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SHORT NOTE

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First report of reproduction in captivity of the Central American bushmaster (*Lachesis stenophrys*) in a European zoo

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The Central American bushmaster (*Lachesis stenophrys*) (Fig.1) is a large species of pit viper. The males are a little longer than the females, averaging of 2-2.10 m and 1.9-2.05 m respectively (Solórzano, 2004). The species lives along the Caribbean versant of Nicaragua to western and central Panama (Campbell & Lamar, 2004), and in Costa Rica is found in tropical wet and subtropical rainforest on the Caribbean versant (Corrales et al., 2014) where the rainfall is very high (3500-6000 mm annual range). Although it can be found at altitudes of 100-700 m its preferences are thought to be 200-400 m (Ripa, 2002).

Lachesis spp. are the only pit vipers on the American continent that lay eggs instead of giving birth to live young (Campbell & Lamar, 2004; Solórzano, 2004). The eggs are deposited in subterranean cavities and the shelters of other animals, with clutches of up to 18 eggs (typically 9 - 13), hatching in 75 – 90 days (Solórzano, 2004). In captivity, females reach sexual maturity at approximately 1.6 m in total length and at about 3.5-5 years of age (Ripa, 2002). There have been several publications describing the husbandry and breeding of *L. stenophrys* in captivity (Ripa, 1994; Ripa 2002; Hohmeister, 2004; Chacón & Valverde, 2004; Corrales, et al., 2014) but we give here the first description of captive breeding in a European zoo.

MATERIALS AND METHODS

Breeding Pair

The breeding pair in this study arrived at Faunia zoo, from Costa Rica, in 2012, as members of a future 1:2 (males/females) breeding project. Their addition to the collection was a great opportunity for us to have a new blood line. The individuals were healthy and growing slowly, the male reached a weight of 4.5 kg the male and the female 3.7 kg immediately before the breeding started in 2019.

Husbandry

The breeding pair were kept separately from each other in two identical glass terraria measuring 200 x 65 x 85 cm (L x W x H). Ventilation was provided by a longitudinal metallic mesh on the top and two circular meshes either side of the terraria. The female was kept behind the scenes, while the male was exhibited to the public. The terraria were equipped mainly with big tree roots, and live plants (*Epipremnum* sp. and *Monstera deliciosa*) offering hiding places. The female´s terrarium also had a restraint area (45 cm x 65 cm x 85 cm) which provides a dark and dry place to rest, destined to become the future nesting site. The substrate consisted of coconut fiber, mulch and a layer of 1-2 cm of dry leaves.

Figure 1. *L. stenophrys*, Parque Nacional Braulio Carrillo, Costa Rica

Ambient lighting was provided by two fluorescent tubes (58 W) and for basking one halogen lamp (50 W) during the spring-summer and a GU10 LED 6500 k during the mid-autumn and winter. The basking lights were on an approximately 12:12 h on/off cycle, however the ambient lights (fluorescent tubes) were timed independently, turning on and off simulating natural cloud cover and sunset. Ambient temperatures fluctuated between 21-24 ˚C during the autumn/winter and 27- 29 °C with a maximum temperature up to 30 °C directly beneath the basking lamp in spring/summer. The temperature and humidity were measured by a TFA 30.5013 digital thermo-hygrometer.

In order to trigger sexual behaviour, we simulated the fluctuation in the environmental conditions described by Corrales et al. (2014). The ambient humidity was held at

around 50-60 % during the coldest months but in spring a fogger was installed that increased relative humidity to 90-95 % and, at the same time, the temperature was increased by 5-6 °C. The fogger used was an Orieme Airsano 3L, located above the terrarium, modified with two large plastic tubes which conducted the moist air inside. In addition, a daily misting was made with a 5 L hand sprayer for the care of the plants but the animals themselves were only sprayed when shedding. An IR nocturnal camera (WiFi Cube Tenda C5S HD 30fps 120 degrees) was installed to record any mating behavior during the night.

Diet

At a frequency of about every 20 days (Ripa, 1994; 2002), the snakes were fed with 1 or 2 small rats (120-200 g, to avoid regurgitation) that were either defrosted or had been freshly killed. However, when the female was younger (about 1 m length) it refused anything other than chicks.

Veterinary monitoring during the gestation period

During gestation the embryos were monitored by both X-ray and ultrasound. The X-ray equipment used was a portable Uni -Travel 70/100, power rating 70 mA-99KV. The snake was held 40 cm from the machine giving 50 mA / 70 KV y 8 mA/s. The ultrasound machine was an Edge II Sonosite system, with a linear transducer that works from 6 to 13 MHz.

Incubation of eggs

The eggs were placed in an incubator (35 x 45 x 60 cm) adapted from one that had been in a wine bar. It had heating as well as an effective cooling system, which is essential during the hot conditions of the Madrid summer time. Within the incubator three eggs were held in a plastic box (28 x 17 x 8 cm) half-filled with vermiculite that had been moistened with water in a 1:0.6 (volume/weight ratio). The eggs were maintained at 26-26.5 °C and the humidity ranged between 75-85 %. Eggs were not turned and were partially embedded in the vermiculite (Fig. 2). In addition, as the eggs shells were somewhat decalcified, to help reduce dehydration we dusted the whole eggs with calcium carbonate powder, a procedure we use frequently when incubating the decalcified eggs of other species.

Figure 2. Eggs of *L. stenophrys* being incubated in vermiculite and dusted with a calcium carbonate to reduce dehydration

OBSERVATIONS

Mating behaviour and copulation

Mating started when the ambient humidity and temperature were increased in mid-February. A change in behaviour was noticed in the male, who was abnormally nervous, moving around the terrarium and flicking his tongue morning and evening. Consequently, on 20 February we decided to put him together with the female. The male was observed approaching the female following her for some hours, rubbing his ventral scales over her body in a fierce way, as previously described Corrales et al. (2014) and Ripa (1994). The first copulation was recorded on the same day inside the restraint area of the female´s terrarium. Early in the morning and for the next three days the pair was seen resting close to each other. The male and the female were placed together twice more (13 March and 3 April) to ensure successful mating. On 13 March we observed the same behavioural pattern seen before, but no copulation was recorded and on the third occasion neither snake appeared interested in the other.

Gestation period

During gestation, the female was as usual resting and only moved when needing to drink, feed, defecate or shed. Four years previously, a breeding female had died due to egg retention (dystocia), so the gestation period this time was highly controlled with reference to previous published studies (Ripa, 2002; Hohmeister, 2004; Corrales et al., 2014) and the opinions of other specialists. The female was fed every 20-25 days during pregnancy and only 5-6 days before oviposition did she refuse food. Clues indicating gestation, or that forthcoming oviposition was imminent, included the female looking for the best place to make a nest and gathering leaves.

Veterinary monitoring

After 100-115 days of gestation, X-rays and ultrasound scans were used to check for signs of dystocia. X-rays were taken of the distal third of the animal, which showed the presence of four masses, presumably eggs that were 4.2-4.8 cm by 3.1-3.5 cm oval shapes with perfectly defined profiles (Fig. 3). Although they had relatively high radio-opacity they are overshadowed by the more radio-opaque intestine. The presence of granular radio-dense material compatible with food in transit was seen in the intestine. The egg masses alternated with each other in order and had a smooth surface but not always perfectly regular. Ultrasound scanning of the distal third of the animal, as expected, showed the presence of several eggs.

Egg laying and incubation

On 26 June (126 days since 1st copulation) a necklace-like group of five tiny follicles (Fig. 4) were seen on the substrate suggesting pre-ovulatory follicular stasis in one of the ovaries. On both the 3th and 4th of July, normal sized but infertile egg was found. Then on 11th July (141 days since 1st copulation) the female was found to be guarding a clutch of five eggs (Fig. 5). A trans-illumination procedure with a

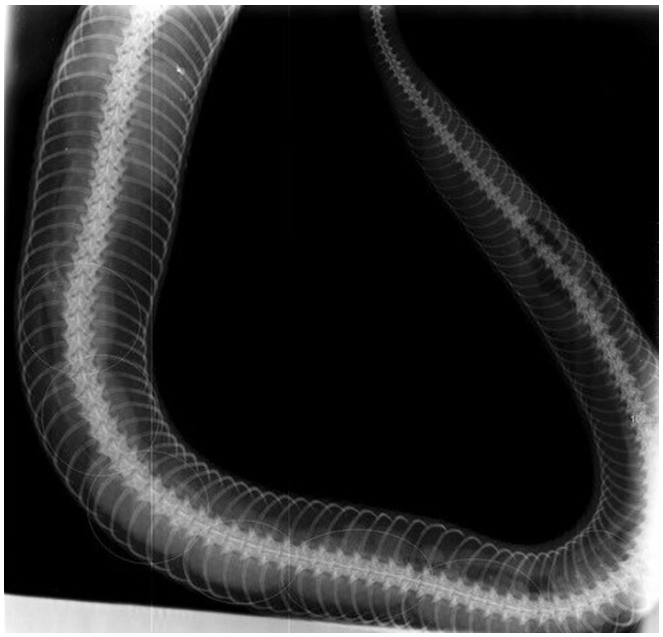

Figure 3. X-ray examination of the distal third of the female *L. stenophrys*, with elipses drawn to show the position of the eggs against the relatively radio-opaque intestine

mobile flashlight revealed a small group of capillaries in two eggs suggesting they were fertile, the other three of poor appearance. All eggs were white to yellowish in colour, and had a soft, but irregular and decalcified shell. For incubation, three of the eggs were removed, coated in calcium carbonate powder, and then carefully placed in the incubator in the plastic box with vermiculite. One week later one egg began to rot (148 days since 1st copulation) and then after 184 days (23rd August) one of the two eggs identified as fertile was found to have become rotten, leaving only a single egg to be incubated. Unexpectedly, the female apparently laid two more eggs on 9th September (201 days after 1st copulation) but these were discarded because they were dehydrated, probably due to long retention (Fig. 6).

Figure 4. A necklace-like group of very small follicles ejected from the female *L. stenophrys*, 126 days after first recorded copulation

Figure 5. The female *L. stenophrys* protecting a newly laid clutch of five eggs

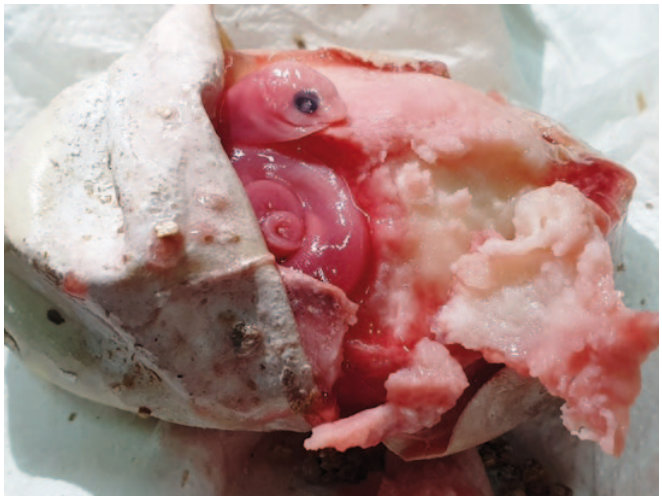

Figure 6. Dead embryo of *L. stenophrys*, note the dehydration of the vitellus, in an eggs that was retained for 60 days beyond the time that other successfully fertilised eggs were laid

After 78 days of incubation, on 26th September (218 days after 1st copulation), hatching from the single remaining egg began. Longish slits were made at the head end of the egg (Fig. 7), the snout of the hatching neonate protruded for some hours, and hatching was completed during the night. The neonate weighted 45g and was 41 cm long.

Figure 7. At early hatching stage, only the snout of the juvenile *L. stenophrys* is protruding

CONCLUSIONS

Ex situ breeding of *L. stenophrys* proved to be a difficult task as evidenced by the low fertilisation rate, long retention of some eggs, and apparently some pre-ovulatory follicular stasis associated with one ovary. At least in the case of pre-ovulatory follicular stasis the cause often relates to problems with husbandry (DeNardo, 2006). For future breeding attempts, veterinary monitoring of the process, especially between copulation and egg laying, is recommended as it enabled us to confirm that the female was pregnant. Consequently, we were able to decide when to take husbandry decisions such as to stop feeding, provide a bigger nest, and leave her undisturbed. We could also 'estimate' the egg laying date and if there been signs of distocia then surgery could have been undertaken while the female was still healthy. The high proportion of infertile eggs observed on this occasion could be related to this being the first breeding attempt of this female or low fertility of the male. The final number of eggs (9) laid is well within normal limits for this species (Ripa, 2002; Corrales et al., 2014) but the single neonate obtained had a weight and length at the lower end of the normal range (Hohmeister, 2004; Corrales et al., 2014).

The timing of mating may fluctuate between February to April (Corrales et al., 2014) and the snakes are best paired at some time in this period. Mating can be stimulated by increasing ambient humidity and temperature and the timing of pairing made more precise by watching out for changes in male behaviour. Normally, eggs are deposited from June to August and birthing takes place from August to October. Consequently, the typical period from copulation to hatching takes about 180 days. In our case the single neonate that emerged did so 218 days after first copulation, somewhat later than might be expected.

The humidity and temperature of the incubation substrate used in this study would seem to be the best recommendation for this species (Ripa, 2002; Corrales et al., 2014). Covering decalcified eggs with a thin calcium layer could help to protect them against natural dehydration and opportunistic pathogens, but further tests with a larger sample size are required before any definite recommendations can be made.

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