HERPETOCULTURE

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Reproduction of *Lachesis stenophrys* (Central American Bushmaster) at ZSL London Zoo

Lachesis stenophrys, the Central American Bushmaster, is a species of pit viper native to Nicaragua, Costa Rica and Panama, inhabiting hilly forested environments from ca. 100–1000 m elevation (Ripa 2002; Campbell and Lamar 2004). It is among the longest species of vipers in the world, with adults averaging over 200 cm total length (TL; Solórzano 2004) and frequently over 4 kg in weight (Corrales et al. 2014). The genus *Lachesis* is unique among new world pit vipers (except possibly *Bothrocophias colombianus*) in that it lays eggs rather than giving birth to live young (Campbell and Lamar 2004; Solórzano 2004). In captivity, females may attain sexual maturity at around four years of age, or 160 cm TL (Ripa 1994; Solórzano 2004). Herein we present the first documented breeding of the species in the United Kingdom and provide additional insights into the successful husbandry and reproductive biology of this uncommon species.

Origins of Animals and Husbandry

The Zoological Society of London, London Zoo acquired three one-year-old, captive-bred *L. stenophrys* in June 2016 from a facility in France. These snakes were kept individually in off-exhibit naturalistic housing in a climate-controlled room set to 25.0°C during the day and 23.0°C overnight, with Arcadia 6% T5 exhibit lighting and fluorescent T8 room lighting on a 12:12 cycle creating a thermal and UVB gradient in each exhibit. From an initial weight of 230–265 g (mean: 248 g) in June 2016, the group reached 1263–1438 g (mean: 1348 g) by March 2019. At this time, a male and a female were moved to a display exhibit in the Reptile and Amphibian House.

The exhibit was an irregular pentagon in shape; three sides had large viewing windows and the other two were internal walls. The ground area measured approximately 4.5 m^2 and was themed in a natural way using a variety of substrates, plants, and pieces of wood to create structure and hiding places (Fig. 1). An automatic misting system (MistKing Starter v5.0, Jungle Hobbies Ltd, Ontario N9E4R3, Canada) controlled precipitation and a hot air blower controlled by a digital thermostat with day and night set points controlled ambient temperature; both systems were adjusted throughout the year in accordance with natural seasonality. Diurnal and nocturnal ambient temperatures ranged from 21.0 and 20.0°C, respectively from January to March, to 24.0 and 22.0°C from April to September, to 22.0 and 21.0°C from October to December. Enclosures were sprayed with water throughout the year, usually for 1–2 min at a time several times per day between 0900 and 1700 h, but more heavily from November to March.

Ambient lighting consisted of twelve 1150 mm T5 lamps, comprising two 12% Arcadia T5 lamps and various brands of non UVb-emitting T5 lamps, as well as a clustered pair of Arcadia 160W mercury vapour lamps. Together, these created an ambient UV index (UVi) of 0.5 at ground level. While the exhibit did initially have a basking zone creating surface temperatures of 31.0°C with a UVi of 2.0, the mercury vapour and fluorescent lamps were often switched off to assist in maintaining appropriate ambient temperatures. The mercury vapour lamps were replaced with two single 18W LED lamps in April 2020 to limit heat input to the exhibit. These lights were maintained on an approximately 11:13 h photoperiod during the

DANIEL KANE*
BENJAMIN TAPLEY
CHESKA SERVINI
UNNAR KARL AEVARSSON
CHRISTOPHER J. MICHAELS
ZSL London Zoo, Zoological Society of London, Regent's Park, London, United Kingdom, NW1 4RY

**Corresponding author; e-mail: daniel.kane@zsl.org*

Fig. 1. *Lachesis stenophrys* exhibit at ZSL London Zoo: January 2021.

Table 1. Comparative reproductive data for *Lachesis stenophrys*. Snake weights reported from this study are from five individuals, length is from three individuals. All values are reported as mean (range). Mass is reported in g and lengths are reported in mm.

Female mass	Clutch size Size	Egg length	Egg width	Egg mass	Clutch mass	Incubation temp. (°C)	Incubation period (d)	Fertilization (%)	Hatchling weight	Hatchling length	Observation type	Reference
1600	7	87 (80–92)	41 (39–42)	–	526	24.5–26.0	77–79	71	67 (64–70)	467 (461–484)	Captive	This study
3300–3700	9 (7–13)	70	–	82	–	26.0–30.0	–	–	63.6 (45–70)	447.9 (380–480)	Captive	Corrales et al. 2014
3700	9	–	–	–	–	26.0–26.5	78	–	45	410	Captive	Camina et al. 2020
–	16	76	63	–	–	–	74–76	–	45	406	Captive	Ripa 1994
–	11	64.15 (60.3–70.9)	40.3 (40.1–40.5)	69.7–74.2	–	–	106.6 (105–108)	100	55.1 (52.6–59.3)	486.7 (480–490)	Recent captive	Chacón & Valverde 2004

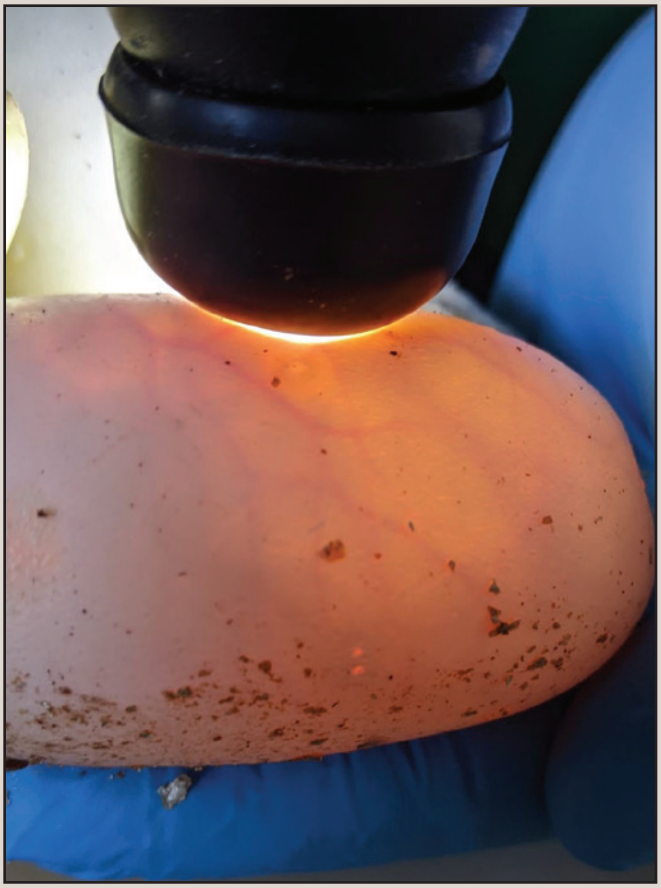

Fig. 2. Illuminated egg of *Lachesis stenophrys* showing healthy vascularization at seven days post-oviposition.

'cool season' and 13:11 h during the 'warm season' cycle. Snakes were fed every 7 to 28 d exclusively on defrosted, pre-warmed commercially-bred rodents which comprised ca. 6–10% of the snake's body weight, never exceeding 16% (Ripa 2002).

Reproduction

We did not observe any copulation and animals cohabited continuously for a period of 16 months prior to egg laying. The breeding female, which weighed around 1600 g when we suspect she mated, consumed a meal 26 days prior to laying eggs and was not offered a subsequent meal prior to laying eggs, so may have continued to eat as normal as reported in Camina et al. (2020). This snake sloughed her skin 37 days prior to laying and again 24 days post-lay.

During the weeks prior to egg laying, the female appeared restless and would become active around one hour before the lights turned off in the exhibit. At 1800 h on 21 July 2020, the female was observed beneath a cork tube that was partially buried in the substrate, coiled around four eggs; body contractions indicated that more eggs were due to be laid. The following morning the female was very alert while being removed from her eggs but did not strike. Seven eggs which adhered together were collected from the exhibit for artificial incubation: total clutch mass was 526 g. The eggs' dimensions were larger than previously reported records in the literature (Table 1). Pre- and post-laying body weights for the female were 2376 and 1770 g, respectively, representing a relative clutch mass of 26%.

Fig. 3. Hatching *Lachesis stenophrys* at ZSL London Zoo, October 2020.

Medium-grade vermiculite mixed with reverse osmosis water at a ratio of 1:1 by weight was used as the incubation media, and all eggs were placed in the same translucent plastic container within a SciQuip Incu-90C incubator (SciQuip Ltd, Shropshire, UK) set at a constant 26.0°C. Due to issues with space, the eggs were later transferred to another incubator set to 26.0°C that featured heat pads mounted on the walls and floor that were controlled by HabiStat thermostats (Ely, United Kingdom). However, night-time temperatures dropped as low as 24.5°C most nights due to the poor temperature control of this unit. Eggs were incubated together in a clump as they were laid, with those on the bottom being half-buried in the vermiculite. Shortly after incubation began, two eggs were discarded as they were not viable and started to discolour, whereas the remaining five eggs showed strong vascularization when illuminated with a torch and had a healthy off-white external coloration (Fig. 2). Incubation period ranged between 77 and 79 d (Fig. 3), with the time individual snakes took to pip and leave the egg varying from one to two days. Upon their removal from the incubation container, each hatchling was weighed with a digital balance (Salter 1160BKDR). Weights ranged between 64–70 g with a mean weight of 67 g; these are substantially heavier than values reported in the literature (Table 1).

Each hatchling was housed individually in a 45 cm^3 Exo-Terra vivarium furnished with a substrate of peat-free compost, a layer of mixed leaf litter with a hide, a small water bowl, live *Philodendron* or *Epipremnum* sp., and a cut *Ficus* sp. branch to provide shade. Enclosures were lit with a single Arcadia 6% T5 fluorescent lamp with reflector, creating an ambient UVi at ground level of 0.7. Enclosures were situated in a climate-controlled room set to 25°C in the day and 21°C at night. Hatchlings sloughed their skin between 17 and 22 d post-hatching and each juvenile accepted a first meal of defrosted and warmed day-old mouse with little encouragement between 29 and 31 d post-hatching. Husbandry was free of problems provided that the substrate where the snakes sat remained dry while ambient humidity was high; wet substrates rapidly led to erythema of ventral scales, which resolved quickly after substrate was changed or dried out.

Discussion

This reproductive event represents the second time *L. stenophrys* has reproduced in a European zoological institution. As with Camina et al. (2020), we experienced less than total fertilization (71%); however, all viable eggs hatched successfully. The clutch size of seven eggs falls within the normal parameters for this species (Table 1), albeit at the low end, and all neonate weights were toward the upper end of what was reported by Corrales et al. (2014) and above what was reported by Ripa (1994) and Camina et al. (2020). Maximum individual egg size was larger than what had previously been reported in the literature (Ripa 1994; Chacón and Valverde 2004). Incubation period fell between previously reported values of 74–108 days (Ripa 1994; Chacón and Valverde 2004). At the time of mating, the female weighed considerably less (ca. 1600 g) than what has been reported for breeding females (Corrales et al. 2014; Camina et al. 2020) despite producing a normal clutch size, large eggs and large neonates. Good nutrition was a potential factor influencing this. As noted in other reproductive accounts (Ripa 1994; Corrales et al. 2014; Camina et al. 2020), an increase in precipitation in the cooler part of the year seems to have encouraged mating activity.

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HERPETOCULTURE NOTES

CAUDATA — SALAMANDERS

***EURYCEA RATHBUNI* (Texas Blind Salamander). Cannibalism.** Although diminutive (<135 mm SVL), *Eurycea rathbuni* is the top predator in the San Marcos Pool of the Edwards Aquifer in Hays County, Texas, USA. It feeds primarily on small invertebrates such as amphipods, shrimp, and aquatic snails. In captivity, *E. rathbuni* has been observed to indiscriminately consume all invertebrate prey types offered (Longley 1981. Int. J. Speleo. 11:123–128). The broad snout, long jaw, and wide head of this species suggests that it may be well-suited to eating large prey. It is plausible that they are opportunistic feeders that utilize their large heads not only to locate invertebrate prey, but also to consume conspecifics. While oophagy has been reported in *E. rathbuni* (Mendyk et al. 2018. Herpetol. Rev. 49:485–486), cannibalism among adults has not been previously reported. Herein, we report the first documented observations of adult cannibalism in both captive and wild *E. rathbuni*.

The San Antonio Zoo Center for Conservation and Research (SAZ CCR) maintains a population of ex-situ *E. rathbuni* for research purposes. SAZ CCR began trapping for this species at four sites (Johnson's Well, Primer's Fissure, Rattlesnake Cave, and Rattlesnake Well) in San Marcos, Texas, USA in January 2016 (USFWS permit numbers: TE800611-7 and TE081884-0, and TPWD permit numbers: SPR-0116-022 and SPR-0513-065). Half of the salamanders captured from each location were collected according to permit requirements and brought to SAZ CCR. Sex was visually determined for all individuals. Eighteen salamanders were brought to the zoo between January 2016 and January 2017 and were housed individually in 20-gal high Aqueon® aquaria with glass lids (63.5 cm in length, 33.66 cm in width, 43.82 cm in height) or in 13-gal Carlisle® food-safe containers (66.04 cm in length, 45.72 cm in width, 22.86 cm in height) with lids. Each enclosure contained pieces of limestone rock and artificial plants.

From January to March 2017, ten *E. rathbuni* were divided among three 20-gallon high Aqueon® aquaria in preparation for a breeding trigger assessment. Cannibalism was presumed in one of these aquaria, which contained three salamanders of both sexes from Primer's Fissure. Salamanders in this group were photographed before being placed in the tank together. As with other central Texas *Eurycea*, *E. rathbuni* have a unique pattern of pigmented spots on their heads that can be used to identify the individual (Bendik et al. 2013. PLoS ONE 8:e59424).

On 28 October 2017, one *E. rathbuni* was found missing from its tank. The tank contained salamanders with the assigned individual identification numbers: ER013, ER004, and ER008. The missing salamander, ER004, was identified by comparing photos with those of the two remaining salamanders. Staff members were uncertain if all three salamanders were accounted for the day prior to the disappearance of ER004 but noted that all three were accounted for at least two days prior to the disappearance. The tank was thoroughly inspected, but no trace of ER004 could be found. After a thorough search of the entire building and a review of the security camera outside of the building to rule out theft, it was assumed that ER013 had consumed ER004 after a staff member noticed that ER013 had a noticeably distended abdomen with a dark blue coloration. Radiographs were taken of ER013 to look for evidence that it had consumed ER004, ca. 1 h after discovering that ER004 was missing. No extraneous bones were observed in the stomach, which would appear to indicate that ER013 had not eaten another vertebrate. ER013 was transferred to an individual tank following this event and monitored. In the following days, it passed at least three large feces, which was highly unusual for this species at SAZ CCR on the normal feeding schedule of twice per week. There was a 17.72 mm difference in SVL between the presumed cannibal and the victim.

On 27 March 2018, five *E. rathbuni* from Rattlesnake Well were placed together in a 100-gallon Rubbermaid® tub (134.62 cm in length, 78.74 cm in width, 63.5 cm in height) for breeding purposes. The tub did not contain a lid, and the water depth in the tub was ca. 15.2 cm. Artificial plants and layers of window screen were added to provide surface area and refuge. Salamanders in this group were administered visible implant elastomer tattoos consisting of a red and/or blue dot at specific points (right/ left, shoulder, pelvis, tail) prior to being placed

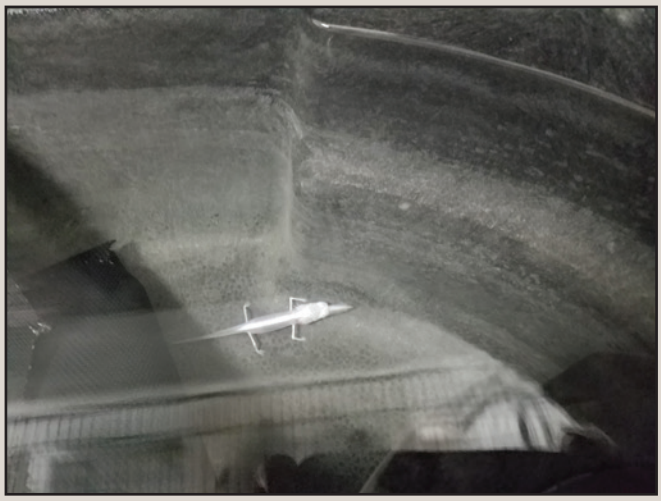

FIG. 1. ER015 found in the process of consuming ER019.

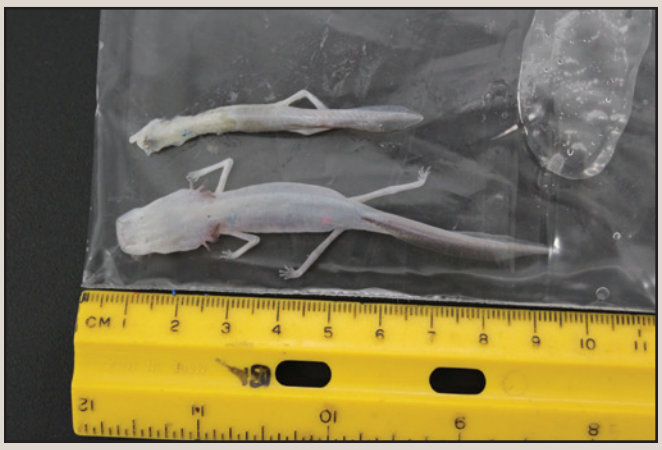

FIG. 2. Measurements taken of ER015 (bottom) and ER019 after it was extracted from ER015's mouth. There was a 15.68 mm difference between the two individuals.

FIG. 3. Female *Eurycea rathbuni* cannibal from Primer's Fissure, Texas, USA attempting to consume her second documented conspecific. The salamander was extracted from her mouth and lived.

in the tank together. In the afternoon of 15 August 2018, one of these five *E. rathbuni* was discovered in the process of consuming another salamander. Approximately 1 cm of the victim's tail was protruding from the cannibal's mouth (Fig. 1). The victim was extricated from the cannibal's mouth, and it was noted that the head appeared to have already been mostly digested.

Both salamanders involved were identified based on their elastomer tattoos. The partially digested salamander was identified as ER019 and the cannibal was identified as ER015. Subject ER019 was preserved in ethanol after measurements and photos were taken of both salamanders (Fig. 2). A thorough search of the tub was undertaken, and it was discovered that another salamander from the same tub, ER020, was missing. A thorough search of the building turned up no evidence of ER020, and it was assumed that this salamander was also a victim of cannibalism. The cannibal and the remaining two living salamanders from that tub were immediately moved into individual tanks. The SVL of the cannibal was 15.68 mm greater than its confirmed victim.

In addition to the observations of cannibalism among captive salamanders, we observed one incidence of cannibalism in the wild. A large female salamander captured in a trap in Primer's Fissure on 1 April 2019 was brought back to the zoo. While collecting morphometric data, it regurgitated a smaller conspecific. The cannibal's SVL was 74.24 mm, and the victim's SVL was 34.22 mm (a difference of 40.02 mm). The sex of the victim was unknown.

On 8 May 2020, the above cannibal salamander was grouped with 1.2.1 other *E. rathbuni* for breeding. Feeding was increased to three times per week. On 8 June 2020, the known cannibal salamander was found with the head and shoulders of another smaller female (30.27 mm SVL) in her mouth (Fig. 3). The animals were netted, and the smaller female was released alive. All salamanders were then housed separately.

Factors that may trigger cannibalism include resource scarcity, density, and opportunity. Cannibalism may be a widespread occurrence in this species, given its life history (long lifespan, slow growth, low metabolism, and low reproductive rate) and the stable, low energy environment in which it occurs. Institutions that house this species should take care to prevent cannibalism from occurring, including housing only salamanders of similar size together, maintaining low tank densities, and ensuring that adequate food is always provided.

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MASON LEE (e-mail: masonmlee3@gmail.com), **ANDY GLUESENKAMP** (e-mail: andy.gluesenkamp@sazoo.org), and **BEKKY MUSCHER-HODGES**, San Antonio Zoo Center for Conservation and Research, San Antonio, Texas 78212 USA (e-mail: bekky.muscher-hodges@sazoo.org).

CROCODYLIA — CROCODILIANS

***CAIMAN YACARE* (Yacare Caiman). TRIPLETS.** The Broad-snouted Caiman (*Caiman latirostris*) and Yacare Caiman (*Caiman yacare*) are the two crocodilian species that inhabit Argentina (Prado et al. 2012. Cuad. Herpetol. 26. Suppl 1:403–410). They can be easily differentiated based on species-specific morphological features (e.g., cranial and nuchal structures). These two species are broadly sympatric throughout their distribution in Argentina, although the *C. latirostris* appears to be allopatric across a larger area.

In Argentina, ranching occurs for both caiman species, with wild, field-collected eggs placed into artificial incubators until hatching. Once hatched, caiman are raised in captivity until they reach 1.2 m in total length, at which point they are harvested for their skins and meat; additionally, some individuals are released back to the wild. Results from these monitoring programs have indicated that the harvest of eggs from both species is sustainable, with populations continuing to increase over time (Larriera et al. 2008. Publicación Asociación de Amigos de Doñana 18:141–180). Argentinian ranching programs are recognized for their contribution to the conservation and biological knowledge base of both species (Villamarín et al. 2021. *In* Zucoloto et al. [eds.], Conservation Genetics of New World Crocodilians, pp. 1–30. Springer, Cham).

An important part of the program involves recording nesting details (e.g., GPS coordinates, number of eggs) and incubation and hatching data (e.g., incubation temperatures, time taken to hatch). After eggs arrive from the field, they are measured (length and width) and placed into plastic incubation containers inside an incubator. Most eggs from the same nest are usually of a similar size, unless they contain more than one embryo.

Although generally uncommon, multiple offspring hatched from the same egg has been reported for several oviparous reptiles. Twinning is more common than triplets, with very few cases of triplets documented in reptiles (Krauss and Horn 2004. Reptiles Austral. 1(4):14–15). Twinning has been reported in snakes (Marion 1980. Kansas Acad. Sci. 83:98–100), lizards (Hartdegen and Bayless 1999. Herpetol. Rev. 30:141; Mendyk 2007. Biawak 1:26–28; Leaché et al. 2013. Genome Biol. Evol. 5:2410–2419), turtles (Tucker and Janzen 1997. Copeia 1997:166–173; Cooper 2009. Introducción a la Medicina Forense Veterinaria Comparada México: Ed. Acribia; Piovano et al. 2011. Folia Zool. 60:159–166) and crocodilians (Webb et al. 1998. Crocodiles of Australia. New Holland Publishers,

FIG. 1. *Caiman yacare* eggs of varying sizes from the same nest. Red line = 10 mm.

Australia; Platt et al. 2011. Zoo Biol. 30:1–12). Here, we report a case of triplets in *C. yacare*.

On 31 January 2020, 26 eggs were collected from a wild *C. yacare* nest as part of the egg ranching program in Banco Payagua, Formosa, Argentina (26.7069°S, 58.3363°W). Seven of these eggs were of an unusual size and elongated in shape (Fig. 1). The eggs were artificially incubated at 30–32°C in a moist vermiculite substrate. After 32 d, 25 hatchlings emerged from 22 of the 26 eggs. Twenty eggs produced single hatchlings, whereas one egg produced triplets, and another egg produced twins. The remaining four eggs did not hatch, but one of them also contained three incompletely developed embryos. Hatching success (# of hatchings / # of eggs collected and incubated) for this nest was 0.91. The eggs that contained triplets were much larger than those that contained single embryos, but not the egg containing twins. The hatchling triplets were also noticeably smaller than single hatchlings, although measurements were not taken.

As it is a seemingly rare occurrence, limited information is available on the hatching of multiple offspring from single eggs in oviparous species. Unless these events occur in captivity, it will be difficult to determine how often this phenomenon occurs in a species because it cannot easily be observed in nature. As far as we can determine, this is the first record of triplets in a crocodilian species.

GERARDO G. VEGA, CAECE University and Caimanes de Formosa S.R.L. Formosa Industrial Park CP3600, Formosa, Argentina (e-mail: vgerardo78@hotmail.com); **PABLO SIROSKI**, Laboratorio de Ecología Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet Litoral) UNL - CONICET, Santa Fe, Argentina (e-mail: pablo.siroski@icivet.unl.edu.ar).

SQUAMATA — LIZARDS

***GARTHIA GAUDICHAUDII* (Chilean Marked Gecko). INCUBATION PERIOD.** *Garthia* is a genus of relatively small (<40 mm SVL) nocturnal geckos (Infraorder Gekkota) and the only genus of reptile endemic to Chile. It is composed of two species: *G. gaudichaudii* and *G. penai* (Demangel 2016. Reptiles en Chile. Fauna Nativa Ediciones. 619 pp.). *Garthia gaudichaudii*, the largest species (39 mm SVL), inhabits semiarid coastal environments of central-northern Chile (Demangel 2016, *op. cit.*). The reproductive biology of this species is scarcely known and is based on anecdotal observations. Females can lay one or two semi-spherical calcareous eggs in communal clutches, which can be found under boulders, rocks, or vegetation (Donoso-Barros 1966. Reptiles de Chile. Ediciones Universidad de Chile. 458 + CXLVI pp.). The eggs are laid between October and December (austral spring) and hatching begins in March (end of the austral summer; Donoso-Barros 1966, *op. cit.*). Under captive conditions oviposition of a single egg was observed during November and the hatching of a juvenile; however, there is no information on the time to hatching (Marquet et al. 1990. J. Herpetol. 24:431–434). Here, we report the incubation period of an egg of *G. gaudichaudii* under laboratory conditions.

On 13 November 2019 during an ongoing study of *G. gaudichaudii* near the coastal area south from the town of Tongoy, Coquimbo Region, Chile (30.3045°S, 71.5101°W; WGS 84; 60 m asl), we manually captured an adult female (32 mm SVL) found under a boulder. The female was transported in a plastic box to the laboratory, located in Santiago, Chile, and upon arrival at the laboratory it was determined to be gravid and relocated to an individual plastic box (20 x 15 x 15 cm) containing an absorbent paper as substrate, a folded piece of cardboard as shelter, and a 6 ml water container. Water was provided ad libitum, and the female was fed three times per week with flour beetle larvae (*Tribolium* sp.) that were dusted with vitamins and calcium (SERA Reptimineral C). The box was placed in an indoor vivarium with natural light from a nearby window and continuous ventilation, with conditions mimicking those recorded in the field site during normal spring days, photoperiod 14:10 h light:dark, and temperatures ranging between 24° and 30°C. Additionally, an infrared light (150 W) was provided.

On 21 November 2019, the female laid one egg. Immediately after, the female was placed in a new individual box to prevent potential damage to the egg. The egg was left undisturbed for the remaining incubation period and visually inspected daily. Temperature during the entire incubation period ranged from 27–32°C. On 4 February 2020 (76 days post-oviposition), the hatchling was observed for the first time (Fig. 1). Its body

PHOTO BY NICOL QUIROGA

Fig. 1. One-day-old hatchling of *Garthia gaudichaudii*.

presented a black coloration and had transverse orange bands only on the tail decreasing in size from the tip to the base (Fig. 1), as documented for juveniles of *G. gaudichaudii* observed in the field (Demangel 2016, *op. cit.*). The hatchling had the following measurements: 16.89 mm SVL, 19.11 mm tail length, and 0.15 g. After measurements, the hatchling was euthanized for further studies by an intracoelomic injection of sodium pentothal at 5% (100 mg/kg).

The authors believe this is the first documentation of the incubation period for *G. gaudichaudii*. This piece of information will be relevant in studies on its reproductive biology as well as in future breeding and ex situ conservation projects of this endemic species.

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CLAUDIO REYES-OLIVARES, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70005, Correo 7, Santiago, Chile (e-mail: creyeso@ug.uchile.cl); **SOPHIE DE BONA** (e-mail: sophie.debona@gmail.com), **FRANCISCA FARÍAS** (e-mail: fcafariasp@hotmail.com), **ESTEBAN SAN JUAN** (e-mail: egsanjuan@gmail.com), **NICOL QUIROGA** (e-mail: nicol.quiroga@ug.uchile.cl), **CAREZZA BOTTO-MAHAN**, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile (e-mail: cbotto@uchile.cl); **JUANA P. CORREA**, Facultad de Medicina Veterinaria, Universidad San Sebastián, Concepción 4080871, Chile (e-mail: juana.correa@uss.cl).