

## Clutch size, morphology, hatching time and offspring behavior of *Pseudoboa nigra* (Dipsadidae: Pseudoboini) from Caatinga

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**Abstract.** Reproductive traits play a central role in the life history tactic theory. In ectotherm animals, traits like offspring number and size can be affected by each other and by environmental conditions. Here we provide information about clutch size, incubation duration and behavior of freshly hatched *Pseudoboa nigra*. We incubated eight eggs laid in the laboratory by a wild-caught *P. nigra* from the Caatinga biome. We obtained morphometric measurements of the female, eggs and offspring. The clutch size was similar to that described for individuals from the Cerrado biome; hatchlings were more similar in size to those described for the Atlantic Forest than for the Cerrado. To our knowledge, this is the first report of exact incubation days and hatching time for *P. nigra*.

**Key words:** clutch size, life history traits, reproductive effort, oviposition, Squamata.

Species may deal with environmental pressures by adapting to use different life history tactics. These tactics mainly relate to changes in life span, growth, body size, survival and reproduction (Alonzo & Kindsvater 2008). Reproductive traits (i.e. clutch size, newborn size, age at reproduction, reproductive effort and behavior) play a central role in the life history theory (Stearns 1976, Godfray et al. 1991). These traits can be correlated, such as the trade-off between clutch size and clutch number per year (Pizzatto 2005, Cox et al. 2007, Pizzatto et al. 2007). Reproduction aspects can be affected by external aspects such as the climatic conditions at the species habitat (Mesquita et al. 2016).

In Brazil, the tribe Pseudoboini comprises 37 species of Dipsadidae, which are medium-sized terrestrial snakes and feed mainly on lizards (Gaiarsa et al. 2013). The Black False Boa *Pseudoboa nigra* (Duméril, Bibron & Duméril, 1854) has a maximum snout-vent length = 1261 mm, is mostly terrestrial, specialized on a lizard diet, distributed in open vegetation areas in the Atlantic forest, Amazon, Caatinga, Cerrado, Chaco, and Pantanal natural regions of Brazil (Orofino et al. 2010, Gaiarsa et al. 2013, Marques et al. 2016). Reports on offspring of *P. nigra* are available only from the Cerrado and Atlantic forest (Orofino et al. 2010, Marques et al. 2016). Here we provide the clutch size, duration of incubation, hatching behavior, and body size at hatching of *P. nigra* offspring from a wild-caught individual from the Caatinga biome in northeastern Brazil.

On 14 March 2019 WPS was doing fieldwork at the municipality of Lajes (-36.3020°, -5.6728°, WGS84), Rio Grande do Norte state, northeastern Brazil, and manually captured an adult female *P. nigra*. The region comprises caatinga bush vegetation, composed of sparse shrubs and woody grasses (IBGE 2012). We identified that the individual contained oviductal eggs after manual palpation. The animal was transported to the laboratory at the Universidade Federal do Rio Grande do Norte (UFRN). It was kept in a wooden box; the temperature regime of the room followed external temperature. We provided fresh water ad libitum. The snake was not handled or disturbed except for water change. We observed the individual for two days following egg laying and released it at the capture site in visibly healthy condition.

The female laid eggs on 26 March 2019. We measured the female's head length with a digital caliper (0.01 mm accuracy), snout-vent length (SVL) and tail length with a measuring tape (0.1 mm ac-

curacy), and its body mass with a digital scale (0.01 g accuracy) after oviposition to avoid disturbance. The female measured 23.24 mm head length, 830.2 mm SVL, 279.8 mm tail length, and weighed 150 g mass. Eggs were individually labeled with a hydrographic pen. We did not measure individual egg mass as eggs were laid clustered as one. Despite this, we carefully handled the egg aggregation and measured each egg's length and width using digital caliper. After measurements we transferred the eggs to a well-ventilated plastic box with the bottom covered with sand substrate (~3 cm depth) and placed the eggs on it. We kept the plastic box in the same room as the female and monitored the air temperature and humidity inside the plastic container once a day during the incubation period with a DRS digital thermohygrometer.

We checked the eggs three times a day for hatching signs. From the moment we observed slits in the eggshell (i.e. piping), we monitored the hatching process. We defined the incubation period as spanning from oviposition to piping, and the hatching period as the time from piping to the moment the offspring completely left the egg. We recorded the duration of both total hatching duration and emergence (time individuals took to completely leave the egg after they have extended a third of their body outside). We used a Canon T5i with a 18-55 mm lens to film the emergence of snakes. The recording aided the behavior description with later information of hatchlings morphology. After hatching was completed, we killed the hatchlings to provide vouchers and individually labeled them according to the correspondent egg. Snakes were killed with an intraperitoneal injection of thiopental (1 ml) (Hawk & Leary 1999), fixed in formalin (10%) and preserved in 70% alcohol. We obtained the following measurements: total length, SVL, tail length, head length, head width, head height, eye diameter, body width, body height, distance between nostrils, ocular-nostril distance, distance between eyes, ventral-symphysial distance, position of yolk insertion (number of ventral scales posteriorly from the anal plate), and body mass. We used a Leica EZ4 stereo microscope for all head measurements, a measurement tape for SVL and tail length, and a Pesola scale (10 g accuracy) for body mass; we provide the mean  $\pm$  1 standard deviation (SD) for these measurements. Vouchers are housed at the Coleção Herpetológica da Universidade Federal da Paraíba, at the Universidade Federal da Paraíba (CHUFPB 12780-12786).

Seven eggs were laid in a cluster (Fig. 1A); one egg was laid separately (38.5 mm egg length, 16.46 mm egg width), but rotted by the 30<sup>th</sup> day of incubation and was discarded. Eggs' average length and width were  $37.0 \pm 1.84$  mm and  $16.33 \pm 0.21$  mm, respectively. Hatchlings' mean SVL was  $220.0 \pm 2.23$  mm (range: 217-223 mm); body mass was  $5.45 \pm 0.26$  g (5-5.8 g). The SVLs of the smallest and biggest speci-



Figure 1. Clustered eggs (A) and observed behaviors during *Pseudoboa nigra* hatching: nostril and head limited to eyes outside the shell (B), tongue flicking from inside the egg (C), jaw adjustment (D), body leverage (E), third of the body outside (F), seeking substrate under eggs (G), and buried (H).

mens were 26.13% and 26.86% of the adult's SVL (Table 1).

Offspring started hatching on 6 June 2019, after 73 days of incubation. The mean air temperature and humidity during the incubation were  $29.4 \pm 0.9$  °C (26.2–29.6 °C) and  $86 \pm 2.2\%$  (78–86%), respectively. Hatching lasted 10 h 55 min  $\pm$  3 h 35 min (range: 4 h 50 min – 15 h 25 min), while emergence lasted from 8 min to 1 h 20 min (Table 2).

After piping, all hatchlings kept the rostral portion of the head outside the egg shell, limited by its nostrils for about 2 h, then they extended the whole head or up to the eyes outside the egg (Fig. 1B). Then, animals alternated either totally retrieving the head, or leaving the rostral portion outside, or extending the whole head outside the egg until they completely left the egg. Just before leaving the egg, with the head outside, individuals started tongue flicking (Fig. 1C), slowly

pulling out the body while inspecting the surroundings and often opening the mouth to adjust the jaw (Fig. 1D). Some individuals used nearby eggs to leverage their body outside the egg (Fig. 1E). After the snakes pulled out about a third of the body outside the egg (Fig. 1F), they usually did not retrieve back inside. Following complete hatching, some individuals buried in the sand under the egg shells (Fig. 1G-H). No hatchling left the egg while another individual was emerging; if a hatchling extended its head outside the egg while another was leaving the egg, the former retreated inside the egg.

The overall clutch size for *P. nigra* varies from 3 to 24 eggs (Gaiarsa et al. 2013). Similar to our observation, Orofino et al. (2010) reported clutch sizes of 6–8 eggs for *P. nigra* from the Cerrado based on three clutches; they also reported

Table 1. Morphological measurements (in mm, g) of seven *Pseudoboa nigra* hatchlings: Voucher numbers, egg length (EgL), egg width (EgW), total length (TL), snout-vent length (SVL), tail length (TaL), head length (HL), head width (HW), head height (HH), eye diameter (ED), body width (BW), body height (BH), distance between nostrils (DBN), ocular-nostril distance (OND), distance between eyes (DBE), ventral-symphysial distance (VSD), ventrals of yolk insertion (YI), and body mass.

Voucher	EgL	EgW	TL	SVL	TaL	HL	HW	HH	ED	BW	BH	DBN	OND	DBE	VSD	YI	Mass
12780	36.66	16.23	248.0	220	28.0	11.16	4.57	2.82	0.91	4.73	4.40	1.49	1.33	2.49	5.81	29–30	5.5
12781	39.23	16.2	248.8	221	27.8	10.76	4.81	2.57	1.00	4.48	4.81	1.66	1.49	2.57	6.56	30–31	5.7
12782	35.62	16.45	248.4	221	27.4	10.73	4.65	2.49	0.75	4.23	3.65	1.66	1.33	2.66	6.56	30–31	5
12783	37.49	16.25	250.2	223	27.2	11.39	4.73	2.57	1.00	4.07	3.82	1.66	1.08	2.57	6.56	32–34	5.5
12784	39.48	16.32	243.2	217	26.2	10.8	4.57	2.57	1.00	4.73	4.57	1.58	1.41	2.49	6.23	33–34	5.8
12785	34.94	16.75	247.3	221	26.3	10.6	4.48	2.82	0.83	4.15	4.15	1.58	1.33	2.66	6.31	30–31	5.3
12786	35.35	16.13	244.2	217	27.2	10.63	4.40	2.74	0.91	4.15	4.15	1.58	1.49	2.66	6.14	31–32	5.4

Table 2: Hatching of *Pseudoboa nigra* offspring: piping order (PO), total hatching time (THT) in hours:minutes, egg emergence time (EET) in minutes, days of incubation (INC), and piping date (PD).

PO	THT	EET	INC	PD	Voucher #
1	15:25	8	73	06/07/19	12782
2	14:00	80	73	06/07/19	12780
3	12:00	N/A	73	06/07/19	12786
4	11:00	12	73	06/07/19	12781
5	08:50	14	74	06/08/19	12783
6	11:20	19	74	06/08/19	12785
7	04:50	15	74	06/08/19	12784

reproductive individuals throughout the year. We cannot infer yet on *P. nigra* reproductive cycle in the Caatinga. Vitt & Vangilder (1983) reported *P. nigra* hatchlings from Caatinga in August during the dry season, but did not provide hatchlings' size. Differences between populations in reproductive cycles and clutch size occur in other squamate species from the Caatinga and Cerrado biomes (Vitt 1982, Colli 1991, Vitt & Colli 1994, Mesquita & Colli 2003); further data should clarify if this also applies to *P. nigra*.

In squamates, there is frequently a correlation between the female's body size and mass and the offspring body size and mass (Shine 2003, Cox et al. 2007, Pizzatto et al. 2007), related to the amount of yolk and egg volume that will influence the young body size (Vitt & Price 1982, Qu et al. 2019). The SVL of hatchlings from Caatinga differed slightly when compared to other biomes. Offspring reported from Cerrado varied from 234 to 324 mm (Orofino et al. 2010), and offspring from Atlantic forest varied from 214 to 274 mm (Marques et al. 2016). The smallest hatchling in our study from the Caatinga was 7.3% smaller than the smallest from the Cerrado and 2.4% bigger than the smallest from the Atlantic forest; the largest hatchling from Caatinga was smaller than the biggest from the Cerrado and Atlantic forest (31.2% and 18.6%, respectively). We highlight that our dataset is small when compared to that for the other biomes and additional data should clarify our inferences.

Only a few Neotropical studies report newborn snake morphometry and size (Santana et al. 2017, Morais et al. 2018, Silva et al. 2019), but information on their hatching behavior is unavailable. Hatching behavior similar to what we observed was reported for *Heterodon nasicus* Baird & Girard, 1852, a dipsadid snake distributed from southern Canada to northern Mexico (Munro 1949). Compared to other Pseudoboini snakes, the incubation period showed similarities with *Oxyrhopus guibei* Hoge & Romano, 1977, from southeast

Brazil, while its hatchlings were often smaller (incubation: 57–94 days, clutch of 3–20 eggs, 137–230 mm SVL, 2.9–4.9 g mass; Pizzatto & Marques 2002). *Boiruna sertaneja* Zaher, 1996 inhabits the Caatinga and is phylogenetically closer to *P. nigra* than *O. guibei*, and has a longer period of incubation (111–120 days), more eggs (13–19), and larger hatchlings (316–367 mm SVL, 20–26 g mass; Guedes & Guedes 2020). Despite being phylogenetically close, these species differ in size: *O. guibei* maximum SVL is 1080 mm (Pizzatto & Marques 2002), *B. sertaneja* maximum SVL is 1940 mm, while the maximum SVL of *P. nigra* is 1261 mm (Gaiarsa et al. 2013). Clutch size and offspring size are associated with female body size in closely related species in the tribe Pseudoboini (Pizzatto 2005, Cox et al. 2007, Pizzatto et al. 2007). Therefore, incubation and offspring data for these three species reinforce that Pseudoboini usually show conservatism in many traits (Vitt & Price 1982, Pizzatto 2005). Future research should address this with more species and reproductive data to compare how species phylogenetic relatedness affects these traits.

We observed that individuals emerged in a short period (two days) from the clustered eggs, similar to *B. sertaneja* (three days; Guedes & Guedes 2020), both observations based from single clutches. Clustered eggs and communal nests (oviposition by different females in the same place) allow synchronous development of the embryos mediated through heartbeating, leading to synchronous hatching, although younger eggs are affected producing smaller hatchlings (Aubret et al. 2016). Total hatch time may vary greatly among reptiles due to individual egg's location within the environment and within the clutch, as well as non-developmental factors such as stimulation by other hatchlings and size of residual yolk (Gutzke et al. 1984).

*Pseudoboa nigra* is predominantly terrestrial, with few individuals climbing on vegetation (Gaiarsa et al. 2013). Hatchlings did not attempt to climb the plastic box, but we observed individuals burrowing themselves after emergence. This can be interpreted as an antipredator behavior, as reported for other hatchling snakes (Burger 1998). We highlight that burrowing might have been the only option due to the limitation of the box, as well as offspring antipredator behavior may have been elicited by the presence of the observer. Despite this, antipredator response may vary with snake size and age (Roth & Johnson 2004). Adult *P. nigra* hide their head under body and exhibit erratic movements (Marques et al. 2017). To our knowledge, this is the first report of incubation length and hatching time and behavior for

*P. nigra* and the first to offer specific information from the Caatinga.

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