 2013; Marques et al. 2013; Panzera and Maneyro 2013; Siqueira et al. 2013; Sousa et al. 2014).

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The genus *Bothrops* has a biennial, seasonal reproductive cycle, exhibiting an active phase of follicular growth, mating, and gestation in one year, and a parturition phase followed by follicular quiescence in the next year (Almeida-Santos and Orsi 2002; Barros et al. 2014). The reproductive apparatus contains two ovaries and two oviducts (Gomes and Puorto 1993). Copulation occurs during the fall with a gestation between four and five months and offspring are born in the summer (Almeida-Santos and Orsi 2002). It is a widely diversified clade with origin and recent radiation in the neotropical region,

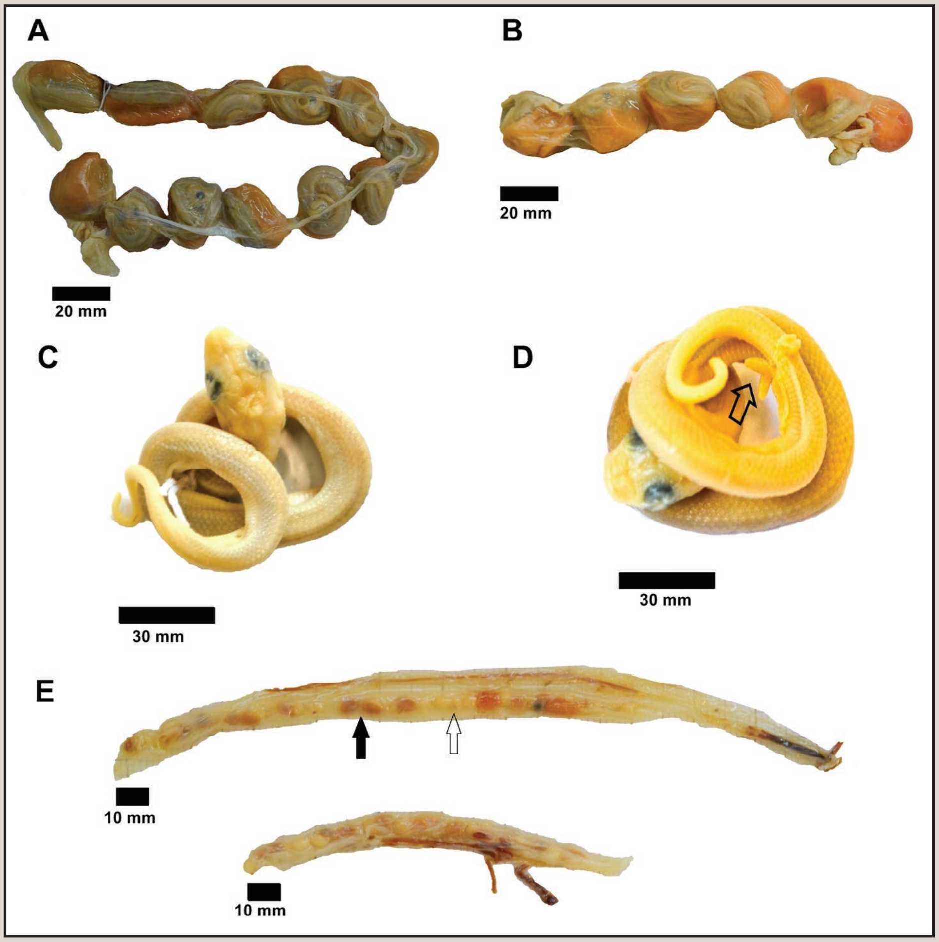

Fig. 1. Reproduction in *Bothrops erythromelas*. Right oviduct (A) with 12 embryos and left oviduct (B) with six remaining. Embryos at stage 31 of embryonic development, according to Zehr (1962): embryo with scales visible only on the body and not on the head (C), and male with hemipenes everted (arrow) visible on trunk coil (D). Right ovary (top) and left ovary (bottom) with transparent follicles without vitellus deposition (quiescent phase) (white arrow) and yellow follicles with vitellus deposition (active phase) (black arrow) (E).

and currently comprises 58 species, distributed primarily in South America (Wüster et al. 2002; Campbell and Lamar 2004; Uetz and Hošek 2014). The species *B. erythromelas*, popularly known as "jararaca-da-seca" or "jararaca-avermelhada," is widely distributed in the Caatinga (Tropical Thorny Deciduous Savanna), in addition to "Brejos de Altitude" (humid forest remnants). These forest fragments are remnants of the cycles of expansion and retraction of the humid forests that were initiated during the Pleistocene and are represented by some "islands" of humid forest that cover some isolated plateaus and mountain ranges varying from 600 to 1200 m of altitude within the Caatinga lowlands (Soares 2001). Previous studies describe the litter size (Lira-da-Silva et al. 1994), courtship and copulation behavior, and fecundity of *B. erythromelas* carried out in captivity (Silva et al. 2013). The present study aims to contribute knowledge on the reproductive aspects of *B. erythromelas* derived from nature. Here we describe the number of days in captivity until parturition, offspring morphology, and the relationship between litter size and female length.

Six *B. erythromelas* females were collected during fauna rescue efforts associated with the São Francisco River Integration Project (PISF) at different locations in the semiarid region of northeastern Brazil, two in Brejo Santo township (7.492778°S, 38,985°W), one in Jati, in Ceara state, and one each in Sertânia, Salgueiro and Petrolina (9.392778°S, 40.507778°W), in Pernambuco state.These areas are semiarid (Ab'Sáber 1974), with a short irregular rainy season concentrated in 2–3 months of each year, between January and March, and rainfall ranging between 500 and 700 mm/year. Mean annual temperatures vary from 28 to 30°C, and maximum temperatures exceed 40°C, whereas the minimum temperatures range between 17 and 20°C. Relative humidity oscillates around 30–50% in the dry season, reaching 80–90% in the rainy season (Nimer 1972).

One of the snakes collected in Brejo Santo, in December 2009, was seriously wounded and was euthanized in the field and deposited in the Herpetology Collection of the Caatinga Fauna Museum – CEMAFAUNA (voucher number MFCH 3000). The dissection was performed in December 2012 and it was found that the specimen was in the gestational phase with the presence of embryos and extra-embryonic structures. The embryonic development stage was estimated according to Zehr (1962). Biometric and body mass data were collected from these embryos. A t-test was used to determine differences in the total length of female and male embryos.

The other specimens, collected at Petrolina in August 2012, Brejo Santo in May 2013, Salgueiro in May 2013, Jati in July 2013 and Sertânia in January 2014, were held in captivity at the serpentarium of the Center for Conservation and Management of Caatinga Fauna – CEMAFAUNA, housed in 100 × 70 × 60 cm plastic boxes under a controlled temperature of 27°C until parturition. During the screening of these snakes in order to record biometric data and sex, dilation was observed in the ventral and paraventral region, suggesting pregnancy. After the offspring were born, biometric data (snout–vent length, tail length, and body mass) were measured. Spearman's correlation was used to correlate litter size with female length. Data were analyzed using SPSS version 13.0. The signicance level adopted for obtaining critical values on the tests was 0.05 (Zar 1999), and descriptive statistics are given as mean ± 1 SD.

A total of 22 offspring from five litters of *B. erythromelas* were analyzed, an average of four per litter. Offspring were born on average 157 days after the females arrived at the serpentarium, with mean length of 200.5 ± 12.61 mm (166.1–224.0 mm) and weight of 4.5 ± 1.01 g (4–6 g) (Table 1).

Twenty-one embryos were found in the dissected female, 12 in the right oviduct and nine in the left. According to Koba et al. (1970), number of eggs in the right oviduct is always greater than the left side in snakes due to its forward positioning in relation to

Table 1. Reproductive and morphological data of *Bothrops erythromelas* from the semiarid northeast of Brazil. Sex was determined only for those embryos from snake VI based on everted hemipenes, as described by Zehr (1962).

Snout–vent length of mother or dam (I–VI) (mm)	Parturition month	Number of neonates / embryos	Sex F: female, M: male	Neonate / embryo data Total length (mm)	Body mass (g)
I. 650	December 2013	3	—	193.0	6
				195.0	6
				195.0	6
				Mean 194.3	Mean 6
II. 526	January 2014	4	—	207.1	6
				207.9	4
				211.4	6
				190.1	4
				Mean 204.1	Mean 5
III. 619	January 2014	4	—	216.0	6
				217.5	6
				209.0	6
				224.0	6
				Mean 216.6	Mean 6
IV. 524	January 2014	5	—	199.5	4
				208.3	4
				206.0	4
				208.0	4
				199.5	4
				Mean 204.2	Mean 4
V. 558	January 2014	6	—	166.1	4
				199.3	4
				187.3	4
				190.7	4
				194.5	4
				190.3	4
				Mean 188.0	Mean 4
VI. 563	____	21	F	120.0	0.45
			F	122.0	0.30
			M	112.0	0.33
			F	118.0	0.43
			M	107.0	0.31
			M	107.0	0.44
			F	115.0	0.44
			M	106.0	0.39
			F	116.0	0.40
			M	107.0	0.55
			F	120.0	0.70
			F	118.0	0.73
			M	111.0	0.59
			F	122.0	0.60
			F	112.0	0.60
			F	124.0	0.61
			M	114.0	0.57
			M	125.0	0.60
			M	110.0	0.62
			F	111.0	0.56
			F	112.0	0.50
				Mean 114.7	Mean 0.51

the left. In *B. jararaca* a larger number of eggs in the right oviduct (69.92%) was observed when compared to the left (30.08%) (Janeiro-Cinquini 2004).

All embryos were enveloped by amniotic sacs separated by tissue constriction isolating each one (Fig. 1A–B). These embryos, in an advanced stage of development, were characterized as stage 31 as described by Zehr (1962). In this phase the scales are visible only on the body and not on the head (Fig. 1C). In males, the hemipenes still everted are also visible on trunk coil (Fig. 1D). The snout–vent length of females (101.2 ± 5.5 mm, 94–110 mm, N = 12) was significantly greater than that of males (92.2 ± 5.2 mm, 87–105 mm, N = 9) (t-test = 3.831, df = 17.89, P = 0.001).

The uterine wall was distended around the embryos and below it was a thin chorioallantoic membrane. It was also observed that the ovaries were asymmetrically disposed, the right more anterior and much larger than the left. Two types of follicles were found in the ovaries: transparent or whitish, without vitellus deposition, in the quiescent phase, and yellow with vitellus deposition in the active phase (Fig. 1E).

Analysis of snout–vent length in females and litter size showed no significant correlation ($r_s = -0.406$, N = 6, P = 0.425), even when disregarding the female with the maximum litter size (21 embryos) ($r_s = -0.616$, N = 5, P = 0.269). Solórzano and Cerdas (1987, 1989), studying the reproductive biology of *Crotalus durissus durissus* and *B. asper* of Costa Rica, found a significant correlation between the total length of females and the number of neonates (r = 0.579, p < 0.05 and r = 0.779, p < 0.01, respectively). Significant correlation was also observed by Janeiro-Cinquini et al. (1990) and Janeiro-Cinquini (2004) between the length of the mother and litter size in *B. jararaca* populations.

The birth and litter records of *B. erythromelas* in December and January, notably in January (four litters), indicate that summer is the preferential reproduction period for this snake, reinforcing the observations made by Almeida-Santos and Orsi (2002) and Silva et al. (2013). The birth of newborns in the wet season is a characteristic of *B. erythromelas* shared with every species of the genus *Bothrops* studied to date (Almeida-Santos and Salomão 2002). According Barros et al. (2014) this similarity in reproductive timing in *Bothrops* species may be attributed to the influence of phylogenetic inertia in the determination of reproductive events for females. The number of days held in captivity prior to parturition for the five *B. erythromelas* at the serpentarium varied from 19 to 236 days. Although these data do not represent the total gestation period, Klauber (1972) and Aldridge and Duvall (2002) argue that variation in gestational period in pitvipers may result from sperm storage, or be influenced by temperature and/or seasonal fluctuations in food availability, making it difficult to determine the standard gestation period of the species.

There was a large difference between the number of offspring in *B. erythromelas* females who laid their litters and the dissected individual (21 offspring), even though the mothers exhibited nearly the same snout–vent length, with a mean of 573 mm. This difference in litter size may be explained by climatic fluctuations at the sites of specimen occurrence, influenced by the availability of food and the nutritional status of females (Fitch 1985; Aldridge and Duvall 2002). The number of embryos here described is much larger than that mentioned in the literature. Lira-da-Silva et al. (1994) reported 11 neonates for one *B. erythromelas* female, and Barros et al. (2014) found two to 13 embryos (average = 8) in the oviducts of five females. Lira-da-Silva et al. (1994) corroborate with the opinion of Klauber (1972) who affirm that intrinsic factors (e.g., physiological) can also interfere in the fertility rate. In this respect, marked reproductive effects that sometimes result in geographic variations can occur.

Silva-Filho (2000) followed the birth of 20 offspring of *B. erythromelas* kept in captivity, observing, based on the biometric data of newborns, that this species can be included among the smaller species of the genus *Bothrops*. However, biennial cycles are common in viperids and, considering the different factors that interfere in determining reproductive frequency in snakes and the size of their litter, it is premature to confirm, based on this sample, the proportion of the population that reproduces each year. Only a more detailed study will be able to determine the proportion of *B. erythromelas* females in the reproductive state per year and the possible fluctuations in reproductive frequency, identifying the existence of years with a larger or smaller number of reproductive females (Vitt and Seigel 1985; Ernst 1993).

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