

**Clutch size, hatching time, and hatchling morphology of Boettger's Sipo *Chironius flavolineatus* Jan 1863 (Serpentes: Colubridae: Colubrini) in the northeast Atlantic Forest**

Knowledge about the reproduction of neotropical snakes has constantly advanced, especially in Brazil (Almeida-Santos et al. 2014). Information about timing and duration of egg-laying, clutch size and egg measures, maternal behavior, incubation time, piping, and offspring morphology is necessary to test the hypothesis of the role of natural selection on clutch size adjustment, and consequences on phenotypes (morphology, locomotor ability) of hatchlings (Aubret et al. 2003). Recent studies describe reproductive aspects of clutch size, hatchling and egg morphology of Brazilian snakes, for example, Pernambuco Worm Snake *Amerotyphlops paucisquamus* (Amaral et al. 2020), Mussurana Boiruna *sertaneja* (Guedes & Guedes 2020), Brown-banded Watersnake *Helicops angulatus* (Amaral et al. 2021), Leopard Keelback *Helicops leopardinus* (Amaral et al. 2019), Green Vinesnake *Oxybelis fulgidus* (Scartozzoni et al. 2005), Falsacoral *Oxyrhopus guibei* (Pizzatto & Marques 2002), Black False Boa *Pseudoboa nigra* (Marques et al. 2021), Amazon Puffing Snake *Spilotes sulphureus* (Morais et al. 2018), Northern Coastal House Snake *Thamnodynastes pallidus* (Medeiros-da-Silva et al. 2019), Pampas Snake *Tomodon dorsatus* (Bizerra et al. 2005), Serra Snake *Tropidodryas serra* (Muscat et al. 2019). This information is very useful for studies on population ecology and the life history of snake species, with high importance in elucidating taxonomic, systematic, ecological, and physiological questions (Amaral et al. 2020).

However, information about the reproduction of *Chironius flavolineatus* is scarce. The genus *Chironius* Fitzinger 1826 (Colubridae) comprises 23 species distributed in South America and the Antilles, with 15 species recorded in Brazil (Costa & Bérnils 2018, Uetz et al. 2021). The Boettger's Sipo

*Chironius flavolineatus* Jan 1863 has a wide distribution in Brazil, occurring in the northeast Atlantic Forest, in Amazonian savannas, Caatinga, Cerrado, and Pantanal (França et al. 2006, Nogueira et al. 2019). The species is a medium-sized snake (reaching 1200 mm in total length), has diurnal and semi-arboreal habits, can be found in forests and open environments, and feeds mainly on amphibians (Dixon et al. 1993, Pinto et al. 2008, Hamdan & Fernandes 2015). The species is oviparous, with reproduction occurring during the rainy season (Pinto et al. 2010).

On 23 April 2021, a gravid female of *Chironius flavolineatus*, measuring 1200 mm in total length and weighing 175g, was found in a forest patch in the northeast Atlantic Forest (-6.84977°N, -34.9281°E; datum WGS 84; 31 m elevation), Municipality of Rio Tinto, Paraíba State, northeastern Brazil. It was captured under permit SISBIO 74327-1. We used a digital caliper (precision = 0.1 mm) and a spring scale (500 g) to measure and weigh the snake at the moment of capture. We verified through handling that the snake carried eggs at an advanced stage of development. We kept it in captivity in a terrarium measuring 40.1 cm long × 45.3 cm wide × 63.5 cm high, with sand, leaves, and a tree branch in the center for perching. We visually monitored the snake's behavior during the days and filmed the egg laying. On 3 May 2021, the snake laid nine eggs (Fig. 1A), starting at 18:25 and finishing at 20:11. Egg-laying occurred with the snake perched on the branch of the terrarium at the height of 20 cm (Fig. 1B). After laying the eggs, the snake was observed for 24 hours; it was exhibiting typical behaviors without signs of stress, and was released at the site of capture, on 5 May 2021. Eggs were removed from the terrarium. They were then incubated at 20–22 °C, at approximately 60% humidity, semi-buried in vermiculite (Fig. 1C) in a rounded glass container (185 mm height and 130 mm diameter). Eggs were measured at the widest and longest point using a digital caliper (accuracy = 0.1 mm) and weighed on a digital scale (accuracy = 0.1 g). Eggs' average measures were 15.45 mm in width (Sd, standard deviation = 0.47), 29.95 mm in length (Sd = 2.79), and 5.11 g in mass (Sd = 0.33)

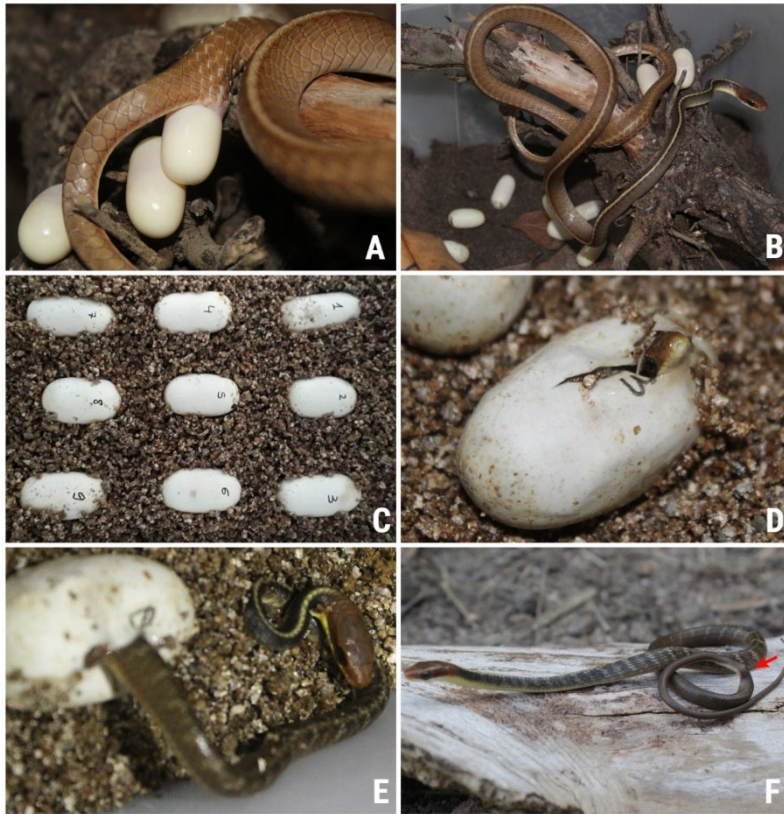


Figure 1: Oviposition, piping, and hatching of *Chironius flavolineatus*. A - Adult female *C. flavolineatus* laying eggs; B - adult female with nine eggs after laying; C - The nine eggs hatching; D - Piping of the first egg; E - A newborn hatching; F - The newborn out of the egg with a tail malformation (red arrow).

On 17 July 2021, after 75 days of incubation, at 12:40, three offspring started hatching (Fig. 1D) and totally emerged in 17 hours. On the same day, at 21:08, another offspring started hatching, the process lasting for 26 hours. On 18 July 2021, the fifth offspring cut the egg at 14:00 and totally emerged after 17 hours (Fig. 1E). On 19 July 2021, one egg started to dry. We dissected the egg and found the offspring dead but fully formed, making it possible to obtain measurements. The other three eggs were not fertilized.

All hatchlings were measured and weighed immediately

after birth (Table 1). The hatchlings' mean total length was 322 mm (Sd = 22.39), with an average weight of 3.78 g (Sd = 0.38). The hatchlings had clear white stripes along the back, differing from adult individuals. One hatchling was born with a tail malformation near the cloaca (Fig. 1F). The dead offspring was preserved in formaldehyde 4% and deposited in the Herpetological Collection of the Universidade Federal da Paraíba (CHUFPB 101). All live hatchlings were released in the forest patch where the mother was captured.

Table 1. Morphometry (mm) and mass (g) of adult female and hatchlings of *Chironius flavolineatus*.

Measures of hatchlings were taken on the day of hatching (17-19 July 2021). S = sex; SVL = snout-vent length; TL = tail length; BW = body width; BH = body height; HW = head width; HH = head height; HL = head length; NW = inter-nasal distance; ED = eye diameter; DN = distance between the navel and the cloaca; M = mass; AF = adult female; Htc = hatchling; Sd = Standard deviation.

#	S	SVL	TL	HL	HW	HH	BW	BH	NW	ED	DN	M
AF	F	750	450	26.3	10.9	6.93	11.43	13.85	5	5.13	-	175
1	F	201	112.28	11.81	5.42	3.54	4.27	4.60	2.25	2.53	30	4.5
2	M	214	124	14.01	5.20	5.21	3.59	5.15	2.94	3.18	30	3.8
3	F	217	115	11.83	5.18	3.73	3.98	4.35	2.44	3.44	27	3.5
4	M	210	120	11.99	5.25	3.90	3.20	3.09	2.80	2.45	23	3.4
5	F	188	94	11.64	5.45	3.56	4.60	5.16	2.66	3.29	22	3.7
6	M	212	126	13.07	5.35	4.17	3.37	5.10	2.51	2.71	23	3.8
Htc Mean		207	115.21	12.39	5.30	4.01	3.83	4.57	2.60	12.93	25.83	3.78
Htc Sd		10.77	11.62	0.94	0.11	0.62	0.54	0.80	0.25	0.42	3.65	0.38

Reproductive characteristics of *Chironius flavolineatus* throughout its distribution are available in a few publications. Pinto et al. (2010) analyzed the reproductive biology of *C. flavolineatus* in the Cerrado of southeast Brazil, with an

average clutch size ranging from 3 to 11 and an average egg length of 34.4 mm (Sd = 6.4) (N = 21). The authors pointed out that the species exhibits a seasonal reproductive cycle with vitellogenic follicles and oviduct eggs occurring mainly

during the rainy season. In the Cerrado of Central Brazil, five clutches were described for the Federal District (F. G. R. França, pers. comm.) with clutch sizes ranging from 3 to 8, with an average egg length of 27.9 mm (Sd = 4.3). Also, all females with eggs were found during the rainy season. Hamdan & Fernandes (2015) indicated an incubation time of 96 days for an adult female captured in the central portion of the Atlantic Forest that laid four eggs. These records show that the species presents a conservative reproductive pattern even in different Brazilian ecoregions, with clutch size ranging from 3–11 eggs, egg sizes close to 30 mm in length, and reproductive cycles occurring in rainy seasons.

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