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First report of the reproduction in captivity of the Chocoan Bushmaster, *Lachesis acrochorda* (García, 1896)

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Abstract. The Serpentarium at the Universidad de Antioquia houses four specimens of the Chocoan bushmaster (*Lachesis acrochorda*) in a vivarium simulating natural habitat conditions. After an acclimatization period of nine months the moisture level in the enclosure was increased artificially to induce reproductive behaviour. One male was observed to court a female for 15 days, but copulation was not observed. The female subsequently refused food and 110 days after the last observation of courting 11 eggs (average weight 57.9 g) were discovered. While three eggs were artificially incubated, the rest were left in the vivarium; both sites were monitored continuously. On Day 74 of incubation, the eggs in the vivarium were moved to an incubator due to a drop in temperature. Hatching began on Day 93 and seven eggs hatched during a 3-day period. Average weight (42.0 ± 3.6 g) and length (40.0 ± 1.4 cm) of the neonates was recorded. This is the first report of captive reproduction for *L. acrochorda*.

Keywords. Courtship behaviour, eggs, hatchling, pitviper, vivarium, *Lachesis acrochorda*, Colombia, bushmaster.

Resumen. El Serpentario de la Universidad de Antioquia alberga cuatro verrugosos (*Lachesis acrochorda*) en un vivario que simula las condiciones naturales de su hábitat. Después de un período de aclimatación de nueve meses, se incrementaron los niveles de humedad en el encierro mediante aumento en la frecuencia del riego, con el fin de inducir el comportamiento reproductivo. Posteriormente se observó el cortejo de un macho a una de las hembras durante 15 días, no obstante no se observó la cópula. La hembra dejó de alimentarse durante las alimentaciones subsecuentes y 110 días después del último día de cortejo se encontró enrollada sobre 11 huevos (peso promedio 57.9 g). Tres huevos se incubaron artificialmente y los demás se dejaron en el vivario, ambos se monitoreaban continuamente. Al Día 74 de incubación los huevos que se dejaron en el vivario se trasladaron a incubación artificial, debido a una caída en la temperatura. La eclosión empezó el Día 93 y siete huevos eclosionaron durante un período de tres días. Los neonatos se midieron (40.0 ± 1.4 cm) y pesaron (42.0 ± 3.6 g). Este es el primer reporte de la reproducción de *Lachesis acrochorda* en cautiverio.

Palabras clave. Cortejo, huevos, eclosión, víbora de foseta, vivario, *Lachesis acrochorda,* Colombia, *verrugoso*

Introduction

Vipers of the genus *Lachesis*, commonly known as bushmasters, or *verrugosos* in Colombia, are the longest members of the family Viperidae (de Souza, 2007). As currently recognized, there are four species of bushmasters, two in Central America, *L. stenophrys* (Cope, 1876) and *L. melanocephala* (Solórzano and Cerdas, 1986), and two in South America, *L. acrochorda* (García, 1889) and *L. muta* (Linnaeus, 1766); the latter is an Amazonian taxon comprising two subspecies, *L. m. muta* and *L. m. rhombeata* (Wied, 1824). Zamudio and Greene (1997) confirmed this taxonomic arrangement using mitochondrial DNA data. These snakes have specialized habitats and are found almost exclusively in primary forest, which makes nearly inaccessible to humans and difficult to find. Their diet usually consists of small mammals, which makes them selective predators

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(Campbell and Lamar, 2004). Additionally, they are oviparous vipers, depositing their eggs in underground cavities or the burrows of other animals (Ripa, 1994; Campbell and Lamar, 2004; de Souza, 2007).

Natural History of Lachesis acrochorda.—*Lachesis acrochorda* is a large snake (Ripa, 2001; Campbell and Lamar, 2004), reaching up to 3 m in total length (TL). It is characterized by an arabesque colour pattern on the head, a ventral scale count intermediate between *L. stenophrys* and *L. muta*, as well as a variety of additional distinguishing features (Ripa, 1999, 2001). Its preferred habitat is tropical wet forest from sea level up to 1000 m (Ripa, 1999; Campbell and Lamar, 2004). *Lachesis acrochorda* is distributed in Panamá, Colombia, and Ecuador. In Colombia populations exist along the northwestern Atlantic Coast, along the Pacific Coast and to the south of Antioquia and Caldas in the valleys of the Magdalena and Cauca rivers (Campbell and Lamar, 2004). Although this species is found in remote areas and primary forest, the fragmentation and destruction of their natural habitat and the largely negative reaction of humans to venomous snakes impacts the population. In addition, it is known that bushmasters do not easily adapt to climatic variations caused by deforestation (Vial and Jimenez-Porras, 1967; Ripa, 1994, 1999). For these conservation-oriented reasons, but more importantly to produce antivenom, it is important to maintain and propagate these species in captivity.

Maintaining bushmasters in captivity poses a significant challenge because they are very susceptible to stress, may refuse to feed, and can suffer from maladaptation syndrome (Turner et al., 2008). Simulating their natural environment, absent the stress that arrives with human interactions in the form of keepers, is hard to accomplish, but their reproduction depends on the conditions in which these animals are housed to be optimal and free from stress (Dowling, 1960; Burchfield, 1975; Boyer, 1989; Ripa, 1994, 1999; Turner et al., 2008). Successful captive reproduction has been reported previously for three species of *Lachesis*: Ripa (1994) reported on *L. stenophrys* and *L. melanocephala,* and Boyer et al. (1989) and de Souza (2007) on *L. muta*, the latter on the subspecies *L. m. rhombeata*. The junior author recently participated in a study of *L. stenophrys* in Costa Rica, where successful reproduction occurred (Corrales et al., 2014).

The aim of this report is to describe the reproduction of *L. acrochorda* in captivity, including the process of gestation, oviposition, and hatching of eggs and the growth of the neonates. To the best of our knowledge, this is the first time captive breeding was achieved for this species.

Materials and Methods

Specimens.—Four wild-caught adult specimens (2M, 2F) were donated from E.S.E. Hospital Maria Toro de Elejalde (Frontino) and E.S.E. Hospital San Camilo de Lelis (Vegachí) to the Serpentarium at the Universidad de Antioquia (Medellín, Colombia) between 2007 and 2013 (Table 1). They were collected at La Blanquita, Frontino, Western Antioquia, Colombia (6°45’00” N, 76°18’00” W) and Las Águilas, Vegachí, Northeast Antioquia, Colombia (6°46’23” N, 74°48’06” W).

Initial Preparation.—Each specimen was assigned a collection number (e.g., M1 = male No. 4074, F2 = female No. 4230), weighed, measured, and sexed. They were housed in individual boxes and maintained in the environmental conditions of Medellín, Colombia, with a temperature between 16–28°C and relative humidity between 50–65% (IDEAM, 2005). Snakes remained in quarantine for a period of 90 days, during which they were under constant veterinary supervision. They were dewormed based on the results from stool sampling.

Table 1. Descriptions for the four individuals of *Lachesis acrochorda* used in this study. Specimen numbers indicate sex (M = male, F = female) and breeding attempt (*).

Sex	Length (cm)	Weight (g)	Origin	Time in captivity
M1*	243	4706	Vegachí	5 yr
F1*	168	2569	Frontino	1 yr
M2	154	1483	Frontino	10 mo
F2	162	1859	Frontino	4 mo

Figure 1. *Lachesis acrochorda* female, specimen F1, coiled around her eggs. Photo by Ana M. Henao

Establishment.—At the end of the quarantine period, the animals were moved to an enclosure with length, width, and height dimensions of 3.58 m x 2.51 m x 2.67 m, respectively. The enclosure had a filter-covered drain and a substrate composed of gravel, soil, and rice husks. It included four shelters constructed from roots and tree trunks, a source of water with 40-liter capacity, larger rocks, plants (Araceae, Cannaceae, Ciclantaceae), controlled irrigation twice weekly, a temperature between 19 and 28°C, and relative humidity of 75–95%, all of which simulates the natural habitat of this species.

General maintenance.—Once the animals were established in their enclosure, they fed voluntarily and no dysecdysis was observed. Every 20 days prey was offered, consisting of guinea pigs (*Cavia porcellus*) and rabbits (*Oryctolagus cuniculus*), each weighing 90–180 g. Prey animals were delivered alive using hooks, and were presented individually to snakes according to body size.

Stimulation.—Several authors described that a sudden drop in temperature (Boyer et al., 1989; Ripa, 2001; de Souza, 2007) and an increase in humidity can induce reproductive behaviour in bushmasters (Ripa, 1994). For this reason, the watering frequency was increased to three times weekly in February 2013 so that humidity could be maintained above 85%. At this time the four specimens were housed together in the enclosure.

Observations.—Animal behaviour was directly observed during three 20-min observation periods every day. No cameras were used. Results from all observations were compiled and evaluated.

Results

Courtship Behaviour.—During the first days of June 2013, we observed M1 engaging in courtship behaviour with F1. This consisted of using its head to rub scales against and gently tap the head and neck of F1, while she was coiled. Courtship began on 6 June after 1600 hrs and was also recorded on 15 consecutive days. No copulation was observed. Courtship was intermittent and always started in the afternoon. During the courtship period, which included the time when mating must have occurred, all animals continued to feed voluntarily. M1 never courted F2, and M2 never showed any courtship behaviour because it was still sexually immature.

Gravidity and Oviposition.—Beginning in early July F1 refused to eat, which we suspect indicated that she was gravid. About one week before we found eggs, F1 began to move actively around the vivarium using several different places to rest. On 23 September 2013, ca. 110 days after having observed the last courtship behaviour, F1 was found coiled around 11 eggs (Fig. 1). During the entire next day, F1 remained beside the clutch. Four days later, we attempted to remove the eggs from the vivarium following the recommendations of Boyer et al. (1989), to avoid the potential loss of the eggs if there was a problem with the incubation conditions. However, only the three eggs on the periphery could be extracted singly since the others were adhering to each other in an underground cavity; this made their intact removal one-by-one impossible. These eggs were therefore left in the vivarium under the previously described conditions to avoid any breakage (Fig. 2).

Eggs and Incubation.—The extracted eggs weighed on average 57.9 g and were kept in 1:1 vermiculite : sterile water. The three extracted eggs were incubated at 24.6–25°C temperature and 77.1–80.1% relative humidity. However, none of the three eggs hatched. The characteristics that identified these eggs as non-developing included the presence of wrinkles, compressions, and hardening after 22, 81, and 85 incubation days, respectively. Dissection revealed a reddish spot of what appeared to be the embryo, surrounded by caseous yolk.

The eggs that incubated under more natural conditions in the vivarium did not experience changes in incubation condition and were monitored twice daily. The temperature ranged from 21.3–27.1°C and relative humidity from 78.7–95.6%. Due to low temperatures

Figure 2. Eggs left in the vivarium. Photo by Ana M. Henao.

around 20°C for a one-week period in Medellín, on Day 74 of incubation, the eight adhering eggs were excavated and moved as a unit into an incubator (Model 12-140, Quincy Lab Inc., Chicago, Illinois, USA), in order to provide optimal temperature conditions for the development of the embryos. In the incubator, the temperature remained between 24.5 and 27.7°C, and relative humidity between 78.0 and 91.4%.

Hatching and Post-Hatching.—On Day 93 of incubation (24 December 2013) hatching began and lasted until Day 96. Seven of the neonates hatched in good health (Table 2), but the eighth never fully emerged from the egg.

Hatchlings had an average size and weight of 40.0 ± 1.4 cm and 42.0 ± 3.6 g. The first food, consisting of pinky mice (4-day olds), was offered after hatchlings first shed their skin. We established the initial feeding frequency at every ten days, and the amount of food was determined based on 10% of the body weight for each neonate. At the time of this writing, one year after hatching, all neonates are feeding voluntarily.

Discussion

F1 arrived at TL 168 cm and reproduced a year later, whereas F2 arrived at TL 162 cm, just four months earlier and was not courted by M1 (Table 1). Our observations indicate that courtship behaviour occurred only between M1 and F1. Sexual maturity for *L. stenophrys* and *L. melanocephala* were described by Ripa (1999, 2001), as early as 4 yrs of age for females and 3 yrs of age for males in optimal conditions (with one male performing courtship at 2.5 yrs). Ripa (1999, 2001) noted that these early breeding episodes were not always successful and might contribute to injury of the female. Solórzano (2004) and Corrales et al. (2014) reported sexual maturity at age 5 yrs in captivity for *L. stenophrys*, and that a female of that age may present with a total length of 160 cm. At our facility, M1 courted and copulated with F1 but not with F2, even though the difference in TL between the females was only 6 cm. While we cannot be certain, the absence of any reproductive advances of M1 on F2 could be a sign that F2 had not yet reached sexual maturity. With sexual maturity in *Lachesis* occurring near a TL of 160 cm, our F2 of *L. acrochorda* may have not have reached the critical size for *L. acrochorda.* On the other hand, given its small size, we are almost certain that M2 was immature. Multiple matings, as described by Ripa (1994), were therefore not possible and not observed.

Courtship behaviour observed was consistent with that described by Ripa (1994). This behaviour matches

Table 2. Time of hatching, length, and weight at birth of eight neonate *Lachesis acrochorda*.

Duration of incubation (days)	Total Length (cm)	Weight (g)
93	41,4	43,3
93	39,9	40,6
93	39,5	42,5
94	40,6	47,5
94	39,6	42,8
94	40	45,4
94	40,8	38
96	40	36,3

the experience of the junior author with *L. m. muta* and *L. stenophrys*, namely a beginning of courtship by the male without initial female response, followed after several attempts by the male suddenly beginning to rub the female, and coiling its tail around the last third of her body in order to stimulate the cloacal area (Corrales et al., 2014).

Regarding the oviposition behaviour of *Lachesis* species, Ripa (1994) described that the gravid female explores the site in order to find a place that ensures adequate humidity and temperature and that allows her to coil up completely around her eggs. Such behaviour has been reported both *ex situ* and *in situ* (Ripa, 1994). It has also been reported that in the wild *L. acrochorda* uses burrows of "*guagua* in Colombia" (*Agouti paca*) and *armadillos* (*Dasypus* sp.) to oviposit (Ripa, 1994; De Souza, 2007). In our case the female oviposited in a small underground cavity created with a root of a tree and remained next to the eggs during the first four days of incubation.

During the "natural" incubation of eggs in the vivarium, F1 did not remain beside the eggs but changed her place of rest frequently, in contrast to the behaviour reported by Ripa (1994) for *L. melanocephala* and *L. stenophrys*, species known to provide significant parental care for their eggs. Additionally, F1 never showed aggressive behaviour when the keeper entered the enclosure to assess the state of the eggs. In comparison with the incubation time reported for other bushmasters at 74–79 days (Switak, 1969; Boyer et al., 1989; Ripa, 1994, 1999; de Souza, 2007; Corrales et al., 2014), the 96-day period of incubation may be due to the incubation temperature in the "natural" vivarium not being constant and at times below 24°C, possibly the minimum incubation temperature for successful development (Ripa, 1999, 2001; de Souza 2007). However, Solórzano (2004) and Chacón and Valverde (2004) reported hatching periods for *Lachesis* between 75–90 and 105–108 days, respectively.

Eggs size in bushmasters varies considerably, correlating with clutch size as well as the size and nutritional status of the mother (Ripa, 1999, 2001). It was reported previously that the average weight of the eggs of a clutch of *L. stenophrys* (n = 16) and *L. melanocephala* (n = 22) were 82.0 ± 0.2 g and 75.0 g, respectively (Ripa, 1994; Corrales et al., 2014), which are heavier than that deposited by the female of *L. acrochorda* (57.9 g). The smaller egg size can perhaps be attributed to the age of F1, who, based on her length, is a young female; her exact age could not be confirmed since the animal was not born in captivity.

The death of three eggs extracted on the fourth day could be linked to the low humidity of incubation (77.1–80.1%), consistent with that reported by de Souza (2007). Successful incubation conditions are probably similar to those of the rainforest, with a temperature range of 25–28°C and relative humidity of 80–95%. Based on our experience, we do not recommended separating the eggs once they are adhered together in order to avoid trauma or injury that may prevent successful hatching. In this

Figure 3. (Left) Overview of the vivarium at the Serpentarium of the Universidad de Antioquia (Medellín, Colombia) where a pair of bushmasters (*Lachesis acrochorda*) was successfully captive-bred. (Right) Detail of the vivarium, showing one of the mated individuals (M1) on the trunk. Photos by Ana M. Henao.

case, the eggs that were left in the place selected by F1 for the first 74 days of incubation hatched successfully, as the cavity maintained adequate humidity, preventing them from crinkling or compacting. Finally, with regard to the death of the last neonate, we presume that this could be from exhaustion, which was previously reported by Mader (2006).

An important aspect for the captive breeding of this species is to provide large spaces that simulate the conditions of their natural habitat (Ripa, 1994, 1999; de Souza, 2007). We believe that the successful reproduction of *L. acrochorda* was facilitated by the construction and adaption of an enclosure at the Serpentarium of the Universidad de Antioquia (Medellín) that simulated the conditions of their natural habitat (Fig. 3). This is the first report of reproduction of this species in captivity.

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