



HEALTH SCIENCES

Notes on court and copula, fertility, nest, eggs and hatchlings of the Caatinga's black snake *Boiruna sertaneja* Zaher, 1996 (Serpentes: Dipsadidae) from northeastern Brazil

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Abstract: The Caatinga's black snake *Boiruna sertaneja* is a Pseudoboini species, endemic of the Caatinga biome. It is rare and fits in five traits that suggest it deserve more attention in reproduction research and conservation policies. Here we provide information on reproductive biology of *B. sertaneja* by adding new data about court and copula, fertility, nest, eggs and hatchling morphometry and pattern of color based on captivity specimens. The court and copula, as well as oviposition of *B. sertaneja* in the Caatinga are associated to period of high temperature and dry season. Our findings indicate that females of *B. sertaneja*, in nature, could select protected places or actively build their nests. We recorded three oviposture after a single event of copula by the female of *B. sertaneja*. The long time gap between copula and clutches strongly suggests that female *B. sertaneja* can store sperm in their oviducts for long periods or do facultative parthenogenesis. Clutch size and hatchling size of *B. sertaneja* was high. We observed variation on the pattern of coloration among hatchlings of same litter. This study comprises the first description of important aspects of reproduction and can give us some clues about how *B. sertaneja* reproduce in nature.

Key words: Caatinga, conservation, hatchling morphometry, pattern of color, reproduction.

INTRODUCTION

Studies on reproductive biology (which include reproductive cycles of both sexes, fecundity, sexual dimorphism, and reproductive behavior) are crucial to understand the biology of any species and an important tool to define conservation strategies, especially to threatened species (Shine & Bonnet 2009, Almeida-Santos et al. 2014, Braz et al. 2019). Much of what is known about reproductive biology of snakes is the result of studies of species from temperate regions (e.g., Shine 1977, 1980, 1985, 2003, Siegel et al. 2011). Despite of the huge advances on reproductive biology of Neotropical snakes

in the last decade, there are still gaps on the basic knowledge that restrict to determine new trends, hypothesis and patterns of reproduction of the Neotropical snake fauna (Pizzatto et al. 2007, Almeida-Santos et al. 2014, Braz et al. 2016).

Number and size of offspring, as well as reproductive frequency, are the most commonly information found about the reproductive biology of snakes (Pizzatto et al. 2007, Braz et al. 2008, Almeida-Santos et al. 2014, Santana et al. 2017). Number of eggs and size of the newborns are related to the body size of the female, while reproductive frequency is apparently related to reproductive mode (oviparous or viviparous) (Marques 1996, Pizzatto & Marques

2002). Thus, morphometric measurements of the eggs and hatchlings are important data that help parameterize life history analyses (Albuquerque & Ferrarezzi 2004, Braz et al. 2008, Morais et al. 2018). On the other hand, data on fecundity are rarely demonstrated in nature, and the biggest amount of information comes from indirect inferences on the observation of the vitellogenic follicles in specimens preserved in scientific collections (Seigel & Ford 1987, Scartozzoni & Marques 2004, Almeida-Santos et al. 2014, Marques et al. 2014). Another parameter poorly understood in reproduction of snakes, is the nest-sites and oviposition modes (Braz et al. 2008). Since the mothers are so successful at hiding their eggs, data about nest-sites are mostly based on punctual observations in nature or comes from observation of captive specimens (e.g., Albuquerque & Ferrarezzi 2004, Braz et al. 2008, Hrima et al. 2014).

The genus *Boiruna* acomodates two species of Pseudoboini snakes distributed in the Cis-Andean region of the South America, *Boiruna maculata* and *B. sertaneja* (Guedes et al. 2018). None of them were assessed in the IUCN red list of threatened species probably due to lack of data for evaluation; and in the Brazilian Red List of Threatened Species they were evaluated as Least Concern (ICMBio 2018). However, in a study about ecology of Pseudoboini, which included *Boiruna maculata*, Pizzatto (2005) listed five traits that suggest these species deserve more attention in research and conservation policies: low abundance, slow life history (low fecundity and possibly late maturation due to the large size of maturation), larger body size, higher trophic level, females larger than males and absence of combat.

Boiruna sertaneja is widely distributed in lowlands of the northeastern Brazil inhabiting xeric open formations and it is known to be endemic of the Caatinga (Guedes et al. 2014a). It

grows to a snout-vent length (SVL) of 2000 mm (TBG pers. obs.). This snake is predominantly nocturnal and has terrestrial habits (Guedes et al. 2014a, Marques et al. 2017). It feeds primarily on snakes, however other vertebrates like lizards and small mammals can also be taken (Vitt & Vangilder 1983, Gaiarsa et al. 2013, Marques et al. 2017). Despite its wide distribution, *B. sertaneja* is rare in nature and, therefore, scarce in scientific collections (TBG pers. obs., Pizzatto 2005, Loebmann & Haddad 2010, Pereira-Filho et al. 2017). Because of this, only one study summarized the principal aspects of its diet and reproduction, based in just few specimens (N=14; Gaiarsa et al. 2013). All data available about reproduction to date include the length of the smallest mature female 1147mm SVL and smallest male 1074mm SVL; it is an oviparous species with clutch size varying from four to 14 eggs, mean 9.25 eggs (Vitt & Vangilder 1983, Gaiarsa et al. 2013).

In this paper, we provide information about reproductive biology of the Caatinga's black snake *Boiruna sertaneja* by adding new data about court and copula, fertility, nest, eggs, hatchling morphometry and pattern of color based on captive specimens.

MATERIALS AND METHODS

Our data are based on the observation of an adult female *B. sertaneja* (1630 mm SVL and 280 mm tail length, microchip N° 96300800047774) from Campina Grande (Paraíba, Brazil) and kept in captivity at the Museu Vivo Répteis da Caatinga (permit conceived IBAMA N° 2512.9536/2014-PB category Zoo C), municipality of Puxinanã, Paraíba, Brazil (7°10'55"S 35°58'04"W, locality where the species naturally occurs; Guedes et al. 2014a). Since 2011, it is kept alone in a masonry terrarium (3.00 x 1.90 x 1.60 m) with

screen in the front, ground covered by sandy soil and leaf litter, room temperature varies from ca. 28°C during the day and 20°C at night, water provided by a dishpan container, and fed once a week or depending of the activity level of each individual, normally it is offered an adult mouse (*Mus musculus*) or a recently weaned rat (*Rattus norvegicus*).

This female was placed together with an adult male (1580 mm SVL and 240 mm tail length) and reproductive behaviors were directly observed and some portions were recorded using a cellphone camera. Since then, this female laid three clutches. We recorded information on the nest site of two clutches (Clutches #2 and #3). We counted the number of eggs for all clutches, but measured and weighed the eggs for two of them (Clutches #2 and #3). For these we enumerate all eggs using a waterproof pen, collected the mass, as well as measurements of egg length and egg width using a precision balance and digital calliper (precision 0.1 mm). We collected data on hatchlings morphometry of one oviposition (Clutch #2). We took data on body mass of each hatchling and measured SVL, tail length (TL), and head length (HL) using a precision balance, flexible ruler and digital calipers (precision 0.1 mm). Since the hatchlings are part of a living collection, we did not sex them to avoid injuries.

Eggs were incubated in a plastic box partially buried in moistened vermiculite following the room temperature. When hatching started, we isolated each egg in a distinct plastic box that allowed us relate the hatching to the same number gave to the eggs.

RESULTS

In 4th October 2017 at 14:30h, the female was placed together with an adult male. Almost immediately, the male started to court the

female. In the beginning of the court, the female actively moved through the terrarium while the male tried to position itself on the back of the female. After that, the male gets positioned in the female's back (Figure 1a). The female continued moving even having the male on its back. For some time the female was seen vibrating the tail as a reaction to male attempts to start the copula. After few attempts during the court, the male was seen starting the copula with the female (Figure 1b). Court and copula lasted about four hours. After the copula male and female were separated to avoid interspecific predation.

The first oviposition occurred on 26-27 December 2017 (two months after copulation) just after the female change its skin; the second on 6 September 2018 (almost one year after copulation), and the third on 4 November 2018. We do not have data about the time and nest site of the Clutch #1. Egg-laying of the Clutches #2 and #3 started in the morning and lasted about five hours. In the end of both oviposition, the female was apparently inactive (worn or tired) and positioned loosely coiled, having the eggs located nearby its body (Figure 1c, d). The eggs and the female (mother) were found in a hole below the water dishpan container (30 cm width x 50 cm length x 12 cm depth). The coverage of leaf litter was almost nonexistent in the second and substantial in the third oviposture (Figure 1c, d).

The female laid 19, 15, and 13 eggs, respectively, in each oviposture. Average number of eggs 15.66. For the Clutch #2, eggs averaged 48.66 ± 2.58 mm in length (range = 44.68-52.35 mm), 31.21 ± 2.78 mm in width (range = 23.57 to 37.84 mm) and 24.2 ± 3.56 g in mass (range = 12-26 g) (Figure 1e; Table I). For the Clutch #3, eggs averaged 47.69 ± 3.24 mm in length (range = 41.50 to 56.32 mm), 25.88 ± 4.34 mm in width (range = 20.32 to 31.37 mm) and 18.15 ± 5.78 g in mass (range = 10 to 27 g) (Table I).

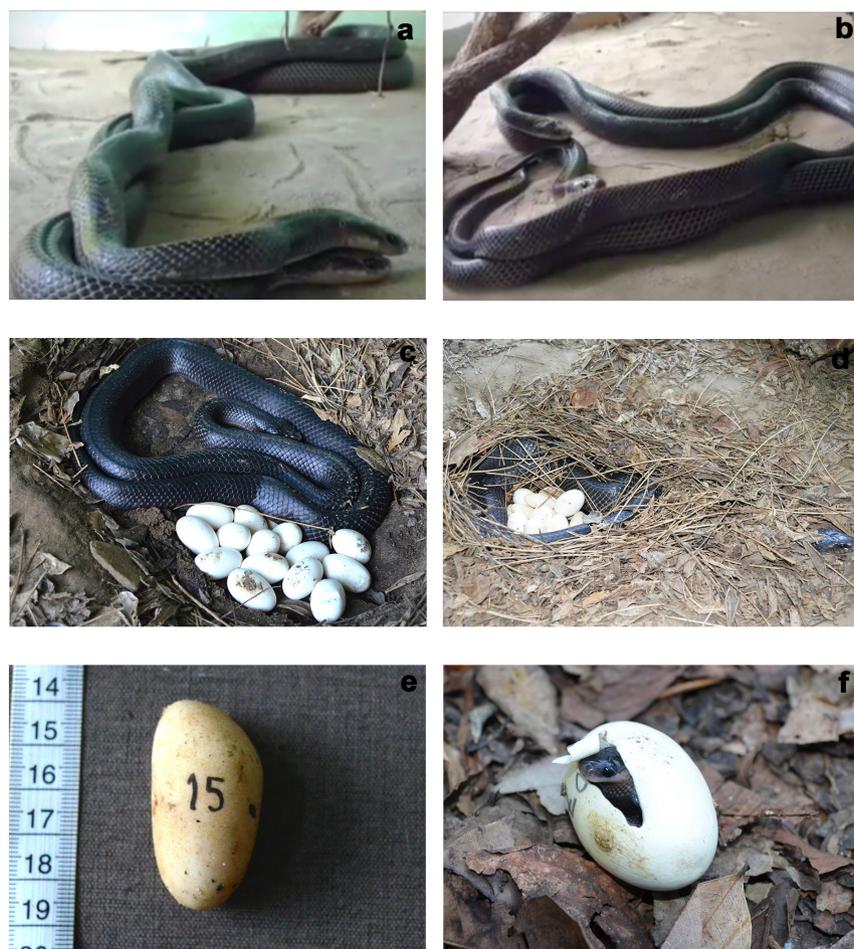


Figure 1. a- Male positioned over the female's back during the court behavior of *Boiruna sertaneja*. b- After few attempts during the court, the male was seen starting the copula with the female. c- Nest-site of the second oviposition of *Boiruna sertaneja* showing the adult female (mother) in a hole on the ground, inactive and curled nearby the eggs. d- Nest-site of the third oviposition of *B. sertaneja* showing the adult female in the same hole on the ground partially covered by leaf litter; the mother is positioned loosely rolled up having the eggs in the middle. e- One of the eggs showing how they were enumerated (it was discarded having no embryo, but just fluids inside); f- Hatchling snake number seven getting out of its egg.

In Clutch #1, 14 eggs hatched after 120 days of incubation. In Clutch #2, 14 out of 15 eggs hatched (Figure 1f) after 111-113 days of incubation (one egg was considered infertile and was discarded having no embryo, but just fluids inside). The period of egg incubation in captivity ranged the average of 116.5 ± 3.5 days. In Clutch #3, all 13 eggs were affected by a fungal infection during incubation process and did not survive. It is important to add that the eggshell of *Boiruna sertaneja* is quite sturdy, with a thickness of 0.49 mm (measurement made using digital caliper). Thus, in some cases, we used a scalpel to help some newborns of second oviposition to continue rip the eggshell (Figure 1f).

Measurements of the newborns are available only for those from the Clutch #2. Average values

are 24.21 ± 1.92 g (range = 20 to 26 g) body mass, 340.57 ± 18.63 mm (range = 316-367 mm) SVL, 63.64 ± 4.18 mm (range = 56-71 mm) tail length, 16.26 ± 0.54 mm (range = 15.38-17.16 mm) head length (Table II). Hatchling color pattern differs from that of the mother and adults in general by presenting a light gray nuchal collar varying from two to four scales width (Figure 2a, b). In eight hatchlings, the reddish/pinkish flanks covered four to seven paraventral scales, being the black longitudinal stripe remained restricted to the vertebral region (Figure 2c, d); in the other six specimens the black longitudinal stripe reaches vertebral and paravertebral region keeping the reddish/pinkish flanks less evident over only 1.5 to 3.5 paraventral scales (Figure 2e). In all hatchling snakes, the gular region and venter

Table I. Measurements collected from the eggs of second and third ovipositions of *Boiruna sertaneja* from Campina Grande, PB, Brazil. Mass is provided in grams (g) and morphometrical data are provided in millimeters (mm). Length = measurement at the longest point of the egg, Width = measurement at the widest point of the egg.

Specimen	Eggs of second Oviposition 6 September 2018			Eggs of third Oviposition 4 November 2018		
	Mass	Length	Width	Mass	Length	Width
1	25	45.67	37.84	27	50.70	31.37
2	25	51.98	30.62	23	51.14	27.89
3	24	46.39	31.78	23	48.77	29.73
4	25	50.00	30.94	23	56.32	30.63
5	26	50.74	31.78	24	51.28	29.05
6	25	52.35	31.56	20	45.23	29.36
7	24	49.21	29.91	21	41.50	29.43
8	26	51.32	30.50	15	49.97	22.47
9	26	50.06	31.14	10	43.15	20.32
10	24	44.68	31.85	13	49.59	20.61
11	23	48.47	31.09	11	44.76	21.43
12	27	50.32	31.15	13	47.22	22.91
13	24	46.20	31.78	13	50.36	21.26
14	27	47.66	32.70	-	-	-
15	12	44.94	23.57	-	-	-
Average	24.2	48.66	31.21	18.15	47.69	25.88
Standard error (±)	3.56	2.58	2.78	5.78	3.24	4.34

were immaculate, sometimes presenting just few subcaudals darkened.

DISCUSSION

Based on our results, the copula and the timing of oviposition of *B. sertaneja* in the Caatinga are associated with the period of

high temperature and dry season (according to the climatic diagram, Figure 3 in Marques et al. 2017), the same known to other endemic species of the Caatinga *Epicrates assisi* (Guedes et al. 2019), and also a congener *B. maculata* in the Cerrado (Pizzatto 2005). The long time gap between the copula and Clutches #2 and #3 (11 and 13 months) strongly suggests that female *B. sertaneja* store sperm in their oviducts for

long periods (see Almeida-Santos & Salomão 1997, 2002, Almeida-Santos et al. 2004). Female snakes store sperm in two portions of their oviducts: the posterior infundibulum and uterus (Siegel et al. 2011). Sperm storage has been reported in some Neotropical snakes, for example *Crotalus durissus*, *Apostolepis gaboi*, *Philodryas patagoniensis*, and *Erythrolamprus*

miliaris (Almeida-Santos & Salomão 1997, Rojas et al. 2015, 2017, Braz et al. 2019). Microscopic examination of the oviducts can confirm sperm storage in female *B. sertaneja* (see Almeida-Santos et al. 2014). Additionally, facultative parthenogenesis can also explain the long term between the copula and clutches, but it is a question poorly investigated (Allen et al. 2018).

Table II. Measurements collected from the newborns of second oviposition of *Boiruna sertaneja* from Campina Grande, PB, Brazil. Mass is provided in grams (g) and morphometrical data are provided in millimetres (mm). Snout-vent length = SVL, tail length = TL, head length = HL.

Newborns of second Oviposition 26-28 December 2018				
Specimen	Mass	SVL	TL	HL
1	20	318	64	15.38
2	26	365	61	16.72
3	23	367	65	17.16
4	22	343	60	16.00
5	26	356	60	16.43
6	26	320	69	16.13
7	25	354	71	15.68
8	26	340	60	16.60
9	24	327	64	17.04
10	23	346	64	15.61
11	25	332	69	16.51
12	25	320	66	16.43
13	22	316	56	15.65
14	26	364	62	16.30
Average	24.21	340.57	63.64	16.26
Standard error (±)	1.92	18.63	4.18	0.54

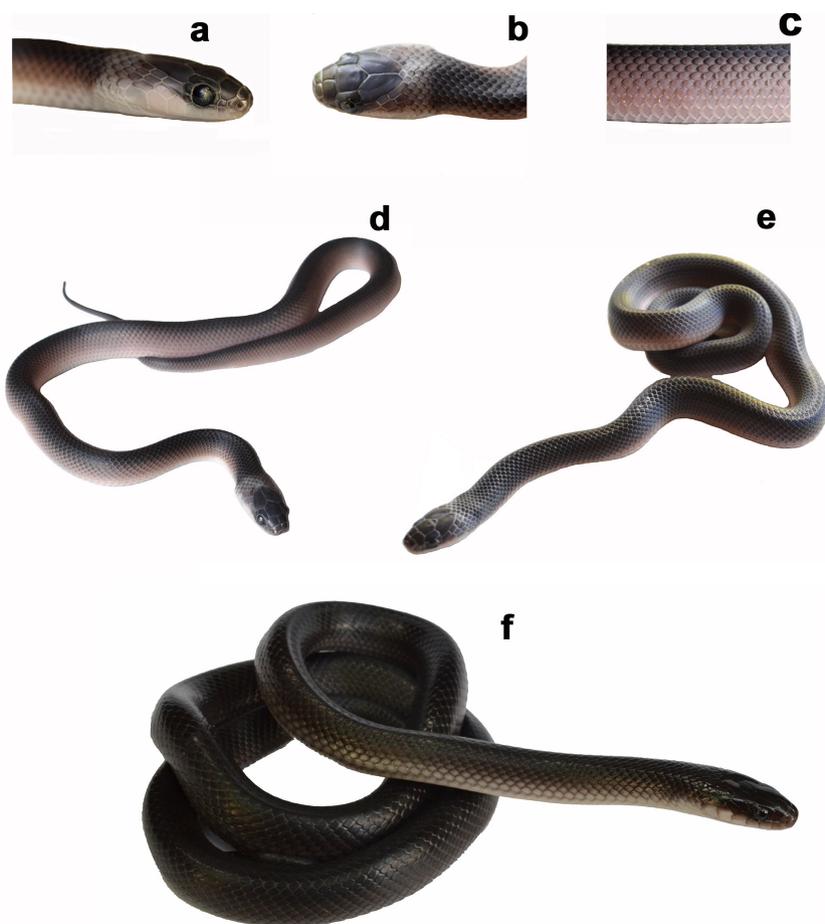


Figure 2. Newborns of *Boiruna sertaneja*. a- Lateral view of the head highlighting the light gray nuchal collar. b- Dorsal view of the head showing the light gray nuchal collar. c- Lateral view of the middle of the body showing reddish/pinkish flanks and black longitudinal stripe covering the vertebral region. d- Hatchling number six showing evident reddish/pinkish flanks covering seven paraventral scales, being the black longitudinal stripe restricted to the vertebral region. e- Hatchling number ten showing black longitudinal stripe reaching vertebral and paravertebral region keeping the reddish/pinkish flanks less evident over only 1.5 paraventral scales. f- Adult of *B. sertaneja* showing the uniform black pattern of color of the dorsum highlighting the differences with the ontogenetic variation presented by the hatchlings.

However, to have the specimen captive and monitored, as is the case, is a perfect scenario to collect DNA samples to confirm parthenogenesis in the future.

In the oviposition #2 and #3, the female of *B. sertaneja* apparently selected the hole already existing below the water dishpan container covered by leaf litter to lay their eggs. Additionally, the female was found coiled around or nearby the eggs after oviposition, although it had not expressed aggressive behavior when the eggs were manipulated by us (e.g., to be transferred to a plastic box). In a previous event of oviposition, the same female of *B. sertaneja* dug its own hole for oviposition in a substrate of soft sandy (Silvaney Medeiros, pers. obs.). Thus, our findings plus S. Medeiros observation indicate that females of *B. sertaneja*, in nature, could

select protected places with an accumulation of debris which she can appropriate for her use or actively build their nests, different of what is known for other Pseudoboini snakes (Braz & Manço 2011).

When oviposit, eggs of snakes are exposed to several environmental pressures like desiccation, overheating, and predation that can limit the survival of the embryo. Thus, the construction of nests, the behavior to guard the eggs by the female and the eggshell thickness are strategies that can guarantee viability of offspring (Oliver 1956, Hrima et al. 2014). Nests of snakes are rarely found in nature and their sites, as well as, their constructions are in general poorly understood. Some nest-sites are reported in preformed subterranean chambers under rocks, logs or other types of coverage

(Packard & Packard 1988, Hall & Meier 1993, Braz et al. 2008, Braz & Manço 2011). For the king cobra *Ophiophagus hannah*, the construction of the nest and the behavior to guard the eggs is well-documented; they used to construct a two-stored chambered nest from leaf litter and other plant material, the eggs are deposited in the lower chamber while the female may reside in the upper chamber (Oliver 1956, Hrima et al. 2014). However, among Neotropical species, reports of nests are scarce, having just punctual observations described for few species (e.g., Albuquerque & Ferrarezzi 2004, Braz et al. 2008).

We recorded three ovipostures after a single event of copula by the female of *B. sertaneja*. The three egg-laying occurred in the same season (not in the same year) in which the temperature was high and there was no rain. There was a long intermission between the two firsts, however the second and third happened in the same reproductive season. There are few evidences that females from tropical regions are able to oviposit multiple times in a single breeding season (Seigel & Ford 1987, Almeida-Santos et al. 2014) and that is related to availability of food and climatic conditions. According to Pizzatto (2005), pseudoboini genera *Boiruna* have the potential to reproduce continuously. However, we are aware that our data comes from specimen kept in captivity, where the stable conditions (especially the constant feed availability) may modify its reproductive potential (Seigel & Ford 1987).

The period of incubation of *B. sertaneja* was longer than the other pseudoboini *Oxyrhopus guibei* (57-94 days, N = 47 newborns from 11 clutches; Pizzatto & Marques 2002). According to studies about fecundity, the number of eggs and hatchling size are both positively related to the size of the female (mother) (Seigel & Fitch 1984) and habits of life (Marques & Puerto 1998, Pizzatto et al. 2007). Clutch size and hatchling

size of *B. sertaneja* was high (N = 4 – 19; this study and Gaiarsa et al. 2013) reaching similar number observed for *B. maculata* (N = 4 – 15; Pizzatto 2005) when compared to other Pseudoboini species *Oxyrhopus guibei* (N = 3 – 20; average 10.9; Pizzatto & Marques 2002). It may indicate a phylogenetic trend shaping the reproductive pattern of both species.

We observed variation on the pattern of coloration among the hatchlings of *B. sertaneja*, however, in general it corresponds to the same pattern already described by Zaher (1996). Species of the genus *Boiruna* share similar traits, as the distinct ontogenetic color variation. Newborns and very young specimens show a black longitudinal stripe covering the vertebral and paravertebral region of the body with a reddish/pinkish flanks as well as a light gray nuchal collar, while adults present subcaudal and ventral scales almost completely black (except for the anterior portion of the body) (Zaher 1996). The ontogenetic variation of color is associated with changes in size, vulnerability and habitat use (Booth 1990), and in some way could guarantee the survivance of the hatchlings in nature (Marques & Sazima 2003). However, the functional significance of this color variation is poorly understood in vertebrates, and especially in snakes (Wilson et al. 2007), and still requires refined quantification of how these color signals are seen by receivers'perspective (Endler & Mielke 2005).

Some traits pointed by Pizzatto (2005) suggest that *B. sertaneja* deserve more attention in reproduction research and conservation policies. Even based in captivity specimens and few observations, the data we provide here comprise the first description of important aspects of reproductive biology of *Boiruna sertaneja*, a rare and endemic species of the Caatinga. Additionally, *B. sertaneja* lives in a threatened biome that is losing their original

vegetation (habitat loss; 50% of the original vegetation was already removed or strongly altered by human activities through logging, fire, agriculture and grazing goats) and because the conservation of the area is neglected by the government since less than 2% of the Caatinga is protected (Guedes et al. 2014b, Marques et al. 2017).

Our results can give us some clues about how this species reproduce in nature and we hope can be useful in future conservation strategies and to boost future interest on the thematic of reproduction of the rare *Boiruna sertaneja* in the Caatinga environment. Reproduction influence energy budget, being some events too costly for the species (Aldridge & Duvall 2002, Pizzatto & Marques 2002, Mathies 2011). Thus, additional studies are needed to assess the hypothesis of continue reproduction for Pseudoboini, as well as the behavioral and physiological repertoire.

Acknowledgments

The information provided in this study is based only in the measurements and photos taken from the snakes (adult male and female, eggs and hatchling snakes). We did not collect or euthanize any specimens reported here, all of them are part of the live collection of Museu Vivo Répteis da Caatinga under responsibility of Silvaney Medeiros. We thank Silvaney Medeiros and Andrea Guedes for all support along more than a year of data collection. We thank Dr. Henrique B. Braz for all suggestions made in the first draft which improved the quality of our manuscript. TBG thanks to Universidade Estadual do Maranhão for the Senior Researcher fellowship.

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Thaís Guedes: conceived the research, led the writing and prepared the first draft of the manuscript. Abimael Guedes: revised the manuscript adding intellectual content. Both authors collected the data and took the photos, read and approved the final manuscript.

