

Pit viper *Bothrops pauloensis*: Reproduction and comments on the colour and pattern of neonates

SILVIA REGINA TRAVAGLIA-CARDOSO*, CIRCE C. ALBUQUERQUE & GIUSEPPE PUORTO

Museu Biológico, Instituto Butantan, São Paulo, Brazil

*Corresponding author e-mail: silvia.cardoso@butantan.gov.br

INTRODUCTION

Bothrops pauloensis Amaral 1925, a species in the *Bothrops neuwiedi* group, is a medium sized terrestrial pit viper that ranges from the central, south-east and central-west regions of Brazil (Silva, 2004; Silva & Rodrigues, 2008) to Paraguay and eastern Bolivia (Nogueira et al., 2019). It is primarily nocturnal and inhabits mainly dry savannah (Cerrado) (Valdujo et al., 2002; Fiorillo et al., 2020) and open areas of the Atlantic Forest (Silva, 2004; Silva & Rodrigues, 2008) where it frequently uses burrows and cavities for shelter, thermoregulation and foraging (Valdujo et al., 2002; Fiorillo et al., 2020). It feeds mainly on small mammals and lizards but has been recorded consuming birds, snakes, amphibians and centipedes, with ontogenetic shifts according to diet (Martins et al., 2002; Valdujo et al., 2002).

Information about the reproductive cycle, litter size and relationship between female size and fecundity has been published (Valdujo et al., 2002). Here we present data on the length, mass and sexual dimorphism in neonate *B. pauloensis* and determine relative clutch mass (RCM) expressed as the total litter mass, including any atretic eggs, divided by the post parturition mass of the mother (after Shine, 1980). The RCM is used as an estimate of the reproductive investment by females, and may be related to the body condition of the female and/or environmental factors during pregnancy.

Two female *B. pauloensis* collected when pregnant were brought and kept in captivity in Museu Biológico, Instituto Butantan (IB). The snakes were housed individually in plastic boxes (650 mm wide; 400 mm in deep; 450 mm high), with a temperature gradient of 24-27 °C, fed regularly with mice (*Mus musculus*), and offered water ad libitum. The females and the neonates were measured with a millimeter ruler and weighed using a semi-analytical balance with a precision of 0.01 g (maximum capacity 3200 g). Statistical comparisons were made using t-tests.

The first female was collected in Uberlândia, MG (18° 55'08" S, 48° 16'37" W) on 20th May 2019. A few days before parturition, the female measured 815 mm snout-vent length (SVL), 115 mm tail length (TL). On 20th December 2019, it gave birth to 4 live young (Litter 1: 3 males, 1 female) and 4 atretic eggs. The mass of the female after parturition was 212 g, and the total litter mass was 48.11 g, so giving an RCM of 0.22. The second female (SVL - 716 mm, TL 85 mm) was collected in Araraquara, SP (21° 47' 38" S, 48° 10' 33" W) on 20th December 2019. On 27th December 2019, this female gave birth to 10 live young (Litter 2: 8 males, 2 females). The mass of the female after parturition was 140 g, and the total litter mass was 113.8, giving an RCM of 0.81.

Biometric information of the neonates was obtained on the day of birth and summaries of each litter, and the litters combined, are given in Table 1. In *B. pauloensis* the adults are

Table 1. Biometric details of *Bothrops pauloensis* neonates (litters 1 and 2)

Mother (SVL+TL)	Litter 1	Males (N=3)	Females (N=1)
815+115	SVL (mm)	230 ± 8.6 (220 - 235)	225
	TL (mm)	41 ± 1.7 (40 - 43)	32
	Mass (g)	8.2 ± 0.2 (8 - 8.4)	8.13
Mother (SVL+TL)	Litter 2	Males (N=8)	Females (N=2)
719 + 85	SVL (mm)	264.3 ± 12.9 (245 - 285)	250 ± 21.2 (235 - 265)
	TL (mm)	46.2 ± 4.8 (38 - 50)	37.5 ± 3.5 (35 - 40)
	Mass (g)	11.7 ± 1.7 (8.9 ± 13.1)	9.9 ± 2.8 (7.9 - 11.9)
Litters combined	Litter 1 & 2	Males (N=11)	Females (N=3)
	SVL (mm)	255 ± 19.7 (220 - 285)	241.6 ± 20.8 (225 - 265)
	TL (mm)	44.8 ± 4.8 (38 - 50)	35.6 ± 4 (32 - 40)
	Mass (g)	10.7 ± 2.2 (8 - 13.1)	9.3 ± 2.2 (7.9 - 11.9)

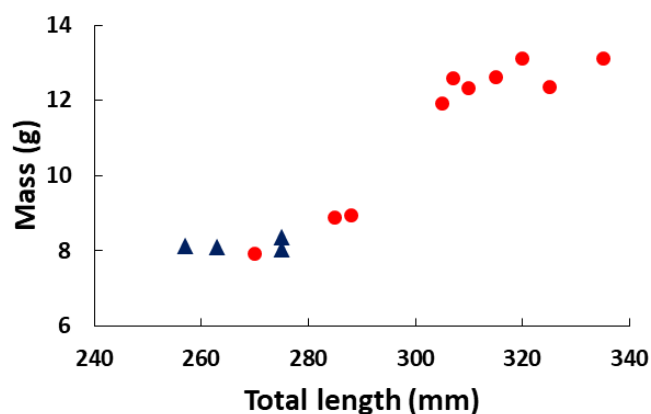


Figure 1. Relationship between total length (mm) and mass (g) in two litters (litters 1 and 2) of *Bothrops pauloensis*. Blue triangles – Litter 1; Red circles – Litter 2

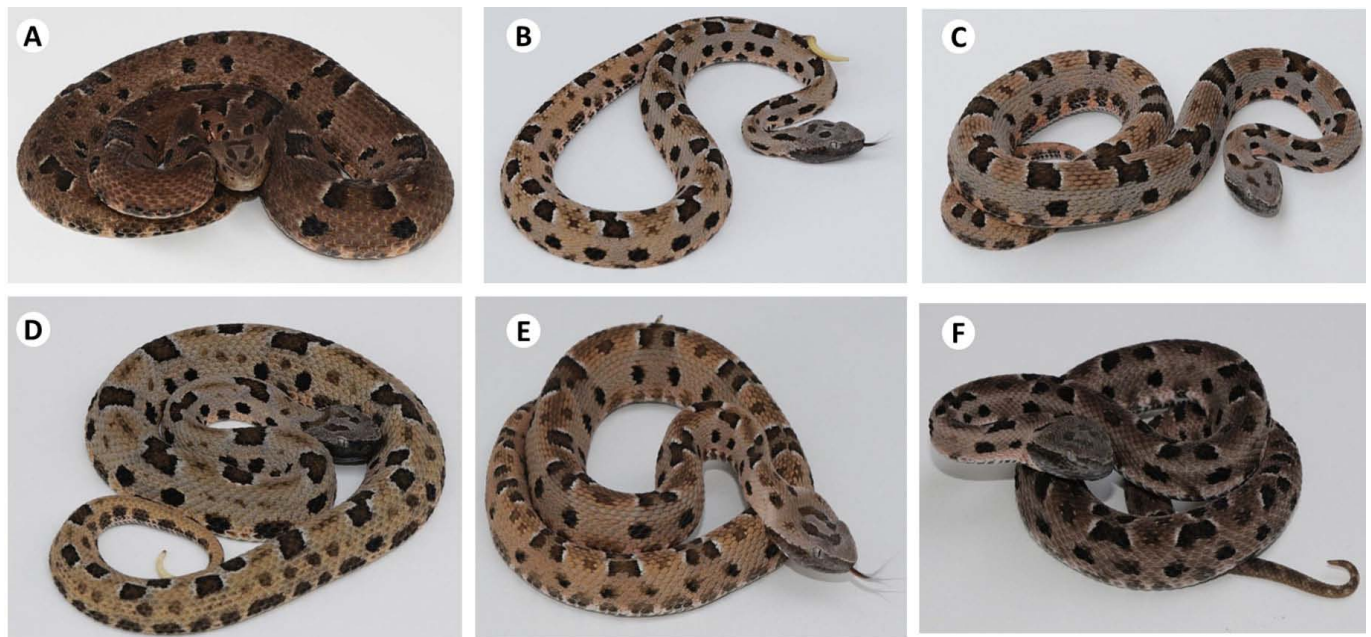


Figure 2. Different skin patterns and skin tones of neonate *Bothrops pauloensis* from a single litter (litter 2): **A.** mother; **B.** orange; **C.** greyish-orange; **D.** yellowish; **E.** light brown; **F.** dark brown

already known to be sexually dimorphic, with females being larger, heavier and with shorter tails than males (Valdujo et al., 2002). Our observations on neonates in the two litters combined, suggests that male and female neonates did not differ significantly in snout-vent length ($t_{0.05(2),12} = 1.034$; $p = 0.3213$) or mass ($t_{0.05(2),12} = 0.9770$; $p = 0.3479$), but were dimorphic in that males had longer tails than females ($t_{0.05(2),12} = 3.017$; $p = 0.0107$) (Table 1).

The RCM differed greatly between the two litters (0.22 litter 1; 0.81 litter 2), and the value obtained for the second litter (0.81) can be considered high for *B. pauloensis*, and other vipers. Despite coming from a larger litter, the young of litter 2 had significantly greater snout-vent lengths ($t_{0.05(2),12} = 4.178$; $p = 0.0013$) and were significantly heavier ($t_{0.05(2),12} = 3.171$; $p = 0.0080$) than those in litter 1 (Fig.1).

The number of neonates per litter is known to vary widely in *B. pauloensis*; 4 to 20 neonates per litter ($N = 15$ litters) (Valdujo et al., 2002). Our observations fit within this range as well as two unpublished records - a litter with 4 neonates and 1 atretic egg from a female collected in Itirapina, SP, that gave birth one day after arriving at the IB (S. Cardoso, pers. observation), and a litter with 6 neonates from a female collected in Alcínópolis, MS on January 2020 (Information Scales of Biodiversity Project, FAPESP: 2016/50127-5).

As for the RCM, other aspects of fecundity (number in a litter, size of the young, and reproductive frequency) in the same species of snake can vary, especially in relation to the size and mass of the females and their geographical origin which likely reflects climatic variation and food availability (Vitt, 1983; Seigel et al., 1986; Seigel & Ford, 1987; Jordão, 1996; Travaglia-Cardoso, 2011). In the case of long-term captive females, the effects of captivity on litter size cannot be ruled out.

For *B. pauloensis*, it is known that there is considerable individual variation in colour and skin pattern, even within

the same population (Campbell & Lamar, 2004). In the litters analyzed, the neonates of litter 1 were similar. However, in litter 2, we identified 5 different patterns and colour tones - orange, greyish-orange, yellowish, light brown and dark brown (Fig.2 A-F). Female snakes may mate with more than one male in a reproductive season, producing litters with multiple paternity and consequently greater genetic variability (Duvall et al., 1992; Madsen et al., 1992). As a possible explanation for the differences in skin patterns and tones between neonates in litter 2 (and the absence of these differences in litter 1), we can suggest that the mother of litter 2 may have copulated with more than one male, generating a litter with greater variability. This can only be confirmed by genetic testing.

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