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Front Cover: The Quindío robber frog *Pristimantis alalocophus*, rediscovered in Colombia after 24 years, photographed by Cristian González-Acosta. See article on p.38.

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Reproductive husbandry of the rat snake *Elaphe moellendorffi*

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ABSTRACT - Möllendorff's rat snake *Elaphe moellendorffi* is a large colubrid snake that has only been propagated a few times in captivity, predominantly in private collections. Chester Zoo successfully hatched 6 rat snakes in October 2019. Prior to introduction, the adult snakes were exposed to a cycling regime that simulated natural seasonality. Eggs were incubated and the young snakes hatched after 80-85 days and had their first slough 13-15 days post hatching. Hatch weights ranged between 19 and 20.6 g. Through a degree of manipulation, the neonates fed successfully 4 days after their first slough.

INTRODUCTION

Möllendorff's rat snake *Elaphe moellendorffi* (Boettger, 1886) (Fig. 1a) is a large colubrid. Adults are typically 180 cm to 250 cm long, weigh up to 2 kg (Gumprecht, 2004) and are sexually dimorphic with males being significantly larger and with much wider heads than females (Gumprecht, 2004). The species is confined to extreme southern China (Zhao & Alder, 1993; Zhou et al., 2012) and northern Vietnam (Schulz, 1996) being found at 30 m to 300 m a.s.l. (Zhou et al., 2012). They inhabit deciduous forest hillsides, the edges of rice fields, meadows and bamboo thickets near water (O'Shea, 2018; Zhou et al., 2012; Gumprecht, 2004) and favour crevices, rocky outcrops and karst limestone cave systems, particularly during the brumation period; this begins in November (Gumprecht, 2004). The distributional range of the species lies in the transition area between the tropics and sub-tropics with a climatic profile of long, warm summers and short, cool winters (Gumprecht, 2004). In January, temperatures are as low as 8 °C whereas during

the warmest months, May to August, temperatures can be as high as 30 °C (World data, no date). These rat snakes have a dietary preference for mammals (O'Shea, 2018), although, adults have also been reported feeding on birds, lizards and frogs (Mehrtens 1987; Zhao, 1998; Gumprecht, 2004). Little is known about the reproductive biology of this species (Gumprecht, 2004), but like most other rat snakes it is oviparous (O'Shea, 2018) with clutch size ranging from 5-15 eggs (Gumprecht, 2004; Köhler, 2005; Zhao, 1998).

In 2012, the species was assessed by the IUCN as Vulnerable (Zhou et al., 2012). It is heavily traded with its meat used for food and medicinal liquor, whilst the skin is used for making clothing accessories (Zhou et al., 2012). In the 1950s the estimated population, in China, was 600,000, which had dropped to 50,000 in the 1990s (Zhao, 1998; Zhou, 2004). In captivity, the species has only rarely been propagated, mainly in private collections. Here we describe the captive husbandry and management of this species and provide the first documented breeding of the species within a European Zoo.

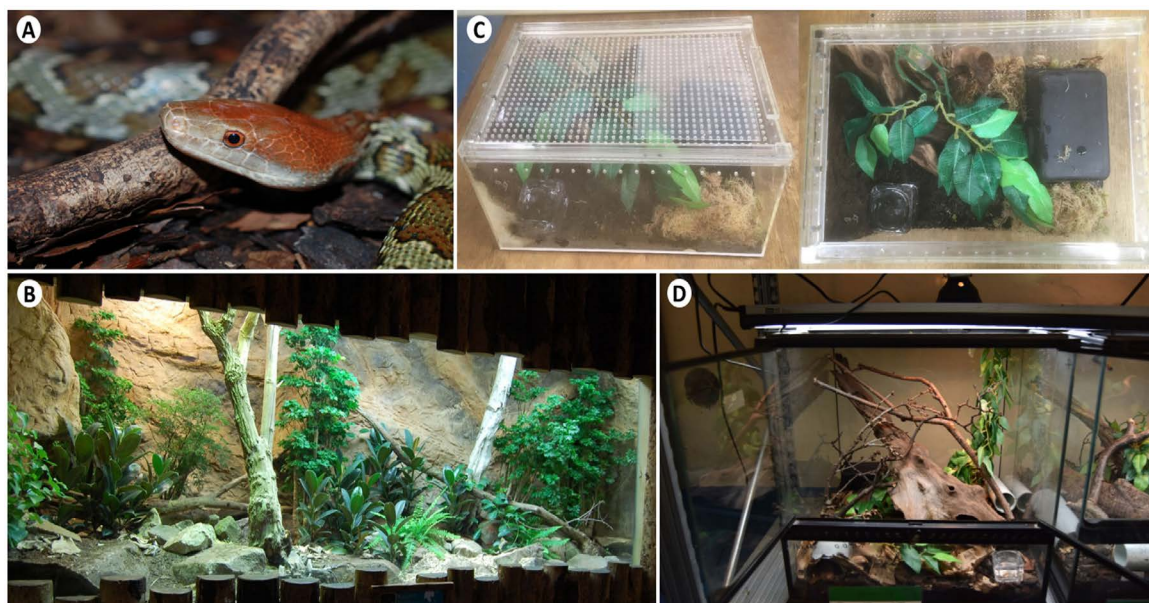


Figure 1. Captive care and husbandry of *E. moellendorffi* - **A.** Adult female *E. moellendorffi* at Chester Zoo, **B.** Display exhibit for adult snakes, **C.** Initial housing used for six *E. moellendorffi* hatchlings, **D.** Final set up of a 45 x 45 x 45 cm Exo-terra to house the hatchlings individually

Table 1. Enclosure parameters for the on-show breeding facility, the off-show brumation facility (male only), and the two neonate enclosures for the captive *E. moellendorffi*

	Display (breeding), Fig. 1b	Off-show (brumation)	Neonate Enclosure 1, Fig. 1c	Neonate Enclosure 2, Fig. 1d
Size (L x W x H)	350 x 120 x 150 cm	'Herptek' vivarium, 180 x 90 x 50 cm	Acrylic boxes 30 x 20 x 15 cm (reptiles.swelluk.com)	Exo-terra terrarium; two size 45 x 45 x 30 cm, two size 45 x 45 x 45 cm and two size 60 x 45 x 30 cm (Exoterra.com)
Substrate	Bark mulch and sand (70:30) and leaf litter	Composted bark fines and leaf litter	Soil with 50% sphagnum moss over	Bark fines (5 cm deep) and leaf litter and turned when overly damp.
Furnishings	Rocks to create multiple refuges, 4 large branches, rear and side rockwork and a pool for water	Wood, branches and commercial reptile hides large enough for full retreat, plus large water bowl	Plastic hide, tube and piece of wood. Small water bowl refreshed daily	Three hides (two with sphagnum moss), wood and branches. Small water bowl refreshed daily.
Planting	<i>Ficus elastica</i> , <i>F. lyrata</i> and <i>F. triangularis</i> (up to 1.5 m) and <i>Stephanotis floribunda</i> for ground cover	None	Plastic plant	Sphagnum moss and plastic plant
Lighting	2 x LightWave T5 Units (Growth Technology, UK) 8:16 (timer setting) but actually 10:14 due to natural daylight from the building roof.	54 W D3+ 12% Arcadia T5 lamp (Arcadia, UK)	Arcadia 54 W D3+ 12% T5 lamps (Arcadia, UK)	Arcadia 54W D3+ 6% T5 lamps (Arcadia, UK)
Photoperiod	10:14 due to natural daylight from the building roof.	9:15	9:15	9:15
Solar gradient*	0-3 UVI	0-3 UVI	0-1.5 UVI	0-2.8 UVI
Basking area	26-35 °C, Arcadia 160 W mercury vapour basking lamp (Arcadia, UK). UVI range 2-3	26-35 °C, 35 W UV-HID Solar Raptor lamp (Econlux GmbH, Germany) and a 50 W Arcadia 'Deep Heat Projector' (Arcadia, UK).	27.5 °C, Heat mat on between 09:30-16:30 h	27-32 °C, 54 W D3+ 6% T5 lamps (Arcadia, UK)
Temperature	Changed seasonally (see Fig. 1)	Autumn: 16-19 °C daytime, and 14-16 °C night time; Winter: 4-17 °C daytime 10-13 °C night time	23-25 °C daytime, 18-20 °C night time	20-26 °C daytime, 18-20 °C night time

*measured with Solarmeter 6.5R, Solar Light Company Inc. USA

METHODS & RESULTS

Study individuals

The breeding pair in this study were held at Chester Zoo in England. The male was captive bred and arrived at Chester Zoo in 2018 and was 13 years old. Two females were hatched in 2011 at Chester Zoo from eggs donated from a private collection. One female was selected for the breeding trials, the second female had poor body condition following a period of brumation and was therefore removed from the exhibit to an off-show area.

Enclosure design and parameters

Initially, both female specimens were housed in a large exhibit on display to visitors in Chester Zoo's 'Tropical Realm' (Fig. 1b, Table 1). The building ambient temperature was created by an air-handling unit and provided a temperature range of 18-25 °C. However, the building was also influenced by the external temperatures. Therefore, the enclosure itself was also affected by the external air temperature, especially as the back wall formed part of the buildings external wall. Within the enclosure, there were additional heat sources that increased the ambient and basking temperatures (Table 1). This, alongside turning on and off the hot air vent, enabled an effective seasonal fluctuation in temperature (Fig. 2a). The temperatures were monitored and recorded using an ETI 'Therma-Hyrometer' and an ETI Mini Ray-temp infrared thermometer (Electronic Temperature Instruments LTD). In the summer, the enclosure was misted twice daily with tepid water. From September onwards, misting was reduced to

once per day. At this time, care was taken only to mist the foliage and ensure the substrate did not become constantly damp. The frequency of misting was reduced further as the temperatures dropped until November when misting was suspended altogether for the brumation period.

The female snakes were fed one small weaner rat, weighing approx. 40 g, per week during the active season (March to October). They were offered food until they voluntarily refused at the start of the cool season (Table 2). After completing an isolated quarantine period of three months the male specimen was placed within the same display exhibit as the two female specimens. This was to ensure he was exposed to the same gradual drop in temperatures and to prepare for the brumation period. The male was last fed one large weaner rat, weighing approx. 75 g, one week prior to transfer from quarantine. After one month co-housing and following a gradual decline in temperature, the male was transferred to an off-show enclosure to complete his brumation period (Table 1). This was to ensure all specimens were healthy and ready to breed following brumation and the introduction could be controlled and observed.

Breeding

The male and breeding female each had eight feeds before they were introduced for breeding (see Fig. 2b for their weights). They were introduced late morning when the female was hidden in a burrow. Within one hour both animals were interacting with increased tongue flicking. The female exited the burrow to the middle of the enclosure where the male followed. Once the male reached the female,

he began more courtship behaviours such as chin rubbing and body jerking on the female, whilst she remained in the same position. Both animals then moved into a more open space and the male aligned his body on top of the female. The tails intertwined and hemipenes were seen everted. The animals were left within the same enclosure from then on. Following this potential mating event, the female refused all food offered. It was confirmed by x-ray that the female was gravid with 10 eggs (see Table 2 for full timeline).

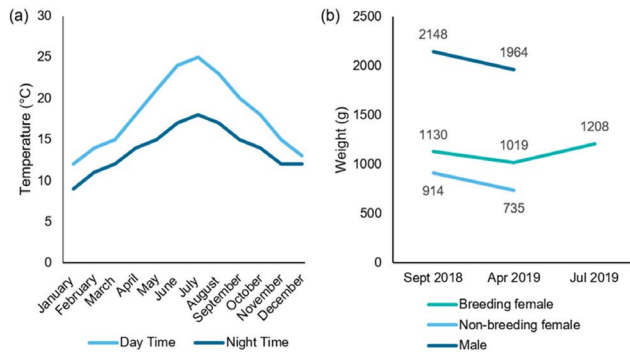


Figure 2. Physical conditions and body condition (weight) during the reproductive husbandry of *E. moellendorffi*. (a) Ambient temperatures of the display exhibit housing two female *E. moellendorffi*, recorded monthly in 2019, (b) Weight (g) for each snake pre-brumation (Sept 2018) and post-brumation (April 2019), and pre-laying (Jul 2019) for the breeding female

Egg deposition and incubation

A plastic storage box (L40 x W26 x H14 cm) with a lid was provided as a nesting site. The lid had a single entrance hole on the top. The substrate inside the nest box was damp sphagnum moss which filled approximately half of the box. The nest box was placed in the middle of the enclosure to be exposed to an ambient temperature of approximately 24 °C in the day and 21 °C at night. After 80 days, seven eggs were laid (Table 2), five of them adhered together (total weight 145 g) and the remaining two were joined (total weight 55 g). The eggs' mean dimensions were about 5.7 x 2.7 cm.

The eggs were transferred to a Pro hatch incubator (<http://pro-racks.com/prohatch.html>) set at a constant 27 °C where the substrate was a vermiculite water mixture (1:1 ratio). The incubation period was 80 to 85 days. Six neonates hatched successfully; the final egg contained a deformed specimen with a section of the lower body fused together in a fold.

Neonate management

After hatching, the neonate snakes were weighed (range 19–20.6 g) and then moved to individual rearing tanks (Table 1, Fig. 1c) within 24h. They all had their first slough 13–15 days after hatching. Subsequently, two individuals developed slight skin lesions. In one specimen a small patch of scales appeared worn away on the top of the head. As this was on the top of the head and not the tip of the snout, it was considered that it was not due to excessive rubbing on the lid or sides of the tank. A further two individuals developed similar patches. It

Table 2. Timeline of breeding events for the captive *E. moellendorffi*

Date	Breeding Event
26/07/2017	Females moved to display exhibit
28/09/2018	Females final feed
02/11/2018	Male added to display exhibit
02/12/2018	Male moved off-show for brumation
04/04/2019	All animals feeding
22/05/2019	Male introduced to breeding female
08/07/2019	Breeding female confirmed gravid with 10 eggs
25/07/2019	Female began oviposition
29/07/2019	7 of 10 eggs laid, final 3 eggs removed by veterinary intervention and discarded
12/10/2019	Neonates began to hatch
16/10/2019	Neonates emerged from 6 eggs
27/10/2019	First skin slough reported
31/10/2019	All neonates had fed

was decided to re-house the animals in different tanks to offer a wider thermogradient as well as a better humidity gradient (Table 1, Fig. 1d). This change in set up seemed to address the skin issues as there were no subsequent problems.

Neonate feeding

A day after the first slough a single new born mouse (pinkie), weighing about 1.6 g, was left outside the neonates' hides but no snake ate them. Two days after the first refusal, a pinkie was offered using forceps, which was successful. The remaining three specimens had completed their first slough by this stage. The successful strike method from forceps was used immediately this time and was successful in eliciting a feeding response. All neonates had eaten their first meal by 31st October. Although feeding could be achieved in this way, the methodology had to be further developed to ensure consistent success. The feeding process was found to be easier with the furnishings removed from the enclosure. The pinkie was offered from a distance via forceps to prevent the keeper's hand from intimidating the snake and hindering focus on the food. It was found that the best way to elicit a strike response was to poke the body and move towards the head with the food item. The most sensitive area to apply a slight pressure with the food item was around the neck. This frequently prompted the snake to strike sideways at the food item with mouth wide open. The pinkie was quickly orientated anteriorly into the snake's mouth. The neonates were never observed constricting their prey. Whilst in the process of swallowing the food item, the young snakes would frequently vibrate their tails. A few further techniques were developed for reluctant feeders. The front legs of the pinkie were cut off, as some individuals would snag their jaws on the pinkie's limb causing them to spit the food out. Some specimens had poor accuracy with their strikes which resulted in them biting at the substrate. To combat this issue, a layer of kitchen roll was placed on top of the substrate and dampened down before feeding. After a few weeks of feeding, they were offered two pinkies per feed. However, some animals regurgitated the prey. To avoid this, the neonate snakes were observed after feeding until they were active again, i.e. had digested their meal, which took 3

days. On the following day another pinkie would be offered. On occasion a pinkie would be left in the enclosure for a day to see if the snakes would start picking the food up on their own. At 7 months of age, 4 specimens began to feed in this way (at this stage the largest neonate weighed 25.1 g).

DISCUSSION

Here we have documented the first successful breeding of *E. moellendorffii* in a European Zoo. If others are to replicate this success, there are some important considerations for husbandry and management, namely seasonal fluctuations in environmental parameters and specific juvenile feeding techniques.

Most reptiles exhibit some seasonality in reproductive activity (Licht, 1972) and we believe that exposing the breeding *E. moellendorffii* to a natural environmental cycle, as they would experience in their home range, was key to this success. Although milder, the climate in Yunnan follows a similar seasonal pattern to that of Britain and therefore the influence of this seasonality on the breeding enclosure would have promoted reproductive cues for this species. This further supports the notion that captive management should be influenced by field data (Tapley & Acosta, 2010; Michaels et al., 2014), and follow an evidence-based approach to husbandry (Loughman, 2020). Where environmental field data are lacking for a species, other resources exist which can be used to close the knowledge gap, such as the work of Baines et al. (2016) on environmental microhabitat data from different biomes and other literature outlining regional environmental information taken from weather stations.

The seven-egg clutch observed in this study concurs with previous reports on both egg number; 6 to 12 eggs (Gumprecht, 2004; Köhler, 2005) and 5 to 15 eggs (Zhao, 1998), and egg size 5 to 7 cm (Zhao, 1998). The incubation period of around 80 days also corresponds with information from both Gumprecht (2004) and Köhler (2005) for eggs incubated at 27 °C.

Two main challenges were encountered with the offspring produced; skin lesions which occurred in the initial housing setup, and difficulties in getting the neonates to feed consistently. In the initial enclosures for the neonates the ambient humidity may have been too high with insufficient airflow which might have caused the skin lesions (Mitchell, 2004). These enclosures also had limited choice of places to hide. The larger enclosures to which the neonates were then moved appeared to resolve the problem by providing more space with better ventilation and wider thermo and photo gradients, giving the animals better opportunity to regulate within this environment.

The feeding of the neonates proved to be challenging. Security and environmental parameters are likely important contributing factors to success. The dietary preference of other neonate colubrid species, including *Elaphe* species, is frogs and lizards (Ernst & Ernst, 2003); it is likely to be similar for *E. moellendorffii*. Pinkies, however, were accepted following the techniques detailed in the results section (see 'Neonate feeding'). The snake's vision is very sensitive and any slight body movement from the keeper would prompt the snake to vibrate its tail and cease feeding or even regurgitate a partially swallowed food item. These defensive behaviours are suspected

to be brought on by the presence of the keeper (Greene, 1988).

E. moellendorffii is poorly represented in the literature, particularly its reproductive biology (Gumprecht, 2004). This study offers an introductory contribution and reference point to the husbandry and breeding of this beautiful and interesting species.

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First reproduction of Panha's crocodile newt *Tylototriton panhai* in captivity, with a description of the courtship behaviour, eggs and larval development

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ABSTRACT - Amphibians are facing extinction on a global scale and maintaining small populations of threatened or endangered species in captivity is essential. In connection with potential captive breeding of Panha's crocodile newt, *Tylototriton panhai*, we report a detailed husbandry protocol and describe breeding and mating behaviour. After six years in captivity, a group of two adult females and six males, tentatively identified as *T. panhai*, successfully reproduced for the first time on 24th June 2020 after a heavy rainstorm and two subsequent days of precipitation when water temperatures in a large aquaterrarium placed outdoors were 18-21 °C. The complete courtship behaviour consisted of five main stages: i/ approach; ii/ nuptial dance; iii/ amplexus; iv/ spermatophore deposition; v/ fertilisation. The two females laid a total of 84 eggs (41 and 43 eggs each) that were deposited on land in clutches of 4-22 eggs or even singly. Egg size averaged 12.6 ± 0.4 mm and the mean size of the embryo capsule 5.1 ± 0.1 (n=84). The egg hatch success rate was ~80% and on hatching the larvae moved to the water at the bottom of the aquaterrarium. By 7-9 days after hatching the total larval length was 11.89-13.78 mm (n=67). Diagnostic morphological characters are provided for stages 30-46. Metamorphosis occurred at 99-102 days and efts started to move to land at an average total length of 56.2 mm and weight of 0.6-0.9 g.

INTRODUCTION

The newts and salamanders of south-east Asia are amongst the most threatened in the world due to high rates of deforestation, alteration of habitats and human consumption including food and traditional medicine (Hernandez et al., 2019; Pomchote et al., 2020; Bunjerdruk et al., 2021). Thus, the captive maintenance (ex situ) of threatened salamander populations originating from this region is becoming increasingly important (Gagliardo et al., 2008; Browne et al., 2011). However, the husbandry requirements for keeping newts and salamanders in captivity are complex due to their biphasic life cycle, complex ecological needs and reproductive triggers, which may account for why to the present very few conservation programmes have made successful reintroductions into the wild in Southeast Asia (Pasmans et al., 2014; Hernandez, 2016a). For most of the known species, the breeding ecology is still unknown either because most species have only been recently described or because descriptions lack natural history details (Phimmachak et al., 2015; Bernardes et al., 2017). Many species fail to breed under artificial conditions, consequently recording appropriate parameters and protocols that lead to breeding success are crucial for conservation programmes (Hernandez, 2016a,b).

In north-eastern Thailand, several previous attempts have been made to study the biology, distribution and ecology of the poorly known Panha's crocodile newt *T. panhai* Nishikawa, Khonsue, Pomchote & Matsui, 2013 (Hernandez, 2016b,c; Hernandez et al., 2019; Hernandez and Pomchote, 2020; Peerachidacho et al., 2021). Herein, we describe for the first time the breeding success of *T. panhai* in captivity, with a description of courtship, eggs and larval development. *Tylototriton panhai* is a threatened and elusive species with a range that appears to be restricted to the southern parts of Luang Prabang mountain range ecoregion from Phitsanulok, Phetchabun, Uttaradit and Loei provinces, north-eastern Thailand up to Xaignabouli district in Laos at middle elevations between 1,150 m and 1,688 m a.s.l. (Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2016b; Hernandez et al., 2019). The main habitat is composed of *Dipterocarpaceae*, *Lauraceae*, *Fagaceae*, and *Ericaceae* in evergreen hill forests and montane evergreen broad-leaved forests near various bodies of water (Hernandez and Pomchote, 2020).

Tylototriton panhai is listed as vulnerable (VU) by the IUCN and has been added recently to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendix II (IUCN, 2020). Unfortunately, only *Tylototriton verrucosus* is protected by the Wild Animal Protection Act B.E. 2562 (2019) (Department of National

Parks, Wildlife and Plant Conservation, 2021) in Thailand. All known populations of *T. panhai* are small, ecologically restricted and threatened by habitat destruction, pesticide usage, and tourism (Hernandez & Pomchote, 2020; Peerachidacho et al., 2021). The species also shows significant geographic variation as there are three distinct phenotypes isolated on plateaus (Hernandez, 2016b).

MATERIALS & METHODS

Origin and identity of specimens

In July 2012, a group of two females and six males *T. panhai* were purchased from a pet store in Spain that was selling them as *T. verrucosus*. They were acquired for conservation programmes and biological studies at Thoiry Zoological Park (Paris) as previously established for the Critically Endangered Kaiser's mountain newt, *Neurergus kaiseri* (Hernandez, 2016a). The Marcel Bleustein-Blanchet Fund in Paris helped the authors to develop and manage the conservation plan for the genus *Tylotriton* including *T. verrucosus* and its relatives. At the start, animals were housed by the first author during his internship at Thoiry Zoological Park. Then, the specimens were gifted to the first and third authors due to the sale of the conservation area of Thoiry Zoological Park to another administration. Species identification was congruent with the morphological description of *T. panhai* (phenotype II) from northwestern Loei province, Thailand by Hernandez (2016a,b) although their exact geographical origin remains unknown. The eight adult specimens were characterised by wide dorsolateral bony ridges on head, prominent and large rib nodules, spine not quadrate, vertebral ridge distinct and not segmented (Nishikawa et al., 2013). However, morphological identification of crocodile newts is often difficult due to the phenotypic variation known within taxa but *T. panhai* shows distinct characters among *Tylotriton*. All the specimens acquired differed from all other congeners by having widely developed dorsolateral bony ridges on head, black limbs and tail except the edges as previously studied (Nishikawa et al., 2013; Phimmachak et al., 2015). Individuals of *T. panhai* also have very different colour markings that range from orange, yellow to reddish brown on the head, parotoids, lips, the vertebral and dorsal tail ridges, and rib nodules. This colouration is unique among the subgenus *Yaotriton* to which *T. panhai* belongs (Phimmachak et al., 2015). Additionally, the species identity will be confirmed subject to the publication of an analysis of mitochondrial DNA, based particularly on one fragment covering a 930-bp region that encodes part of the NADH dehydrogenase subunit 2 (ND2) and which suggests that the specimens originated from north-eastern Thailand. The sexes were distinguished following Pomchote et al. (2008) and Phimmachak et al. (2015). Adult males showed a pronounced oval to rounded cloaca (Fig. 1A) while females had a conical cloaca especially during the breeding season (Fig. 1B). Moreover, females tend to be robust, larger in size with a distended abdomen and brighter colouration.

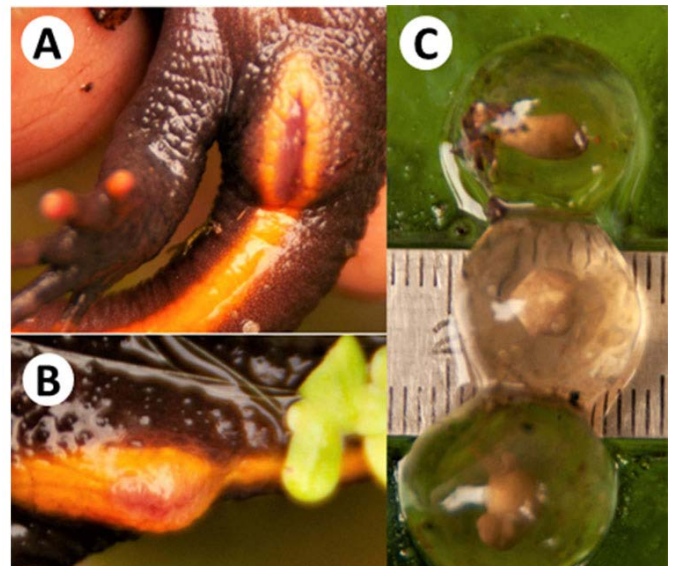


Figure 1. Images of *Tylotriton panhai* - A. Cloaca of the male, B. Cloaca of the female, C. Eggs

Maintenance aquaterrarium

All adult individuals of *T. panhai* were maintained together in a large aquaterrarium (Exo Terra) measuring 120 x 45 x 45 cm (LxWxH) (Fig. 2A). Temperature and humidity were measured with a Bio Dude Digital Thermo/Hygrometer (Houston, Texas), water pH with Expresstech @ LCD PH Medidor Digital (Kingpow Company Limited, China), and other water chemical parameters were taken using drop-by-drop colour tests from JBL (Testlab, Germany). The aquaterrarium was placed in a porch at temperatures between 11–24 °C with a natural circadian rhythm. The substrate was 40 cm deep and 100 cm long in two distinct layers. There was a lower (30–40 cm deep) moist and dark layer composed of clay balls (this substructure ensured good drainage and aeration), loam soil, three large fired-clay bricks, rocks, pieces of cork and other durable bark and an upper dryer layer (10–15 cm deep) consisting of oaks leaves (*Quercus* spp.), mosses (*Kindbergia praelonga*), and plants (*Tradescantia* spp., *Asplenium nidus*). The upperlayer provided a stable humidity of 60–70 % while the lower layer recorded 80–100 %. There was a small permanent pool of water measuring 20 cm long and with a depth of 10 cm. All water used was dechlorinated originating from rainwater at pH 6.6. To increase humidity of the terrestrial substrate, the aquaterrarium was misted with a hand sprayer three times a week. The food source comprised different insects or small invertebrates such as small crickets (*Acheta domestica*), woodlice (*Armadillidium vulgare*) and earthworms (*Dendrobae naveneta*).

From 2012 to June 2020, the crocodile newts showed secretive habits with little crepuscular or nocturnal activity. On 14th July 2019, two male adult specimens showed increased daytime activity and were observed in the pool, they had a lighter appearance; no females were seen. This change in behaviour suggested that a breeding period was imminent and prompted the establishment of an aquaterrarium for breeding as described below.

Breeding aquaterrarium

In June 2020, in an attempt to simulate the natural conditions in which the animals breed during the rainy season, all animals were deposited in a larger aquaterrarium of 200 x 60 x 60 cm furnished with various debris, e.g. rocks, oak leaves and many waterweeds (*Elodea* spp.) (Fig. 2B). This aquaterrarium was placed outdoors for one month. The water depth of the aquatic section was 40 cm with one terrestrial part of 30 x 40 x 30 cm located on the water surface supported by fired-clay bricks, large rocks and bark covered in moss on the upper part for egg deposition. During June 2020, temperatures dropped to 18 °C at night and rose to 24 °C in daytime. Partial water changes of 10-20 %, using cool water, were made every two to three weeks.

Larvae were raised in the same tank as used for breeding except for the addition of a bottom layer of oak leaves in which the larvae could hide. The larvae grew up in stagnant water at temperatures of 18-22 °C. They required feeding once every three days equally spaced with *Daphnia* spp., *Tubifex* spp. and mosquito larvae. After mating and oviposition were complete, all adult specimens were returned to the smaller maintenance aquaterrarium (Fig. 2-A).

All water used in the breeding aquaterrarium was rainwater obtained from empty tanks or water collected from natural springs. No filter was used. Water was acidic to neutral with pH 6.3-7.4, nitrite less than 0.1 ppm, and nitrate less than 10 ppm.



Figure 2. The aquaterraria used for maintenance and then breeding of *T. panhai* - **A.** The aquaterrarium used during the newts' terrestrial phase in September to April placed in a sheltered porch from September to April, **B.** The aquaterrarium used for captive breeding in July/August placed outdoors and with deeper water

RESULTS

Environmental stimuli and preliminary observations of reproductive behaviour

After a hard rainstorm with high air pressure and two subsequent days of precipitation, on a wet day (17th June 2020) two adult males were observed to be more active and showed colourful orange to reddish dorsolateral glandular warts, dorsal ridge, tips of fingers and toes and cloacal parts. They entered the water of the breeding aquaterrarium several times, becoming totally aquatic after two consecutive days at air temperature of 18-20 °C and water temperature 18° C. The four other males followed the first specimens, also becoming partially to totally aquatic between 19th and 21st June 2020. The two females were found to be less active during the first two days in their new aquaterrarium. However, on 20th and 21st June 2020, one or both females were observed entering the water for periods of just a few minutes to 120 minutes during the night time between 21:30-23:00 h. On June 20th and 21st 2020, two males were observed swimming to approach one female during the night. Inter-male competition was observed under water, in which one male attempted to stop the other male from moving in front of the female. Then, the competitor male was engaged in a nudging ritual with one female into the deeper water.

Courtship behaviour

The reproductive behaviour was observed twice and consisted of five main stages as follows:

i) The approach

Upon encountering the female, one male was observed presenting its full lateral view of the body to the female. This tentative approach was repeated several times because the female was trying to escape.

ii) The nuptial dance

When the female stopped in front of the male, the latter repeatedly circled the female very slowly in front of its head, first sniffing the snout and parotoids region and then, the tail, cloaca, flanks and legs. Both were continuing to sniff each other in a circular manner and turned around several times for about 15-30 minutes.

iii) Amplexus

After that, the female pushed the male with its right hindlimb and went under the male (Fig. 3A). The male was seen lying upside down over the female in a submissive manner for few seconds but this attempt failed and was repeated several times (15 minutes to 1 hour), before the male managed to grab the female's forelimbs firmly in its own forelimbs, forming a ventral amplexus position (Fig. 3B).

iv) Spermatophore deposition

The male then deposited a cone-shaped spermatophore on the water substrate.

v) Fertilisation

Fertilisation was achieved when the spermatophore was taken up into the cloaca of the female.

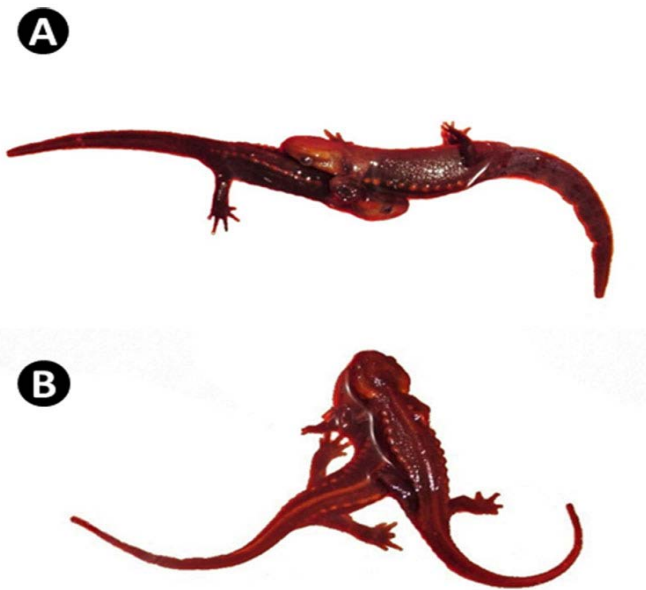


Figure 3. Courtship behaviour of *T. panhai* - **A.** Pre-amplexus of the species, **B.** Ventral amplexus stage

Egg laying and hatch rate

On June 26th 2020 at 22:35 h with water temperature of 21 °C, the first adult female laid 43 eggs, followed two days later by the second female with 41 eggs. The eggs were deposited singly or in small clutches of 4-22 eggs. The eggs were attached under wet moss above the water throughout the terrestrial part of the aquaterrarium. Egg size ranged between 12.6 ± 0.4 mm (n=84) and the mean size of the embryo capsule 5.1 ± 0.1 mm (n=84). In shape the eggs were rounded and they contained a slightly clear gelatinous layer, with some eggs more opaque in colouration. The liquid inside was very clear. The capsular chamber contained an embryo that was a noticeably dark to brownish or with a creamy yellowish to whitish colouration (Fig. 1C). About 80 % of the eggs hatched successfully.

Larval development and morphological characters

Diagnostic morphological characters for larvae are provided for stages 30-46 (see Fig. 4) following Grosse (1997). On 3rd July, 7-9 days after hatching at a water temperature of 19 °C the 67 larvae that had survived were at stages 30-32 and were observed on the bottom of the aquaterrarium. The range in total length of these 67 larvae was 11.89-13.78 mm. The dorsal fin was well-developed and higher than the head, starting at the middle of the trunk; the ventral fin shorter than dorsal fin. When hatching, larvae were thin, long and very slender in appearance; snout short and flat; ground colouration yellowish with a dense dark pigmentation on the whole body; gills well-developed and yellowish to pale orange different colouration, especially on their extremities. At 10-15 days, two fingers were visible on the forelimb of some larvae (stage 33; n=10), while in others the hind limb bud (stage 37; N=10) was already noticeable. At 17-22 days,

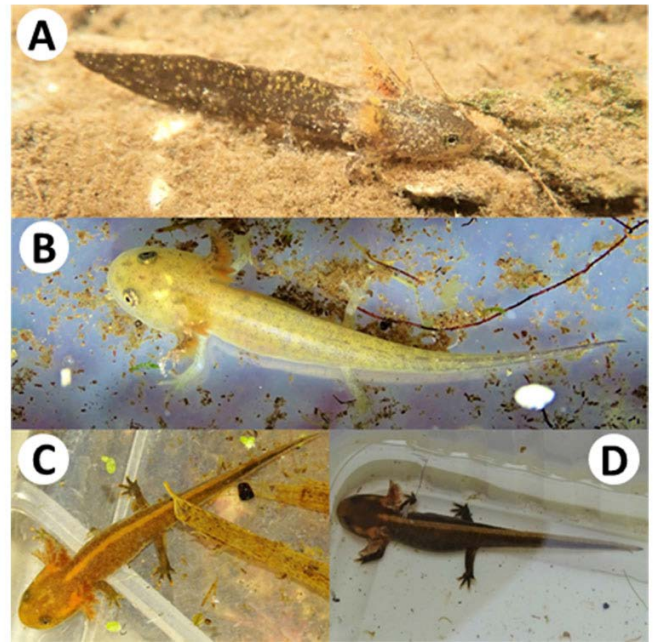


Figure 4. Larval development of *T. panhai*- **A.** At 15 days **B.** At 45 days, **C.** At 65 days, **D.** At 95 days

all four fingers, five toes and joints were fully developed after (stage 43; n=10). At 45-75 days, larvae were totally vivid yellowish to bright orange in appearance with numerous and various dark little spots on the dorsal parts and red gills were slightly high (stage 44; n=10). At 65-95 days, pigmentation was darker turning to black and gills starting to degenerate (stage 45; n=10). At 75-99 days, red gills disappeared (stage 46; n=10). Larvae were fully developed and dark in colouration. Metamorphosis was completed by 99-102 days and eftts started to move to land at an average total length of 56.2 mm and their weight range was 0.6-0.9 g (n=12). The general ground colouration was dark to black on the dorsal parts, tail, venter and head with a pure orange to yellowish orange colouration on parotoids, mouth area and snout, vertebral ridge, tips of fingers and toes as well as ventral ridge of tail; the eyes were golden with black pupils. By 127 days, juveniles developed an orange colouration on the 9-12 dorsolateral glandular warts, reaching an average snout-vent length of 75 mm (n=12).

DISCUSSION

Under natural conditions, adult *T. panhai* enter water at the end of April to early May and remain there until August during the monsoon season, which corresponds to the breeding period (Pomchote et al., 2008). Both males and females of the species develop an attractive and brighter colouration for their nuptial dance, becoming lighter in appearance as observed in other *Yaotriton* relatives such as *Tylototriton asperrimus* (Hernandez, 2016b). According to Khonsue et al. (2010), wild *T. panhai* can reach sexual maturity at 4-8 years in males (n=12) and 4-6 years in females (n=2). Our study

corroborates these results and confirms that the captive specimens were more than six years old at first reproduction.

The various species within the genus *Tylototriton* differ in reproductive behaviours to the extent that there can be either terrestrial or aquatic mating and there are variations in the sites they select for oviposition (Pogoda et al., 2020). In this study, the breeding behaviour of *T. panhai* involved five main stages including i/approach, ii/nuptial dance, iii/amplexus, iv/spermatophore deposition, and v/fertilisation. Similar stages were previously reported in other related species within the subgenus *Yaotriton* in the wild including *T. wenxianensis* (Sichuan province), *T. asperrimus*, and *T. lizhenchangi* (Raffaëlli, 2013; Sparreboom, 2014; Hernandez, 2016b). Within the subgenus *Liangshantriton*, studies in the wild of *T. pseudoverrucosus* and *T. taliangensis* have shown that they employ ventral amplexus (Hernandez, 2018) of the type used by *T. panhai*.

The two females of *T. panhai* deposited a total of 84 eggs with egg viability of about 80 % indicating this species may breed successfully in captivity. The current study confirms previous reports that the species deposits eggs singly but mostly in small clutch of 10-20 eggs on land, particularly in moss with a saturated wet atmosphere (Hernandez, 2016b). Hernandez & Pomchote (2020) reported oviposition sites being on land near ponds, where eggs were attached to twigs, plants and even grasses or tree roots, sometimes more than 20–40 cm above the ground to avoid predators. Thus, *T. panhai* shows a preference for terrestrial oviposition while other congeners among the subgenera *Tylototriton* and *Liangshantriton* mainly lay their eggs in water bodies (Pogoda et al., 2020).

Eggs of *T. panhai* are remarkably large in size and the description of the egg and its embryo is very similar to that of its sister species *Tylototriton vietnamensis* (Bernardes et al., 2017). This study shows that an adult female may deposit a total of 4-43 eggs measuring 12.6 mm, values that are similar to our field observations of this species (Hernandez & Pomchote, 2020); female *T. vietnamensis* may deposit a total of 5-85 eggs, measuring 6.06-13.58 mm (mean 9.73 ± 1.61 mm; $n=133$), deposited either singly or in small clutches between 17 and 188 cm (mean 80 ± 41 cm; $N=41$) from water bodies (Bernardes et al., 2017). Our observations from captive and wild specimens are congruent with most of other species within the subgenus *Yaotriton* depositing an average of 20-99 eggs on land, mainly in clutches or, even singly as previously reported by Fei and Ye (2016).

Larvae are of the pond-dwelling type with red gills and feed on various invertebrates (Hernandez, 2016c). Their total length 7-9 days post hatching was 11.89-13.78 mm ($n=67$) and metamorphosis was completed after 99-102 days. Efts started to move to land at an average total length of 56.2 mm ($n=10$). Certain species of salamander exhibit breeding behaviours and phenology that diverge widely from those of wild populations (Bernardes et al., 2017) and this remains an important area for future study of *T. panhai*.

The current study provides new insights into the reproduction of *Tylototriton* in captivity. The subgenus *Tylototriton* including the species complex, *T. verrucosus* and *T. shanjing*, was the first species group to breed in captivity

with successful results recorded for many years (Mudrack, 1969; Ziegler et al., 2008). During the last decade, several new species have also reproduced in captivity including *T. yangi*, *T. shanorum*, *T. kweichowensis*, *T. lizhenchangi*, *T. vietnamensis*, *T. ziegleri*, *T. wenxianensis* and *T. taliangensis* (Raffaëlli, 2013; Pasmans et al., 2014; Sparreboom, 2014). Studies have shown that in long-term captivity, reproduction may fail in many species but may be successful if the captive newts are in a large aquaterrarium and provided with a dry and cool period from October to April-June and a wet, moist and warmer period from April to August (Raffaëlli, 2013). The first attempts to induce breeding were to keep the newts indoors and then to introduce a homemade rain chamber into the aquaterrarium at the right moment. This approach was superseded by the use of an aquaterrarium with a larger aquatic section that was placed outdoors in a wet and poorly illuminated location (Hernandez, 2016b,c). In this study, we used the second approach which involved providing a large aquaterrarium of 200 x 60 x 60 cm placed outdoors during several rainy days in June, the water at pH 6.3-7.2 and temperatures varying from 24 °C in the day to 18 °C at night.

Although the morphology of the newts investigated in this study is congruent with *T. panhai* (phenotype II), without collection locality or published genetic data, there remains the possibility that the data pertain to another (potentially undescribed) species. Such issues have arisen previously, for example an in almost identical husbandry and breeding article (Sparreboom, 1984) where animals referred to as *Paramesotriton chinensis* were in reality *P. fuzhongensis* a species described 5 years after the publication of the article. In any case, the observations presented in this article represent a step forward in understanding the captive care and breeding of *Tylototriton* spp. that can be employed in any future ex situ conservation programme. However, successful restocking programmes should have in mind the importance of genetic diversity for assuring long-term survival of the populations and must be paired with threat mitigation in the field (Browne et al., 2011).

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Brumation of the clouded monitor lizard *Varanus nebulosus* in north-eastern Thailand

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ABSTRACT - The clouded monitor lizard (*Varanus nebulosus*), is a semi arboreal lizard widely distributed throughout much of South and Southeast Asia. Despite its wide distribution there is almost nothing known about the ecology of this species. During the course of an 11-month radio telemetry study, in a reserve with a tropical savannah climate (Köppen Aw), we made the first records of brumation in this monitor lizard. This contrasts with earlier reports of the same species in a tropical monsoon climate (Köppen Am) where no brumation was recorded. We successfully tracked 10 individuals throughout their inactive period and found that seven of the monitors selected tree hollows within the endangered *Shorea henryana* tree. All tree hollows selected faced between the east and south cardinal points (90 °-180 °). The average brumation period was 100 days (range 86-113 days, standard deviation - 10.7), beginning in November at a time of falling temperatures and humidities and ending in early March when these variables had been restored. Eight of the 10 monitors basked partially or completely out of their shelters on multiple occasions. Of those eight monitors, two individuals moved between shelters during brumation after an extended period in one location. Our observations provide insight into the relationship between *V. nebulosus* and the tree *S. henryana*, in the dry evergreen forests of north-eastern Thailand. Future research should investigate how this tree will be affected by climate change in the coming decades and what that could mean for the future persistence of the clouded monitors that appear to rely on it.

INTRODUCTION

Brumation is a form of hibernation in ectothermic animals, defined as a period of inactivity or dormancy, typically associated with changes in environmental temperatures (Mayhew, 1965). Environmental temperature plays a critical role in the ability of ectothermic species to: capture prey, avoid predation and regulate their metabolism (Mcconnachie & Alexander, 2004; Naulleau, 1983; Stevenson et al., 1985). Brumation and hibernation are well documented in reptiles, with many temperate species spending two-thirds of their lives inactive (Etheridge et al., 1983). Despite these cycles being such a large part of an animal's life-history, there is little understanding of the drivers leading to their onset and subsequent emergence, especially for species in the tropics.

The clouded monitor lizard *Varanus nebulosus* (Gray, 1831) is a semi-arboreal well camouflaged (low detection rate) species with a wide distribution from Myanmar to Vietnam and southern China to Indonesia (Koch et al., 2013). They are a medium-sized varanid reaching a maximum length of 160 cm and maximum weight of 8 kg (Auffenberg, 1994). Their conservation status in the wild has yet to be assessed and the IUCN listing remains under Bengal monitor (*Varanus bengalensis*) as there is uncertainty among researchers pertaining to the species status. Numerous studies suggest two separate but closely related species (sister species) apparent from; distinguishable oblique ventral scale counts (*bengalensis* 88-110, *nebulosus* 70-90; Auffenberg, 1994), distinct hemipenial differences (Ziegler & Böhme, 1997), differences in scale morphology and micro-structures

(Bucklitsch et al., 2016), and mitochondrial DNA (Ast, 2001). It is suggested that since the number of mature individuals is steadily declining, they will require a "vulnerable" listing once properly evaluated (Cota et al., 2021). Despite their large size and broad geographical distribution, the general ecology of the clouded monitor remains little known (but see Traeholt, 1997; Duengkhae & Chuaynkern, 2009 for records on diet and basking behaviour).

Radio telemetry is a commonly accepted method to record seasonal and daily activity patterns, home range sizes, movement trajectories, macrohabitat use, shelter site preferences and ultimately threats to survival (Cagnacci et al., 2010; Malhotra et al., 2021; Ujvari & Korsos, 2000). The objective of this study was to observe whether the clouded monitor in north-eastern Thailand has an annual period of brumation and if so then address the following questions: a) When does brumation occur and what is its duration? b) Do the monitors select specific species of trees for brumation? c) What specific microhabitat features do they select? d) Do the monitors have a dormant or active brumation (dormant equating to no movement at all; active consisting of thermoregulating and possibly moving between sites)? and, e) Does average daily temperature and humidity contribute to either the onset of, or emergence from, brumation?

METHODS

Study Site

From July of 2020, we undertook an 11-month radio telemetry study of the clouded monitor at the Sakaerat Environmental

Research Station in north-eastern Thailand. The research station is a part of the Sakaerat Biosphere Reserve (SBR; 14.44-14.55 °N, 101.88-101.95 °E), encompassing an area of approximately 360 km². The biosphere reserve has three main designations: core, buffer and transitional. The core area is predominantly dry evergreen forest (DEF) with large patches of dry dipterocarp forest (DDF) and has active ranger enforcement; the buffer zone consists mainly of both DEF and plantation forest; the transitional zone lacks official protection and is an agricultural matrix with expanding human settlements (Trisurat, 2010). The DEF at SBR, has a mean canopy height of 35-40 m with two subtypes dependent upon the dominant tree species: *Hopea ferrea* dominates the first type and occurs on level ground creating a closed canopy; *Shorea henryana*, dominates the second type and mainly occurs on slopes, creating a patchy canopy (Bunyavejchewin, 1986, 1999). Both *H. ferrea* and *S. henryana* are classified as endangered species and are at risk from habitat loss and logging (Ly et al., 2017a & b). In contrast, the DDF at SBR, has a mean canopy height of 11-14 m and a more open canopy. Several species, *Shorea roxburghii*, *Shorea obtusa* and *Dipterocarpus intricatus* are dominant trees in DDF (Lamotte et al., 1998). Based on the Köppen climate classification, SBR is a tropical savanna (Aw) with an altitude range between 280-762 m a.s.l. (Köppen, 1931; Rubel & Kotteck, 2010). The SBR has three distinct seasons: dry (November-February), hot (March-May) and wet (May-October; Tantipatanap et al., 2016).

Capture/Tracking Techniques

Monitors were located during road-cruising on forest roads with a motorcycle and visual surveys by scanning large trees with binoculars (Fig. 1). Once found, we captured monitors either with drop traps - placed along the base of the tree with a sheet metal perimeter - or with a noose. Once captured the monitors had a backpack style harness fitted around their pelvic girdle, which contained a radio transmitter and GPS logger. We released all individuals at the exact capture site and began radio tracking them once daily between 05:00 h and 20:00 h. During this study we recorded a considerable brumation period for all study animals - 8 individuals - beginning on 5th November 2020 and lasting until 6th March 2021 (date of last emergence). We defined the onset of brumation as the first date from which a lizard remained in a shelter site for at least 14 days, during a period where we deemed the weather adequate for movement. Although we did record large spans (up to 13 days) of inactivity within our study animals, prior to brumation, these were all during the monsoon season, through periods of heavy rainfall, high cloud cover and lower temperatures.

We also found three new individuals during the brumation period: M11 on 18th November, M12 on 27th November and M13 on 27th December, 2020. The first two monitors were basking outside of their brumation sites, so we placed traps along the shelter-tree base. The third individual was found on a forest road, perhaps moving from one shelter site to the next. We documented two of our radio tracked monitors moving between brumation sites around the same timeframe: F01 on 19th December and F09 on 21st December,

2020. The traps set for M11 and M12, were checked daily during radio tracking protocols and observations of basking were documented, when possible, on these two individuals. Although we do not know what date these two monitors entered brumation we decided to include them in our dataset because we could determine the exit dates, and found each within the range of our other radio tracked monitors entering brumation. However, because M13 was captured late into the brumation cycle we have chosen not to include it in our summary dataset.



Figure 1. Clouded monitor F03 basking completely exposed above its hollow in a *Hopea ferrea* tree

Data Collection

During radio tracking we recorded: brumation site location, the onset date, the frequency of basking observations (recorded during each daily fix if the lizard was visible when we were using telemetry to find the individual) and the date of emergence from brumation of each monitor (Fig. 2). We assessed each monitor's shelter site location by recording: habitat type, species of the tree selected, the diameter at breast height (DBH) using a tape measure, the height of the shelter site and height of the tallest branch (both measured using a Nikon Forestry Pro I rangefinder) as well as the cardinal direction the shelter site was facing measured using the compass feature on a handheld Garmin 64s GPS.

There are five weather stations spread throughout the SBR; each station records the atmospheric temperature and humidity every hour. We collated station weather data and averaged all of the stations together to plot the average temperature and humidity for our study period. We then overlaid the monitor's dormancy duration, to see if there were any relationships between these two climatic factors and the onset of, or emergence from, brumation (Fig. 3).

We used R studio version 4.0.3 (R Core Development Team, 2020) for data manipulation with reshape2 package version 1.4.4 (Wickham, 2007) data visualizations including

ggplot2 version 3.3.5 (Wickham, 2016). To produce temperature and humidity graphics we employed ggpubr version 0.4.0 (Kassambara, 2020) for final visuals, lubridate version 1.8.0 (Grolemund and Wickham 2011) to control date formats, tidyverse version 1.3.0 (Wickham et al. 2019) for functionality, and viridis version 0.6.2 for colour-blind friendly palettes. We have additionally made all data and R scripts available on Open Science Framework (<https://osf.io/xd243/>).

RESULTS

Brumation sites

For the 10 monitors we followed through brumation, we recorded 14 different brumation sites. All study animals spent the duration of brumation within tree hollows, despite differing habitat types. Of the 14 shelter sites documented, 64% were on *S. henryana* trees. We were unable to identify two tree species

Table 1. Characteristics of the brumation sites used by *Varanus nebulosus*. Shelter height is the height of the tree hollow utilized by each monitor. Tree height is the height of the tallest branch. DBH is the diameter breast height (girth) of the bole of each tree. Shelter direction is the cardinal direction of the tree hollow selected by each monitor.

Monitor ID	Forest habitat	Tree species	Shelter height (m)	Tree height (m)	DBH (cm)	Shelter direction
F01	MDF	<i>Shorea henryana</i>	19.1	27.3	254.4	E
F01*	MDF	<i>Lagerstroemia calyculata</i>	21.4	29.8	287.3	E
F03	DEF	<i>Hopea ferrea</i>	14.4	17.5	198.6	ESE
M04	HS	Unknown	2.7	2.7	102.5	E
M06	DEF	<i>Shorea henryana</i>	26.3	35.7	274.2	ESE
M07	DDF	Unknown	11.4	11.4	190.6	E
M08	DDF	<i>Pterocarpus macrocarpus</i>	11.8	21.5	263.2	E
F09	DEF	<i>Shorea henryana</i>	19.0	26.7	298.8	SE
F09*	DEF	<i>Shorea henryana</i>	37.9	41.2	301.3	ESE
F09*	DEF	<i>Shorea henryana</i>	18.3	28.8	243.9	E
F09*	DEF	<i>Shorea henryana</i>	21.2	33.2	260.1	ESE
M10	DEF	<i>Shorea henryana</i>	38.3	43.9	326.7	SE
M11	DEF	<i>Shorea henryana</i>	39.6	45.6	312.5	SE
M12	PLT	<i>Shorea henryana</i>	20.6	31.1	216.0	SSE

Habitat Abbreviations: DEF= Dry Evergreen Forest, DDF= Dry Dipterocarp Forest, MDF= Mixed Deciduous Forest, PLT= Plantation Forest and HS= Human Settlement
Shelter Direction Abbreviations: E = East, ESE = East by Southeast, SE = Southeast, SSE = South by Southeast Asterisks (*) indicate when individual monitors moved from one shelter site to another during the brumation period.

(used by M04 and M07), because both trees were already dead with no identifiable features left. The average shelter site height was 21.6 m above the ground (range - 2.7-39.6, standard deviation - 10.8; Table 1). The orientations of the tree hollows were all within the east and south cardinal points (90 °-180 °) and occurred within gaps or clearings of the canopy, allowing open access to direct sunlight (Table 1). All shelter trees were large (DBH mean - 252.2 cm, range - 102.5-326.7, standard deviation - 59.6; Table 1). We were unable to take measurements on the depth, width and internal features of the tree hollows due to the heights of shelter sites.

Brumation observations

There was a distinct relationship between the onset of brumation with lower average temperatures and humidity. We observed that the first study animals entered brumation shortly after the average daily temperature dropped below 22 °C (Fig. 3). Brumation peaked during the lowest average temperature and humidity for the year. Individuals brumated on average for 100 days (range - 86-113 days, standard deviation - 10.7; Fig. 2). All clouded monitors entered brumation within 29 days of each other and emerged within a 12-day span (Fig. 2). We observed that all but two individuals (M07 and M10) basked either partially or completely out of their tree hollows (Figs. 1&2).

Both monitors F01 and F09 moved to different shelter locations during the course of brumation. Monitor F01 moved once on 19th December 2020, from a *S. henryana* tree to a *Lagerstroemia calyculata*. Monitor F09, moved three times through the brumation cycle, moving to a *S. henryana* each time, on: 21st December 2020, 29th January 2021 and 12th February 2021 (Table 1; Fig. 2).

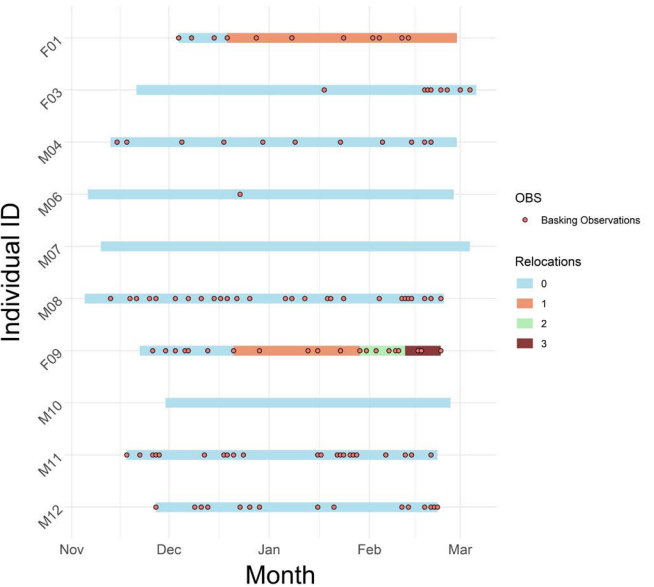


Figure 2. Brumation observations of clouded monitor (*Varanus nebulosus*). Most monitors entered brumation in November 2020 and all individuals had completely emerged by early March 2021. Relocations are annotated for monitors which moved to different shelter sites during brumation. Basking observations are listed for each visual observation we made during this period.

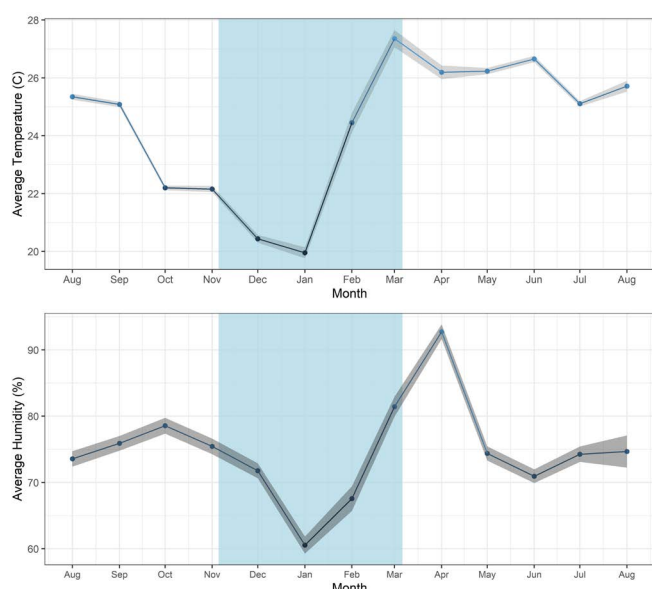


Figure 3. Climatic conditions at the Sakaerat Environmental Research Station from August 2020 to August 2021. Top-Mean daily temperature with upper and lower confidence intervals, Bottom - mean daily relative humidity (with upper and lower confidence intervals). In both cases the shaded blue area is the total brumation period, 6th November 2020 to 6th March 2021.

DISCUSSION

Brumation behaviour

Walter Auffenberg, in his seminal work on the Bengal monitor (*V. bengalensis*), recorded individuals from Trang province in Thailand (now considered *Varanus nebulosus*), that did not enter a period of inactivity and instead remained active throughout the year (Auffenberg, 1994). The climate of Trang province by the Köppen classification is tropical monsoon (Am), with distinct wet and dry seasons and an almost uniform temperature throughout the year (Rubel & Kottek, 2010). We have potentially demonstrated variation between *V. nebulosus* populations within the same geographical region. It is likely that *V. nebulosus* goes through a brumation period throughout the northern part of its distribution where there is a tropical savannah climate (Köppen Aw) with a distinct cool dry period and that it remains active throughout the year in the southern part where there is a tropical monsoon climate (Köppen Am).

Shelter selection

We saw that throughout the brumation period most study animals selected tree hollows within *S. henryana* trees. Of the five monitors living within the DEF, four selected *S. henryana*. Also, two monitors living in the MDF and plantation forest also sought out this same species of tree within habitats where it is uncommon (Bunyavejchewin, 1999). Monitors within the DDF and HS each selected different species of trees and this may be due to the forest structure: in the DEF the canopy is mostly closed, so *S. henryana* provides a good shelter site for obtaining adequate UV radiation for thermoregulation; the DDF and HS have an open canopy and

therefore monitors inhabiting these areas may have a broad range of potential shelter sites. Within the SBR, mature *S. henryana* individuals are rare and have high mortality rates as young trees (Bunyavejchewin, 1999). However, climate change in the tropical dry forests of north-eastern Thailand, likely will lead to a shift to wetter tropical forests by the year 2100; in this scenario the density of *S. henryana* trees are likely to decrease at a faster rate than at present (Boonpragob & Santisirisomboon, 1996). Based on our results it is likely that *S. henryana* is a critical species for *V. nebulosus*, playing a key role in their brumation cycle and possibly their overall survival in DEF. Future research should investigate how *S. henryana* will be affected by climate change in the coming decades and what that could mean for the future persistence of *V. nebulosus* at this site.

Brumation behaviour

Every tree hollow that the tracked monitors selected faced between the east and south cardinal points and all shelter sites were fully exposed, either above or within canopy gaps. This feature may be a necessity for survival during the cold season as we observed individuals on numerous occasions thermoregulating on warm mornings and afternoons. Clark et al. (2008) found that rattlesnakes selected over-wintering hibernacula on south-facing slopes, which is likely due to southern slopes receiving more solar radiation than north-facing slopes (Hamilton & Nowak, 2009). This same principle is likely the basis for *V. nebulosus* selection of east and south facing tree hollows. East and south basking orientation has also been observed in agamid lizards in Saudi Arabia (Al-Johany, 1995) and in arboreal skinks in Brazil (Maia-Carneiro et al., 2018).

Two individuals moved between shelter sites during the brumation period. F01 moved from a *S. henryana* to a *Lagerstroemia calyculata* tree. F09 changed shelter sites three times and all three occasions F09 moved to a different *S. henryana* tree and never moved more than 100 m. Cummings (2020) documented a single female desert tortoise which also moved between shelter sites during its brumation period, while all other individuals monitored remained stationary. These observations could have been linked to uncommonly warm days, or perhaps insufficient basking area on the original trees.

In most reptile species documented that brumate or hibernate, the males emerge before the females (Etheridge et al., 1986; Winck & Cechin, 2008). Although with *V. nebulosus*, the only two individuals to move between shelter sites were females and based on the emergence dates we saw no clear distinguishing patterns between male or female emergence. We were unable to collect any microhabitat data which could help deduce why these monitors moved during brumation because shelter sites were too high to safely reach without climbing gear (which we lacked). We assessed the average and daily high temperatures between 18th December and 27th December 2020 since we documented three different individuals (F01, F09 and M13) moving in this period. However, we were unable to come to any clear conclusion about what was driving this behaviour, suggesting there are other unknown underlying factors.

Potential drivers of brumation in the tropics

We observed average daily temperature and humidity at our study site during our study period and were able to identify a marker for the onset of brumation - when the average daily temperature fell below 22 °C combined with the average daily humidity dropping below 75 %. However, when the temperature and humidity rose above average in late January, the monitors all remained dormant suggesting that there are potentially other factors at play. These could include daily photoperiod, prey abundance, rainfall, peak daily temperatures, or a combination of factors (Auffenberg, 1994; Ortiz et al., 2016).

It is imperative to understand the underlying drivers of animal behaviours as the effects of climate change are predicted to alter the structure of many forests around the globe (Boonpragob & Santisirisomboon, 1996). Understanding environmental drivers and microhabitat features utilised by different species is important for planning and implementing effective conservation management (Ljubisavljević et al., 2017). We observed a clear relationship between the tree *S. henryana* and *V. nebulosus* at the SBR; it is important to determine whether this relationship persists throughout north-eastern Thailand. If so, what are the implications for populations of *V. nebulosus* in areas where *S. henryana* has been extirpated or severely decreased? It would also be worthwhile identifying the specific factors that lead ectothermic species to brumate when they are living in tropical climates with relatively little annual temperature variation (as in the current study). Despite the limited scope of our study, our preliminary data demonstrate behaviour that has not been widely documented and our findings can be used to further bolster understanding of the life-histories of ectothermic species in tropical environments.

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Collection of vulnerable nests with eggs for the captive incubation of king cobra *Ophiophagus hannah* as a conservation strategy in Mizoram north-east India

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The king cobra *Ophiophagus hannah* (Cantor, 1936) is the world's largest venomous snake inhabiting a wide variety of niches such as grassland, forests, shrubland, wetlands, mangrove swamps, agricultural areas and may occur in the vicinity of rural villages (Whitaker & Captain, 2008; Stuart et al., 2012). It is widely distributed across south Asia, south-east Asia, and east Asia at altitudes up to at least 2,000 m a.s.l. (Waltner, 1975; Das et al., 2008). It is the only snake species where females actively construct a nest from leaf litter or other plant material (Loveridge, 1946; Schmidt & Inger, 1957; Whitaker et al., 2013; Hrima et al., 2014; Dolia, 2018). Nests have been seen between April and July (Daniel, 1983; Whitaker et al., 2013). The females are known to guard the nest, sometimes residing in the nest's upper chamber or coiling on top of the nest (Loveridge, 1946; Whitaker et al., 2013; Vanlalchhuana et al., 2017), and subsequently deserting the nest when the neonates hatch (Kannan, 1993). Females can lay up to 14–53 eggs in a single clutch (Das, 2012; Hrima et al., 2014; Burchfield, 1977). On one occasion, twin nests at a distance of only 6.6 m apart were reported from the northern India (Dolia & Das, 2020).

Destruction of habitats, illegal trade, and persecution by humans are the primary conservation threats to the king cobra (Stuart et al., 2012; Shankar et al., 2013; Marshall et al., 2018). Consequently, the species is considered to be declining throughout its range and is currently listed as “vulnerable” (VU) on the IUCN Red List (Stuart et al., 2012); placed in Appendix II by CITES; and is in Schedule II of the Indian Wildlife Protection Act (1972). In north-eastern India, Das et al. (2008) speculate that king cobra numbers are declining rapidly due to large scale habitat destruction. Sightings of freshly killed king cobra in Mizoram are not uncommon especially in agricultural or plantation areas. Herein, we report the cases of king cobra nests threatened with destruction by local villagers, which we relocated as a preliminary approach for ex-situ conservation of king cobra populations in Mizoram, north-east India.

During the study period (2017–2021), we documented 18 new king cobra nesting sites at elevations ranging from 400 m to 1,450 m a.s.l. plotted using QGIS v3.16.2 in Figure 1

together with 23 previously documented sites from Mizoram. For details of the newly reported nesting sites refer to Table 1S in Supplementary Material. The nests from Chhingchhip (Serchhip District) and Thenzawl IV (Serchhip District) had already been deserted by the female (Fig. 2A) and hatchlings were found inside the nest (Fig. 2B). Unfortunately, while visiting the nest at Muthi (Aizawl District), the female snake was found lying rotten nearby its nest; also, the nest at Tlangsam (Champhai District) was completely destroyed along with the eggs, and the female was found shot dead (Fig. 2C). However, the nest encountered in Dampa Tiger Reserve (Mamit District) had no potential threat at the time of our visit, so it was left in the natural breeding site after photographing the snake (Fig. 2D). Nesting materials from those recorded in Champhai District comprised various leaves of plants such as *Pinus kesiye*, *Quercus griffithii*, *Q. serrata*, *Heteropanax fragrans*, *Lithocarpus pachyphyllus* etc., which are mainly found in the Assam Subtropical Pine Forest (9/C2) (see Fig. 2C), while the remainder of documented nests were constructed of bamboo leaves such as *Melocanna baccifera*, *Dendrocalamus hamiltonii*, *Bambusa tulda* etc., which are found in Secondary Moist Bamboo Brakes (2/2S1) (Figs. 2A, 2B & 2D) (Champion & Seth, 1968).

In total we relocated 13 nests, along with 12 females, that were threatened by local villagers and were close to either human habitations or agricultural land. The eggs and nests were translocated intact, and the eggs successfully incubated using the original mound inside an enclosure (created using a 1.5 m high tarpaulin ‘wall’) under semi-natural condition in the backyard of the Departmental Building of Zoology (23.736734 °N, 92.663347 °E; 796 m a.s.l.), Mizoram University campus. During the incubation period (June to August), the temperature and humidity within the mounds were recorded using a digital hygrometer, ThermoHygroMeter-Temptec (Mextech™) and were observed to fluctuate between 26–30 °C and 80–90 %, respectively. The females and the hatchlings were released back in the nearest reserved forests to within an average distance of 3.8 km (1.1–5.6 km) of the original nesting sites (Fig. 2F, Table 1S).

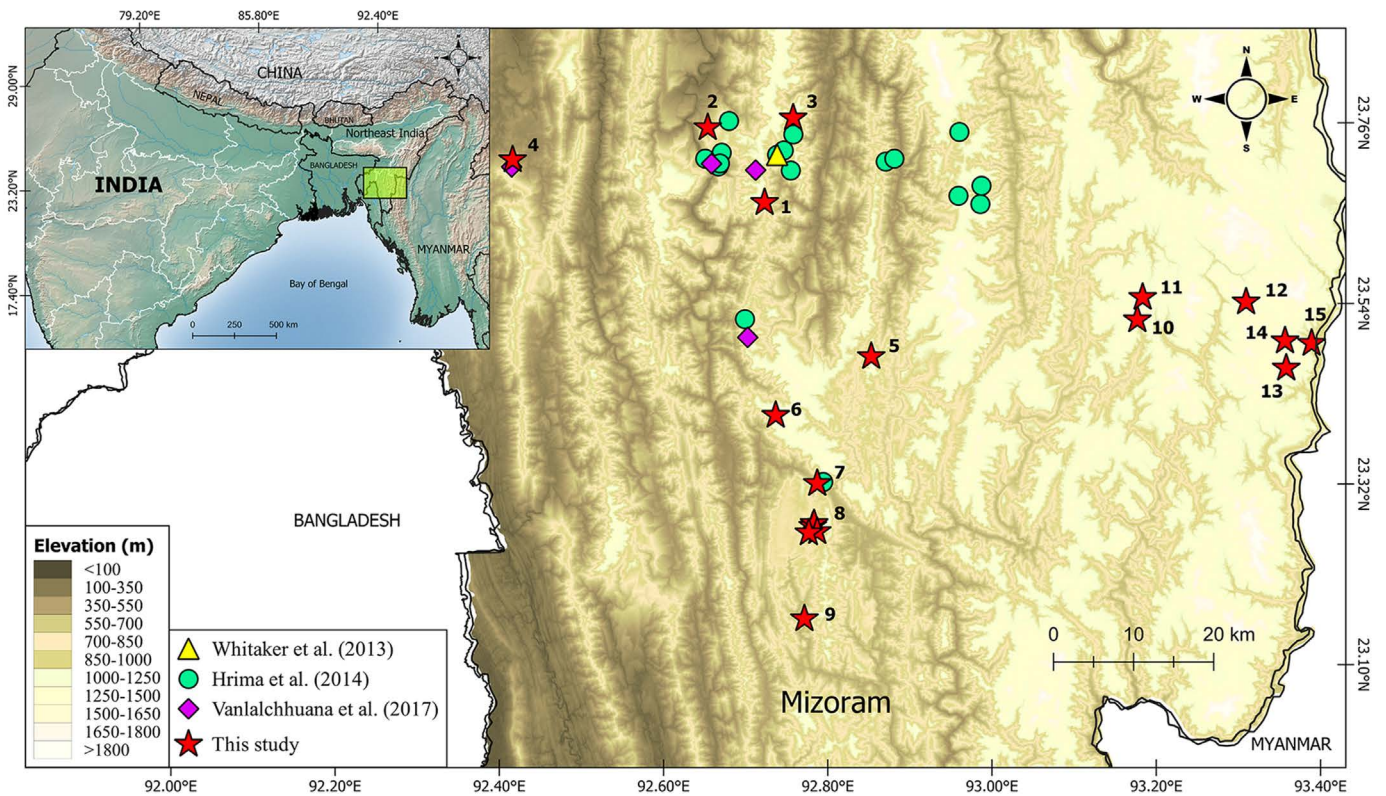


Figure 1. Map showing king cobra nesting sites in Mizoram, north-east India from both previous studies and the current conservation programme (red stars, some red stars refer to more than one nesting site) **1.** Hualngo, **2.** Tanhril, **3.** Muthi, **4.** Dampa Tiger Reserve, **5.** Chhingchhip, **6.** Samlukhai, **7.** Sailam, **8.** Thenzawl I–IV, **9.** Chhipphir, **10.** Khawzawl I, **11.** Khawzawl II, **12.** N. Khawbung, **13.** Tlangsam, **14.** Zote I, **15.** Zote II.



Figure 2. *Ophiophagus hannah* in Mizoram, north-east India: **A.** Deserted nest at Thenzawl IV, **B.** Deserted hatchlings uncovered inside the nest at Chhingchhip, **C.** Completely destroyed nest and eggs with the freshly killed female from Tlangsam, **D.** Female coiling above its nest in Dampa Tiger Reserve, **E.** Hatching from incubated eggs, **F.** Releasing hatchlings at Zongaw Reserved Forests

The local community (Mizo) has a deep-rooted fear of snakes and all are treated as deadly animals, so they are killed without hesitation. Currently, nest translocation is the only available conservation option for the region. We suggest that a more effective long-term conservation management strategy is required that includes an intensive awareness campaign directed at local communities.

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Defensive behaviour by the African snakes *Amblyodipsas unicolor* and *Atractaspis andersonii*

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ABSTRACT - Coiling into a spiral as a way of concealing and protecting the head is described in species of two African snake genera – *Amblyodipsas* and *Atractaspis*, the latter otherwise known for a very different and distinctive ‘snout to ground’ avoidance behaviour. Spiralling and balling are considered in other snakes worldwide and the individualism of animal behaviour warned against. It is to be hoped that recognition of ‘spiralling’ as a distinctive form of ‘coiling’ or ‘balling’ behaviour will lead to more critical analysis of snake defensive behaviour.

INTRODUCTION

Carpenter & Ferguson (1977: 382) list 108 different snake behavioural postures from a survey of almost 1000 publications where they log four of these behaviours (their numbers 24, 39, 71, 95) as ‘balling’. Balling is usually only solicited when a snake is harassed and coils into a compact ball, often with its head at the centre, and this is interpreted as a defensive manoeuvre whereby its head is protected. Here (Fig. 1), I reproduce with permission from Prof. M.S. Kahn (2006) his Plate 126B of the common Indian wolf snake (*Lycodon a. aulicus*) displaying this defensive behaviour. When humans are under assault - as by police with batons, they will attempt to protect their heads by raising their arms - so balling by snakes may reflect a widespread reflex which the limbless snakes carry to perfection. Bustard (1961) provides an account of balling by the Pacific boa and a very informative early summary of this behaviour in other species, including the African ball python (*Python regius*) which gets its name from this habit. When in Ghana, where this diminutive and colourful python abounds, I used to carry a hatchling in my trouser pocket so as to demonstrate to fearful students how harmless some snakes could be.

Spiralling behaviour by *Amblyodipsas unicolor*

In Ghana, in September 1963, I was given a specimen of what Cansdale (1961: 41) refers to ambiguously as “The burrowing snake” (*A. unicolor*) but which Broadley (1971: 646) names the purple-glossed snake, by Mr Brian Harper, an engineer working on Ghana’s first motorway, from near a steelworks at the then new port at Tema (05°37’ N, 00°01’ E). When provoked, the behaviour of this specimen did not result in a ‘ball’ but in a spring-like orderly compaction with the head at the lowest point, not somewhere lost within a knotted ball or a coil in one plane (Fig. 2). This posture is usually maintained when the spiralled snake is gently inverted by human hand but when again upon the ground it would rapidly uncoil/recoil to place its head near the ground. If prodded it would repeat this behaviour, often incompletely: see later with *Atractaspis andersonii*. Similar behaviour would seem to be described for *Alluaudina bellyi* from Madagascar (Domergue 1984: 543) which, it is claimed, can maintain a similar defensive spiral for more than one hour!

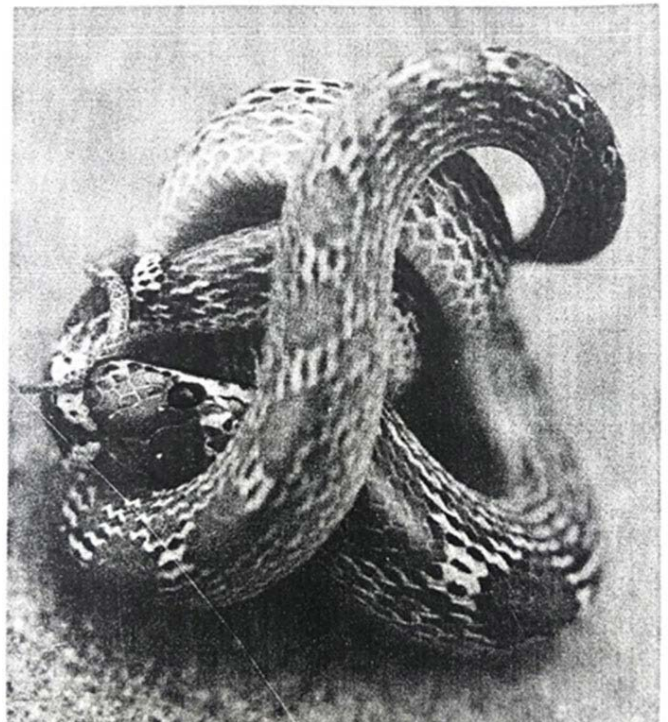


Figure 1. ‘Balling behaviour’ by *Lycodon aulicus*, Plate 126B in Kahn (2006: 198), with permission from Prof. M.S. Kahn

Defensive behaviour by *Atractaspis* spp

Several species have long been known to react to stimulus by arching the neck so as to bring the snout into contact with the ground as though about to try to burrow (Loveridge 1918: 318, 1923: 19, 1928: 1, the last quoted by Pitman 1938: 287, Cansdale 1961: 67, etc). Michele Menegon (in Spawls et al., 2018: 474) has a nice photograph of a stiletto snake (*Atractaspis bibronii*) in a ‘prepare to burrow’ stance, as Pitman (1928: 291) refers to it. Here Steve Spawls has provided a similar picture of an *Atractaspis aterrima* from Wa, Ghana doing the same thing (Fig. 3).

Stationed in the University of Ghana, on the Legon campus since 1960, I was familiar with the stiletto snake (*Atractaspis dahomeyensis*) and could distinguish it on sight from the

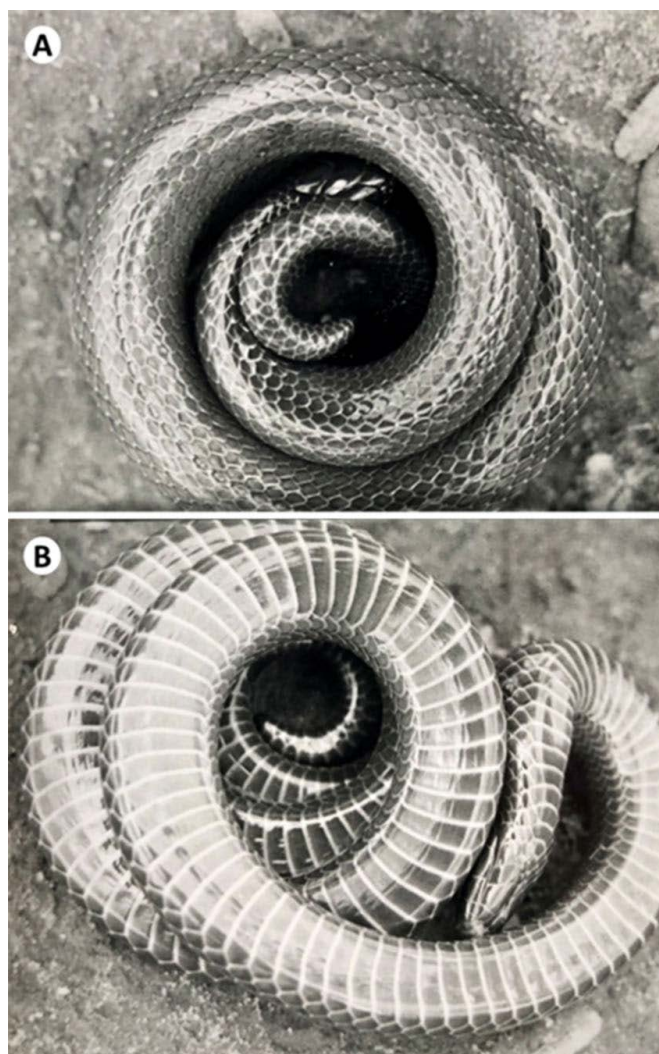


Figure 2. *Amblyodipsas unicolor* ‘spiralled’ specimen **A.** In dorsal view with the tail concealing the head, and **B.** When gently turned over; Ghanaian specimen which later escaped.



Figure 3. *Atractaspis aterrima* from Wa, Ghana displaying typical *Atractaspis* spp ‘arching’ of the anterior body with snout-to-ground; with thanks to Steven Spawls whose specimen (GS 337) later escaped

similar-looking ‘burrowing snake’ (*A. unicolor*) of Candale (1961: 41) because the latter has no more than 17 rows of body scales whereas the stiletto snake has at least 29. In 1963, I collected alive the first *A. aterrima* to be found at Legon but mistook it for Candale’s burrowing snake because it had no more than 23 rows of body scales. Provoking the newly acquired specimen and expecting to be rewarded with a display of spiralling behaviour I ended in hospital for a week with multiple bites on both hands, the result of picking up this unco-operative burrowing viper, as we then called stiletto snakes. A half century later this was a lesson without need of repetition!

Comparable spiralling behaviour by *Atractaspis andersonii*

By contrast Thomas Kowalski (in Grossmann et al., 2018: Abb. 5) and co-author of this account has produced a series of photographs of *A. andersonii* in a defensive spiral where the head is kept near the ground and the tail occupies the apex of the pyramid above it (Fig. 4). Furthermore, when gently inverted the snake will retain this position (Fig. 5) except to attempt to re-position the head out of sight (Fig. 6). If poked roughly, the spiralled snake will rapidly straighten out and as rapidly, recoil into a spiral. This seems to have taken place when the type specimen of *Atractaspis branchi* was collected and it appeared to “jump” when uncoiling and recoiling (Rödel et al., 2018: 114). These behaviours are unexpected from species of a genus of whom several members have long been known to react in a different way, itself thought to be distinctive.

DISCUSSION

The Concise Oxford Dictionary defines ‘coil’ as to ‘dispose in concentric rings’ and ‘spiral’ as ‘coiled’ whether remaining in the same plane like a watchspring or rising in a cone. The writer would here substitute ‘spiral’ from the dictionary definition of a conical coil and restrict coil to one plane (two dimensions) whereas a ‘spiral’ occupies two planes (or 3 dimensions) and it is with these restricted meanings that



Figure 4. *Atractaspis andersonii*, Abb. 5a by Thomas Kowalski (Grossmann et al., 2018: 9) from Oman, ‘spiralled’ position seen from above



Figure 5. *Atractaspis andersonii*, Abb. 5c by Thomas Kowalski (Grossmann et al., 2018: 9) when overturned.



Figure 6. *Atractaspis andersonii*, Abb. 5f by Thomas Kowalski (Grossmann et al., 2018: 9) when attempting to re-spiral.

these terms will be used here. Neither of these concepts can accommodate the posture adopted by a Zimbabwean slug eater (*Duberria lutrix rhodesiana*) as described by Taylor (1970: 18) with a diagrammatic sketch of a horizontally coiled body within which the snake has placed its head. This was a unique event where the subject did not survive attack from a crow and could not be elicited from other specimens of the same species. Balling and spiralling hide and protect the head: the distinction may be academic but both are defensive postures.

It should be noted that animals of both genera considered here spend most of their time in a burrow underground and come to the surface at night to feed, or to breathe when the ground is waterlogged. The behaviours described cannot be effected in underground burrows nor can contact with troublesome humans who produce the duress under which they exhibit this unusual defensive behaviour. The suddenness of spiralling and unspiralling produces uncertainty and

wariness in the observer and may be effective in warning off possible predators.

Another lesson to be learned is that behaviour observed many times by different observers with different species of *Atractaspis* does not mean that such snout to ground behaviour is universal to species of this genus. On the other hand, the second lesson is that whilst none of the second author's photographs show *A. andersonii* performing typical snout to ground behaviour, we do not know if it might not have done so on another occasion under different circumstances. Both of these reservations also apply to the *Amblyodipsas* and show how these casual observations may be misleading as to the kinds of behaviour to be associated with particular snake species. The taxonomy may be exact but individuals are not.

It is to be hoped that recognition of 'spiralling' as a distinctive form of 'coiling' or 'balling' behaviour will lead to more critical analysis of snake defensive behaviour by future observers.

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Records of multiple clutching in captive mountain chicken frogs *Leptodactylus fallax*

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ABSTRACT - Multiple clutching, with two or three successful clutches raised in a single breeding season, is reported from two females of *Leptodactylus fallax* in two European zoos. Previously, only single clutches were known to be raised by this species. Multiple clutching is perhaps unexpected in this species due to its resource-heavy parental care behaviour. Potential drivers of multiple clutching, including food resourcing and timing and size of initial clutches, are discussed.

INTRODUCTION

The mountain chicken *Leptodactylus fallax* (Müller, 1926) is the largest native species of anuran in the eastern Caribbean, and a vital part of the islands' ecology as a native apex predator (Hudson et al., 2016). Historically occurring on six - possibly eight - Lesser Antillean islands, its range is now restricted to Montserrat and Dominica, due to anthropogenic pressures (Hudson et al., 2016), including the arrival of the fungal pathogen *Batrachochytrium dendrobatidis*, one of the causative agents of the disease amphibian chytridiomycosis (Hudson et al., 2016). Now assessed as Critically Endangered (IUCN SSC, 2017), approximately 130 individuals of *L. fallax* remain on Dominica and the species is likely extinct in the wild on Montserrat (Hudson et al., 2016). Until the threat of emerging infectious disease can be ameliorated, a bio-secure, captive metapopulation has been established in European zoos, alongside a non-biosecure population from the same gene pool, with the aims of producing viable stock for future translocation purposes, as well as for research and public engagement initiatives (e.g. Tapley et al., 2015; Jayson et al., 2018a,b; Michaels et al., 2021). Captive breeding success and translocation planning requires further life history data to inform refinement and to tackle current limitations (Jameson et al., 2019) and for this reason, it is important to document new information when unique observations are made.

The reproductive mode of *L. fallax* is unique to the species and was first described from captive animals. Larvae hatch from eggs laid within a terrestrial foam nest created by the male and female frog. The female guards the nest throughout tadpole development and feeds the tadpoles by releasing unfertilised eggs into the nest (Gibson & Buley, 2004). The male likely defends the surrounding area (Gibson & Buley, 2004). Once the tadpoles are ready to metamorphose, they leave the nest and at this point, parental care stops (Gibson & Buley, 2004).

Although *L. fallax* has repeatedly reproduced in captivity (e.g. Gibson & Buley, 2004; Tapley et al., 2015; Jameson et al., 2019), reproduction is inconsistent between individual

animals and between institutions, only single viable clutches per female per annum have been recorded in ex situ populations and there is very little information on reproduction in nature (e.g. Davis et al., 2000). Captive female *L. fallax* are known to produce multiple foam nests containing eggs within a single season (Jameson et al., 2019), but have not been documented to rear more than one of these; others are typically abandoned soon after oviposition (Jameson et al., 2019).

Here we describe the first incidences for the species of multiple successful clutching and rearing events in two European institutions.

METHODS AND RESULTS

Animals involved in observations are referred to by institution (ZSL or NA), sex (F or M) and number, where more than one individual of a given sex is involved. For detail of the backgrounds of each animal and husbandry conditions refer to Table 1S in the Supplementary Material. Multiple clutching was observed separately at the Zoological Society of London, London Zoo in 2017 and 2019, and at Nordens Ark in 2020 and 2021. Environmental conditions are an important determinant of fecundity in amphibians; frogs in both institutions were housed according to best practice husbandry guidelines (Table S1; Jameson et al., 2019). Briefly, animals were kept in large pens of at least 2 x 2 m with a substrate of damp organic mulch, various refugia and nest boxes for reproduction (see below). Temperatures were between c. 21 °C (night) and 30-35 °C (day) with seasonal variation and spatial gradients. Reproduction was primarily stimulated by small increases in temperature, especially at night, combined with increasing frequencies of spraying, simulating the warm, rainy period triggering reproduction in nature (Jameson et al., 2019). Nests were exclusively produced in nest boxes constructed according to Jameson et al. (2019), essentially opaque plastic storage boxes with entrance tunnels and lined with damp clay. Boxes were checked c. weekly for new nests. Nests were left under

Reproductive events at ZSL London Zoo		
Date	Event	Offspring
24.05.2016 06.04.2017	ZSLF and ZSLM1 paired Sprays increased 2x/day	
28.04.2017 15.06.2017 16.06.2017	Nest 1 found (sire: ZSLM1) First metamorphs moved from nest Last metamorph moved from nest	4/6 metamorphosed 2/4 metamorphs developed health conditions requiring euthanasia
30.06.2017 14.08.2017	Nest 2 found (sire: ZSLM1) All metamorphs moved from nest	31/33 metamorphosed 31/31 metamorphs remained healthy
02.09.2017 16.04.2018 30.03.2019 01.04.2019	Sprays decreased 1x/day ZSLM1 moved out of enclosure ZSLF and ZSLM2 paired Sprays increased 2x/day	
07.04.2019 31.05.2019 04.06.2019	Nest 1 found (sire: ZSLM2) First metamorphs moved from nest Last metamorph moved from nest	6/7 metamorphosed 6/6 metamorphs remained healthy
06.06.2019 12.06.2019	ZSLM2 moved out of enclosure ZSLF and ZSLM3 paired	
17.07.2019 30.09.2019 05.09.2019	Nest 2 found (sire: ZSLM3) First metamorphs moved from nest Last metamorph moved from nest	13/13 metamorphosed 1/13 metamorphs developed health conditions requiring euthanasia
Reproductive events at Nordens Ark		
Date	Event	Offspring
29.08.2019 01.10.2019 01.11.2019 01.03.2020	NAF, NAM1 and NAM2 paired Sprays and photoperiod decreased Sprays and photoperiod decreased Sprays and photoperiod increased	
25.04.2020 01.05.2020 01.06.2020 17.06.2020	Nest 1 found (sire: NAM1) Photoperiod increased Sprays increased Last metamorph moved from nest	1/1 metamorphosed
22.06.2020 01.08.2020 15.08.2020 16.08.2020	Nest 2 found (sire: NAM1) Sprays decreased First metamorphs moved from nest Last metamorph moved from nest	4/4 metamorphosed 1/4 metamorphs developed health conditions requiring euthanasia
30.09.2020 01.11.2020 26.11.2020	Nest 3 found (sire: NAM2) Photoperiod decreased Last metamorph moved from nest	10/10 metamorphosed
21.12.2020 01.03.2021 01.04.2021	NAF and NAM3 paired Sprays and photoperiod increased Sprays and photoperiod increased	
04.05.2021 01.06.2021 07.07.2021	Nest 1 found (sire: NAM3) Sprays increase 2/3 metamorphs moved from enclosure	3/3 metamorphosed without further complications
10.07.2021 01.08.2021 19.08.2021	Nest 2 found (sire: NAM3) Sprays decrease Metamorphs (6) are starting to leave the nest	6/6 metamorphosed frogs are kept with parents and sibling

parental care; tadpoles were counted once they became individually discernible. Metamorphs were removed once the tail had shrunk considerably and mouthparts began to transform (Gosner stages 43-45; Gosner, 1960), indicating the cessation of oophagy.

One female, ZSLF, produced two viable nests in 2017 and 2018, while NAF produced three viable nests in 2020 and two in 2021. Temporal sequences of frog pairing, courtship and nest production, environmental manipulation and the metamorphosis of young are recorded in Table 1, along with

the number of tadpoles produced and proportion surviving to metamorphosis. All other aspects of reproduction were unremarkable.

DISCUSSION

Multiple clutching has been reported in a number of other frog species, especially taxa that are asynchronous in oogenesis (Tsuji & Lue, 2000). However, multiple clutching is typically observed in species without extended female

parental care such as those where eggs are abandoned at oviposition (Tsuji & Kue, 2000), or where male, rather than female, parental care is present (Townsend & Stewart, 1994; Rogowitz et al., 2001), although notable exceptions to this include dendrobatid frogs with both multiple clutching and female extended parental care (Meuche et al., 2011). Reproduction in *L. fallax* females, which are likely asynchronous in oogenesis, is particularly costly in that the animal must commit resources to nest construction and courtship, production of both developing and infertile feeder eggs (an estimated 10,000-25,000 feeder eggs per clutch; Gibson & Buley, 2004), defence and maintenance of the nest, and must also have sufficient energy stores to survive almost complete cessation of feeding during larval development. Females frequently lose up to one third of their pre-breeding body mass during the period of parental care (pers. obs., authors) and the short breeding season across c. 3 months of the year leaves little time for replenishment of energy reserves after an initial successful clutch.

Although we report multiple clutching from two separate individuals of *L. fallax*, this is still a rare occurrence in the captive population and has never been documented in the wild. A number of factors common to both individuals may have contributed to this phenomenon. Both females were in very good body condition (scoring 3-4 on the mountain chicken frog body condition score scale; Jayson et al., 2018b) and were well habituated to keeper routines, ZSLF having been established in captivity for more than a decade and NAF having been raised in the Nordens Ark facility since metamorphosis. This allowed animals to capitalise on feeding events to consume numerous prey items and maximise the nutritional benefits of prey items, which lose nutritional quality quickly after addition to an enclosure (Michaels et al., 2014). ZSLF was an especially large animal and had far exceeded average longevity for the species, being an adult when collected from the wild and then having lived for around 16 years in captivity, compared with an average longevity of around nine years (Guarino et al., 2014). Although this may have influenced reproductive capacity, the Nordens Ark female was not similarly large or aged. Both animals produced initial, small clutches early in the reproductive season. This may have resulted in minimal depletion of energy reserves, and sufficient time to replenish these reserves in time to produce further clutches. At least one clutch per animal was within normal numbers for this species in captivity, bearing in mind that clutch size in captivity is substantially smaller than seen in new founder animals (Gibson & Buley, 2004) and appears to be decreasing in time within and between generations (Jameson et al., 2019). Although both animals had access to only one (ZSLF) or two (NAF) males (Table 1), both facilities contained additional males, the presence of which may have stimulated reproductive events. Finally, handling of frogs was kept to an absolute minimum, with animals captured only for necessary veterinary interventions; as disturbance and capture is thought to reduce food intake (Jameson et al., 2019), this low-disturbance management may have facilitated frogs building energy reserves through feeding as well as avoiding the interruption of breeding events.

The ability of *L. fallax* to produce multiple clutches within one breeding season may have implications for captive management and for the recovery of populations in nature, which at least for the time being, is likely to be dependent on the translocation of captive bred individuals. A better understanding of drivers of multiple clutching and of the relatively small clutch sizes associated with some clutches would facilitate this and further research in this area is recommended.

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First record of feeding and defensive behaviour in Thompson's caecilian *Caecilia thompsoni* from the Upper Magdalena Valley, Tolima, Colombia

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The most speciose caecilian genus in the Neotropics, *Caecilia* Linnaeus, 1758, has a total of 34 species distributed in southern Central America and South America. These caecilians are characterised by having an imperforate stapes, inner mandibular teeth, eyes surrounded by the maxillopalatine bone, and monocusped teeth (Wilkinson et al., 2011) and have offspring that develop within the egg so that there is no aquatic stage (Funk et al., 2004; Pérez et al., 2009).

Caecilia thompsoni (Boulenger, 1902) is the world's largest caecilian, reaching 1767 mm total body length (Arredondo-Salgar, 2007). In Colombia, this species inhabits the Magdalena Valley lowlands as well as the eastern slopes of the Cordillera Central and the western slopes of the Cordillera Oriental, ranging from 300-1600 m a.s.l. (Fig.1). According to Lynch (2000), this caecilian can be diagnosed by its range of 187-240 primary grooves, 26-42 secondary grooves, a total body length of 471 to 1767 mm (Arredondo-Salgar, 2007) and its ratio of length/width of 62-100 times. In life, its main body colouration can vary from dark purple, blue or slate on the dorsal surfaces of the body and usually a lighter hue of the same colour on the ventral surfaces of the body (Fig.2 A-H).

The diet, predatory and defensive behaviours of *C. thompsoni* are entirely unknown although it is known that the species is preyed upon by the coral snakes *Micrurus dumerilii* and *M. mipartitus* (Bernal & Palma, 2011; Herrera-Lopera & Ramírez-Castaño, 2018). Unfortunately, little is known about the majority of caecilian species because their fossorial habits keep their natural history a secret (Gower & Wilkinson, 2005); therefore, any studies aiming to document and describe natural history and ecological aspects of caecilians are important.

During the course of our routine fieldwork in Cuatro Esquinas Vereda, Carmen de Apicalá municipality, eastern Tolima department, Colombia (4°5'59.798" N, 74°47'0.221" W, 335 m a.s.l.; Fig.1), we were able to witness and photograph a predation/regurgitation event involving *C. thompsoni* and a large earthworm (Fig.2 A-H). This event took place on the night of 2nd April 2019 inside a highly degraded riverside forest patch, situated among an agroecosystem composed of open grasslands given over to grazing cattle, and the remnants of secondary tropical dry forest patches located in

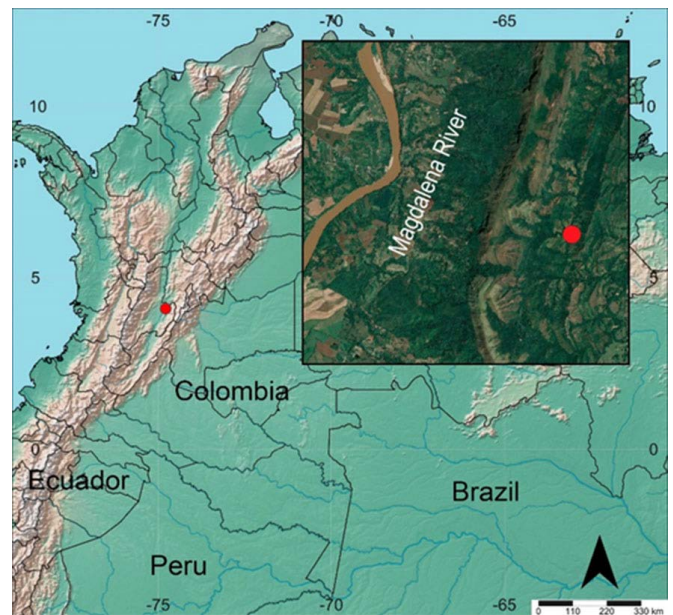


Figure 1. Map of north-western South America indicating the locality of Cuatro Esquinas, Carmen de Apicalá, Tolima, Colombia, with a red dot. The insert is a satellite image taken from Google Earth Pro.

the western foothills of the Apicalá River Valley (Fig.1). The mean annual temperature of this region is 28 °C and its mean annual rainfall is 1630 mm in a biannual cycle (IDEAM, 2017; Gómez-Sánchez et al., 2021).

At 23:10 h on this very humid night, we came across a medium size individual of *C. thompsoni* (ANDES-A 4462; 725 mm total body length; Fig.2) that was actively hunting a long (200 mm) earthworm (class Clitellata, subclass Oligochaeta). The main body colouration of the caecilian was somewhat blueish grey, darker dorsally and much lighter grey ventrally (Fig.2 A-H). The caecilian was observed crawling across a patch of sandy, muddy soil (Fig.2 A-H) partially covered by tree roots located along the bank of a small creek. As soon as it seemingly became aware of our presence it began to crawl backwards, perhaps trying to find shelter while testing the surface of the ground with its terminus. Then it started to regurgitate the earthworm that was half swallowed. This 'prey release' lasted approximately four minutes. Once the



Figure 2. Details of the partial ingestion and then regurgitation of an earthworm by *Caecilia thompsoni* **A.-B.** *C. thompsoni* has bitten the earthworm, **C.** *C. thompsoni* pulls the earthworm out of the soil, **D.** The earthworm is now half eaten, **E.** *C. thompsoni* starts moving backwards slowly, **F.** Regurgitation begins, **G.-H.** *C. thompsoni* moves backwards and finishes regurgitating its prey.

caecilian finished regurgitating the earthworm, it quickly began moving forward seemingly trying to escape from us. At this point (23:14 h) both the caecilian and earthworm were captured and brought to our field station at finca El Albergue to be preserved for future housing at Museo C.J. Marinkelle, Universidad de Los Andes, Bogotá, Colombia.

We identified ANDES-A 4462 as *C. thompsoni* based on the following morphological characters and meristic data. A *Caecilia* with a total body length of 725 mm, width at mid-body of 11.6 mm, an attenuation index (length divided by width) of 62.5 times, 200 primary grooves and 44 secondary grooves found throughout its body length, no terminal shield, with subdermal scales within the connective tissue of the skin and dermal scales within the dermal pockets, dentition arranged as follows: 8-1-8 premaxillary-maxillaries, 9-1-9 prevomeropalatines, 4-4 dentaries and 2-2 splenials. At the moment *C. thompsoni* is the only Colombian caeciliid known to reach and surpass the 200 primary groove count and the number of secondary grooves and dentition falls within the range provided in Taylor's (1968) account for this species, therefore, we are confident of our identification.

Furthermore, the slender and elongated body, the blueish grey body colouration, the "bullet-shaped head" (Lynch, 2000) and its provenance from the Magdalena Valley concur with the identification that we have given to this specimen.

Even though we did not provoke or attack the caecilian - at least not in any way perceivable to our human senses - we can only consider our presence (i.e., the perception of our smell, the flash of the camera, the light coming from our headlamps, and the vibration of our footsteps) in its proximity to have triggered a warning signal that prompted the caecilian to panic and start regurgitating its prey; still, the possibility that the earthworm was 'unpalatable' to the caecilian cannot be ruled out. Considering the high physiological costs associated with foraging (Duellman & Trueb, 1986) and the fact that when caecilians emerge to the surface they make themselves vulnerable to predators, we interpret prey regurgitation as a 'last resort mechanism' that sacrifices the energetic and physiological rewards of a meal for the chance to escape a potential predator.

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Red tail in male spiny footed lizards *Acanthodactylus erythrurus* during the breeding season

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The spiny footed lizard *Acanthodactylus erythrurus* (Schinz, 1833) is a widely distributed lacertid from the Iberian Peninsula and north-west Africa (Sindaco & Jeremcenko, 2008) of which the complex taxonomy continues to be an ongoing topic of discussion (Harris et al., 2004; Fonseca et al., 2009; Miralles et al., 2020). This species complex displays visible polymorphism throughout its range, although its features of sexual dimorphism are considered to be invariable across all phenotypic forms (Schleich et al., 1996; Salvador, 2014). Chromatic dimorphism mainly consists of the accentuation of colouration in males, especially the lateral yellow ocelli, whilst in females the tail and hind legs exhibit a conspicuous red/orange colouration. These characteristics are evident both in the population of north-west Africa (Schleich et al., 1996), as well as in the populations of the Iberian Peninsula (González de la Vega, 1989; Ortiz-Santaliestra et al., 2011; Fresnillo et al., 2015). It has even been suggested that there are no examples of males displaying tails with red colouration (Barbadillo, 1994). Hypotheses that aim to account for this sexual dimorphism contemplate the possibility that the red tail observed in sexually receptive females could be linked to mating, whilst the loss of colouration could be linked to pregnancy (Cuervo & Belliure, 2013).

This report describes various observations of male individuals with intense red colouration of the tail and hind legs during the breeding season in the Campo de Gibraltar region of the Province of Cadiz in the south-east of the Iberian Peninsula.

The first observation took place in May 2004 in Pinar del Rey (36°15' N, 5°25' W; UTM 30S TF 83 14), through the capture of an adult individual with a snout-vent length over 6 cm and unmistakably male characteristics (large and prominent femoral pores and broad tail-base) displaying red colouration of the tail and hind legs. As juveniles display red tails, it was originally thought possible that the individual had retained certain immature characteristics and therefore that this was an anecdotal observation of a persistent juvenile trait.

Subsequent sporadic observations without capture were made in the same location during the period 2010-2012, leading to sampling in 2013 during the months of May and June. On the 31st May, four males and five females were observed, with all nine individuals displaying red colouration of equal intensity and extension (Fig. 1. A, C, E). On 17th June, only two males and two females were observed, due to unfavourable weather conditions during sampling. None

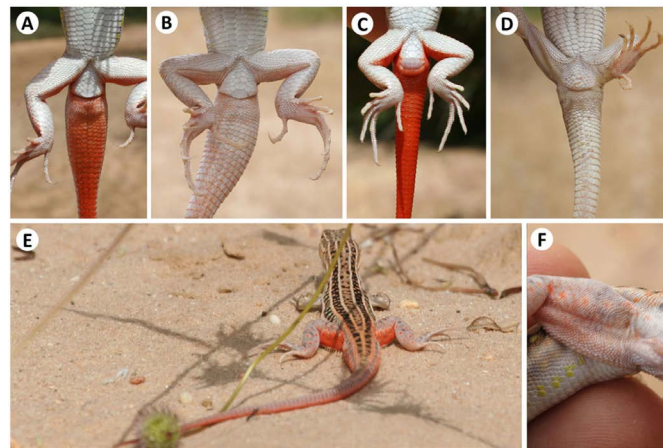


Figure 1. Tail colouration in male *Acanthodactylus erythrurus*- **A.** 31 May, **B.** 17 June; Tail colouration of female *A. erythrurus* - **C.** 31 May, **D.** 17 June; Close-up of hind legs of male, **E.** 31 May, **F.** 17 June



Figure 2. Gravid female *Acanthodactylus erythrurus*, 17 June

of the four individuals displayed conspicuous red colouration (Fig. 1. B, D), with only one male still displaying very indistinct orange traces on the hind legs (Fig. 1. F). One of the females was observed to be gravid (Fig. 2). On 21st May 2019, a male was observed with colouration of the tail in El Chapatal (36°16' N, 5°26' W; UTM 30S TF 82 18), approximately 3.5 km from the site of the first observations.

In principle, the combination of observations made indicate that males and females acquire and lose red colouration simultaneously, as it was in the month of May when maximum colour intensity was observed. Large specimens were not observed during the sampling, so the phenomenon has only been observed in males during their first reproductive year. All individuals were found in the grid 30S TF 81 belonging to the metapopulation present in the eastern area of the Campo de Gibraltar, which covers grids 30S TF 90, TF 91 and TF 92. Mitochondrial DNA analyses have shown the existence of two clades in the Iberian Peninsula. E2 is restricted to the provinces of Cadiz and Malaga whilst E1 occupies the rest of the peninsular area (Fonseca et al., 2009). Further research into the populations located in the Province of Malaga would be necessary in order to determine this as an identifying characteristic of the E2 clade.

These observations rule out red tail colouration as a decisive feature of sexual dimorphism in sexually receptive females. This should be confirmed in other populations and a new hypothesis formulated explaining the evolutionary reason for this characteristic in males.

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First records of Columbus crabs *Planes minutus* associated with loggerhead turtles *Caretta caretta* in Galicia (NW Spain)

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The loggerhead turtle (*Caretta caretta* Linnaeus, 1758) is distributed in temperate and tropical oceans and seas worldwide (Wallace et al., 2010). Most of the loggerheads that appear on the European Atlantic coast are juveniles originating from nesting areas in the western Atlantic (Bolten et al., 1998). These specimens have either deviated from their usual developmental migration within the north Atlantic Gyre system (Witt et al., 2007), or have originated from Cape Verde having been displaced by storms (Monzón-Argüello et al., 2012). Since 1990, in Galicia (NW Spain) an average of 9.7 individuals are recorded yearly by the stranding network: they are juveniles that are found stranded at the coast or accidentally captured in fishing gear, both alive and dead (López et al., 2014).

The Columbus crab (*Planes minutus* Linnaeus, 1758) is a small pelagic species that is found in the north Atlantic, western coast of Africa, Mediterranean Sea and western Indian Ocean, living on floating material, both natural and artificial, and frequently associated with sea turtles (Pfaller et al., 2019). In Galicia, these crabs have been found on beaches, associated with the gooseneck barnacle (*Lepas anatifera* Linnaeus, 1758) (Urgorri et al., 1990), but they had never been observed on sea turtles. However, according to the records summarised by Pfaller et al. (2019) the species has been reported on turtles in nearby areas including the Canary Islands, Madeira, the Azores and France.

From 1990 to 2016 a total of 122 live loggerhead turtles were examined in Galician rehabilitation facilities, and no Columbus crabs were reported. In 2017, the first crab was found on the single live turtle that was examined. From 2018 to 2020 just one live turtle was examined and no crabs were recorded. But in 2021, 4 crabs were found in 3 of 4 examined live turtles, evidencing an increase of the crab's occurrence on turtles. All five reported crabs were observed around the tail and rear flippers of the turtles (Fig. 1), which are the places where they are usually found (Davenport, 1994), although they have also been observed occupying a wound in the carapace (Anjos et al., 2021). The smallest turtle with an associated crab had a straight carapace length of 21.5 cm. It could be thought that this is a very small size to have this kind of epibiont, but crabs have been found on smaller loggerhead turtles in Madeira (Dellinger et al., 1997). Actually, the crab is more frequently present on juvenile turtles, which live in epipelagic habitats, than on subadult and adult turtles that



Figure 1. One of the Columbus crabs found on a loggerhead turtle captured accidentally on 20 April 2021

occupy mostly neritic habitats (Pfaller et al., 2019).

The crabs were measured with a Vernier digital calliper and weighed with a digital scale Pesola MS500 (Table 1). According to body measurements proposed by Dellinger et al. (1997), all the crabs were adults, but none of the three females carried eggs. In previous studies it had also been observed that the vast majority of crabs recorded on turtles were adults (Dellinger et al., 1997; Scaravelli et al., 2008). The reason for this would be that juvenile small crabs colonise floatsam and large ones can move to sea turtles with little or even no swimming involved, when the turtles rest or feed close to floatsam (Dellinger et al., 1997). Two of the crabs, female and male, were found together on the same turtle. Heterosexual couples have been reported previously, even sometimes being more frequent than solitary individuals (Dellinger et al., 1997; Scaravelli et al., 2008).

The relationship between the turtle and the crab could be considered as mutualistic, since both species gain a benefit. The crabs that live on turtles feed on other epibionts, including crabs, or capture food particle while the turtle is feeding, obtaining a higher diversity of prey than crabs living on floatsam; meanwhile, the turtle receives a cleaning service (Frick et al., 2004) which prevents the proliferation of epibionts that can alter the swimming capabilities of the turtle (Anjos et al., 2021).

Table 1. Columbus crabs recorded on loggerhead turtles in Galicia (NW Spain)

Date	Coordinates	Turtle SCL (cm)	Condition	Crab			
				Sex	SCL (mm)	SCW (mm)	Weight (g)
06/12/2017	43°45' N, 007°38' W	52.5	Gillnet by-catch	M	20.54	19.98	3.9
03/03/2021	42°20' N, 008°46' W	21.5	Gillnet by-catch	F	18.74	18.66	3.1
20/04/2021*	43°44' N, 007°50' W	23.0	Gillnet by-catch	F	17.88	17.71	2.3
20/04/2021*	43°44' N, 007°50' W	23.0	Gillnet by-catch	M	15.72	15.53	2.0
09/07/2021	42°11' N, 008°47' W	22.1	Stranded	F	17.06	16.74	2.4

*Crabs found on the same loggerhead turtle, F – female, M – male, SCL – straight carapace length, SCW – straight carapace width

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Foam making behaviour of tadpoles of the pepper frog *Leptodactylus vastus* in north-eastern Brazil

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The pepper frog *Leptodactylus vastus* Lutz, 1930 belongs to the *Leptodactylus pentadactylus* group and is the largest species of leptodactylid to occur in north-eastern Brazil (Heyer, 2005). This species is endemic to Brazil and occurs exclusively in open-formation habitats (Heyer, 2005).

At the time of the first rains, adult leptodactylids produce foam nests near the margins of temporary or permanent pools, or in cavities excavated in the soil. The eggs are enclosed in a dense layer of foam (Silva et al., 2005). In some leptodactylids, foam production is not exclusive to adults, with tadpoles of the species also showing this behaviour. This has been recorded in *Leptodactylus labyrinthicus*, which also belongs to the *L. pentadactylus* group (Kokubum & Giaretta, 2005), in five species of the *Leptodactylus fuscus* group, namely *L. fuscus* (Downie, 1984; Downie, 1989), *L. mystaceus* (Caldwell & Lopez, 1989), *L. furnarius* (Giaretta & Kokubum, 2004), *L. latinasus* (Ponssa & Barrionuevo, 2008) and *L. troglodytes* (Kokubum et al., 2009). It is also known in two species of the genus *Adenomera* (Almeida & Angulo, 2002; Kokubum & Giaretta, 2005).

Foam production by tadpoles assists in obtaining soil moisture, preventing tadpoles contacting the bottom of the nest, preventing compaction and overcrowding, and facilitating respiratory processes and excretion (Downie & Smith, 2003; Kokubum & Giaretta, 2005). Here, we report the results of a laboratory test designed to show whether or not tadpoles of *L. vastus* can produce foam and to what extent the addition of small amounts of water is required for foam production.

For the tests, two egg clutches were collected in a flooded, grassy area on the campus of Universidade Federal de Campina Grande, municipality of Patos, Paraíba State, Brazil (7° 1'32" S, 37° 16'40" W). Species identity was inferred from traits of the foam nests, in particular the large size (>30 cm diameter) and thickness of the foam, also taking into account the species present in the area (Heyer, 2005). Identity was subsequently confirmed by following the tadpoles through to complete metamorphosis. The first clutch (471 tadpoles) was collected on January 18th 2021, and the second (295 tadpoles) on February 5th 2021. In the first clutch, we found that the tadpoles had already hatched and were in stage 27-31 of Gosner (1960), there were also 143 possibly trophic

eggs. The second clutch also had some possible trophic eggs but they could not be counted due to the dryness of the clutch.

The test followed the design of Kokubum & Giaretta (2005). Sixty tadpoles in stages 27-31 (Gosner, 1960) were removed from each clutch, washed in dechlorinated tap water and then placed in 30 cm³ Falcon® tubes (Fig. 1A). For each clutch two replicates were prepared with 5, 10 or 15 tadpoles in the tube. Each group was kept for a period of up to six days in the Falcon® tube (Fig. 1A). The test with clutch 1 was initiated on 18th January 2021 and with clutch 2 on 5th February 2021. At the start, a shallow film of water (two drops with a doser) was added to all tubes just covering the tadpoles, this was to prevent desiccation; the tubes were kept open. In test 1, the water film was replenished as required, when the foam production was stopped, while in Test 2 it was not replenished immediately, to observe the influence of moisture on foam production. We measured the air temperature and air humidity with a digital thermohygrometer. The mean air temperature in Test 1 was 31.5° C (29.0°-33.4° C) and the mean relative humidity 39 % (30 %-48 %). In the second test, the mean temperature was 32.6° C (30.4°-34.4° C) and the mean relative humidity 43 % (29 %-50 %). Once the tests had started the tubes were observed every three hours.

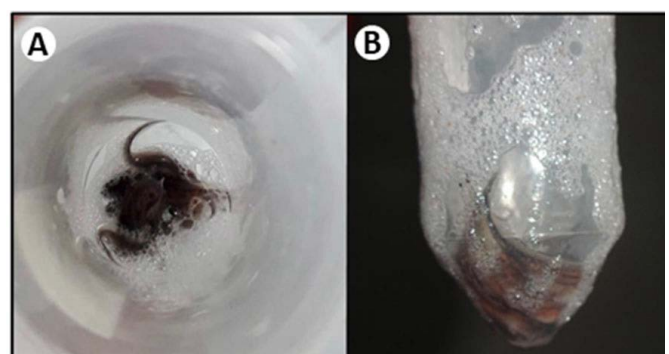


Figure 1. A group of 15 *Leptodactylus vastus* tadpoles in a plastic tube three hours after the beginning of a test to observe foam production-
A. Tadpoles viewed from above, **B.** Tadpoles viewed from the side

The tests were considered successful, since the tadpoles began to produce enough foam to move around in the tube after the first three hours in both tests (Fig. 1B). In those tubes with 15 tadpoles, foam had appeared by 90 minutes, indicating that with greater numbers of tadpoles the foam is produced more quickly. In Test 1, tubes containing 5 or 10 tadpoles required the film to be replenished after 37 h while in the tubes containing 15 tadpoles the foam lasted longer and did not need replenishment until 55 h after the start. This corroborates the observations of Downie & Smith (2003) for the need of a shallow film of water for foam production and that with greater numbers of tadpoles the water film does not need replenishment so quickly.

In Test 2, the tadpoles in the two tubes with 5 individuals had all died after about two days as the water film was not replenished; death occurred about 7.5 hours after the foam production had ceased. The tadpoles in the two tubes with 10 individuals maintained a considerable quantity of foam until three days after the start of the experiment, on the fourth day the foam production had declined and after a further 8 hours the tadpoles had died. The tadpoles in the two tubes with 15 individuals managed to maintain a considerable amount of foam until the fourth day when the foam production declined and within 7 hours the water film was replaced which prevented the death of the tadpoles.

We observed that the production of foam by the tadpoles was caused by the release of small bubbles from the mouth parts of the tadpoles and was facilitated by the movement of their tails, in a similar manner to the tadpoles of *L. fuscus* (Downie, 1989), *Adenomera* sp. and *L. labyrinthicus* (*pentadactylus* group) (Kokubum & Giaretta, 2005). Three leptodactylids foam-producing tadpoles have been shown to have oral epithelial mucous glands - *L. furnarius*, *L. labyrinthicus* and a member of the *L. marmoratus* group (= *Adenomera*) (Giaretta et al., 2011).

The ability of *L. vastus* tadpoles to produce foam, independent of their parents, allows tadpoles to remain in the foam nest for longer, preventing nest desiccation. Additionally, this behaviour occurs before or after periods of irregular rainfall, which allows tadpoles to survive and develop until frequent rains provide temporary water bodies where they complete their development. This is before other species that begin their reproduction only in the period of regular rains and provides an advantage to the foam-producing species. In the species of the *fuscus* group, tadpoles in foam nests reach a specific stage, but do not progress any further until the rain washes them into a pool (Downie, 1984). But those of the *pentadactylus* group that have been studied, continue to develop and grow because their tadpoles are able to feed on trophic eggs (Kokubum & Giaretta, 2005).

The individuals involved in this test were sacrificed using the liquid anaesthetic lidocaine, fixed in 5% diluted formaldehyde and deposited in the Collection of Amphibians and Reptiles of the Laboratório de Herpetologia da Universidade Federal de Campina Grande (LHUFCL0115, LHUFCL0116, LHUFCL0117, LHUFCL0118, LHUFCL0119, LHUFCL0120, LHUFCL0121), municipality of Patos, state of Paraíba, north-eastern Brazil, under the license (#25267-1)

of the Biodiversity Authorization and Information System (SISBIO-ICMBio).

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Rediscovery of the Quindío robber frog *Pristimantis alalocophus* in a new locality in the Department of Quindío, Colombia

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Colombia has a remarkable diversity and endemism of amphibians of the genus *Pristimantis* (Anura: Craugastoridae), hosting more than 230 species (Acosta-Galvis, 2021; Rivera-Correa et al., 2021), of which 149 species are endemic to the country (IUCN, 2021). However, half of this diversity has been listed in a conservation threat category (IUCN, 2021) due to deforestation, agricultural and mining activities, emerging infectious diseases and pollution (Stuart et al., 2004; Lips et al., 2008; Ruíz-Carranza & Rueda-Almonacid, 2008; Isaacs-Cubides & Urbina-Cardona, 2011). Additionally, in expeditions to type localities and/or their surrounding areas to search for some species of the genus *Pristimantis* many of these have not been found, remaining known only from their formal description, which alerts us to the possibility that they may now be extinct (e.g. *Pristimantis lichenoides*, *Pristimantis torrenticola*, Duarte-Marín et al., 2018; *Pristimantis cacao*, *Pristimantis diogenes*, Pisso-Flórez et al., 2018; *Pristimantis anolirex*, Acevedo et al., 2018).

Pristimantis alalocophus (Roa-Trujillo & Ruiz-Carranza, 1991), is an endemic species to the Andean forest and streams of the Central Cordillera, between 2650-3100 m.a.s.l. (Roa-Trujillo & Ruiz-Carranza, 1991; Ruiz-Carranza et al., 1996; Bernal & Lynch, 2008). This species is catalogued as Endangered (EN) due to its restricted distribution (2700 km²), few localities of occurrence (5-6), and a continuing habitat loss in extent and quality caused by deforestation and livestock grazing (IUCN SSC Amphibian Specialist Group, 2019). This species was last seen and collected in 1997 by Taran Grant in the Natural Reserve La Sirena, municipality of Palmira, department of Valle del Cauca (Colección de Prácticas Zoológicas, Universidad del Valle) (CPZ-UV). Considering the above, we report the rediscovery of *P. alalocophus* 24 years after it was last recorded.

On 8 April 2021 at 20:15 h, we found an adult female of *P. alalocophus* (SVL: 45 mm) during a naturalists' photo tour in the Kiraikai stream of Kiraikai Ecolodge (4°38'57.7" N, 75°38'57.7" W, 1780 m a.s.l.; Fig. 1), municipality of Filandia, Department of Quindío, Colombia. The individual was found perched 25 cm from the ground in riparian vegetation where it was photographed but not collected (Fig. 2A). The individual was identified with the help of researcher Gustavo Gonzalez-Durán based on diagnostic characters proposed for *P. alalocophus* according to the original description by Roa-Trujillo & Ruiz-Carranza (1991): dorsum skin smooth with small flat pustules, absent dorsolateral folds (Fig. 2B),

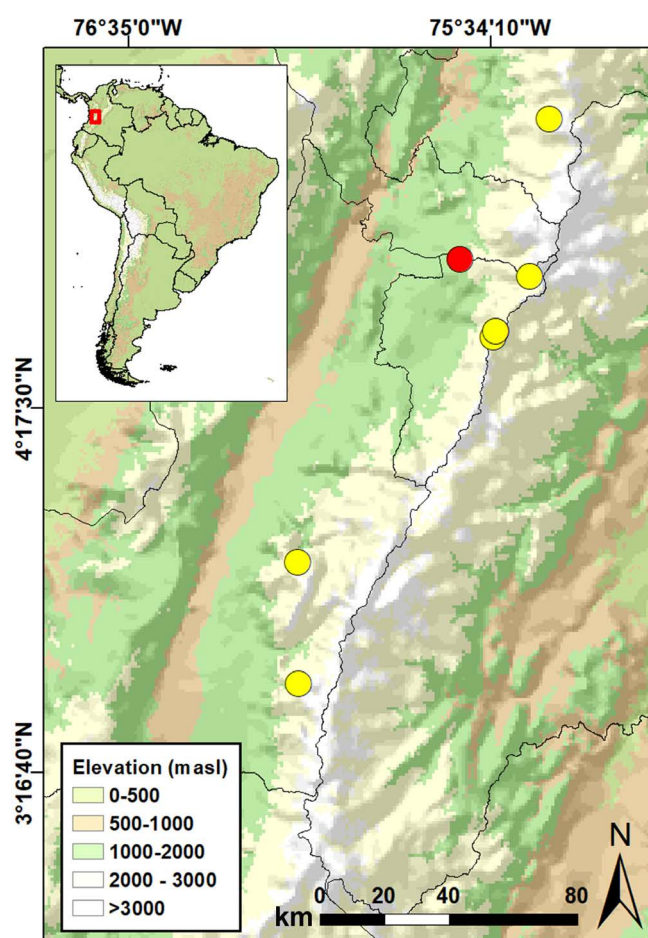


Figure 1. A portion of the Cordillera Central of Colombia showing historical records (yellow circles) and the new record of *Pristimantis alalocophus* (red circle). Historical records obtained from Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN) and Colección de anfibios y reptiles de la Universidad del Valle, Cali, Colombia (UVC).

absent tympanic membrane and tympanic anullus (Fig. 2C), subconical and moderate-sized tubercle on heel, females with black belly with spots and white granules of variable density (Fig. 2D).

Pristimantis alalocophus is similar to the three species of *Pristimantis* that have lack tympanic membranes or concealed and absent tympanic anullus, which inhabit

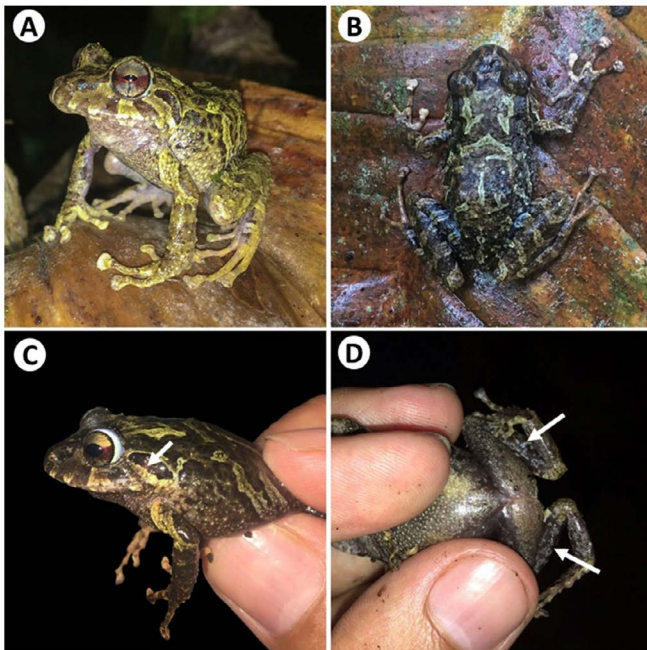


Figure 2. An adult female *Pristimantis alalocophus* recorded but not collected at the Kirakai Ecolodge, Filandia, Quindío- **A.** General features, **B.** Dorsal view, **C.** Lateral view of head, and **D.** Ventral view of hindlimbs. White arrows indicate diagnostic characters, see text for details

Central Cordillera of Colombia (*P. bernali*, *P. gracilis* Lynch, 1986; *P. lichenoides* Lynch & Rueda-Almonacid, 1997), but differs from these by the combination of the following characters -dorsum skin smooth with small flat pustules (granular in *P. bernali*, smooth with postorbital folds in *P. gracilis*), ventral skin areolate (coarsely areolate in *P. bernali* and *P. lichenoides*), vomerine odontophores triangular (oval in *P. bernali* and *P. lichenoides*), males without vocal slits and vocal sac (present in *P. gracilis*), nuptial pad present in males (*P. bernali* and *P. lichenoides*), ulnar tubercles present (absent in *P. bernali* and *P. gracilis*), tarsal tubercles present (absent in *P. gracilis*), heel tubercle present (absent in *P. gracilis* and *P. bernali*), inguinal region brown (grey in *P. bernali*, yellow and orange with white spots in *P. gracilis* and brown with occasional cream flecks in *P. lichenoides*).

Our observations suggest that *P. alalocophus* may have little tolerance for disturbance, which may explain its disappearance from its type locality despite extensive recent fieldwork in the area (Gómez-Hoyos et al., 2017, 2018). In the early 90s, the forest where *P. alalocophus* has previously been reported, had been heavily affected by habitat degradation resulting from intensive agricultural activities (e.g., Livestock) (García-Romero, 2013). However, other factors such as climate change and emerging infectious diseases (e.g. chytridiomycosis) should also be considered as potential threats.

Although *P. alalocophus* is a rare species and has a restricted distribution in the Central Cordillera, this report does not increase its known area of occupancy but does lower its elevation limit by 870 m. Due to the lack of information on the wider distribution, threats, and natural

history for *P. alalocophus*, we suggest that its conservation status should remain as Endangered (EN). For the future, it is important to direct efforts to the collection of basic data on the natural history and the size of populations of threatened species such *P. alalocophus*. Although there are conservation and monitoring programmes for species in the Department of Quindío (Paula Navarro-Salcedo & Sebastián Acevedo-Muñoz. pers. comm.), these efforts focus on charismatic species such as *Centrolene savagei*, *Hyloscirtus larinopygion* and *Andinobates bombetes*. Sampling efforts need to be extended to new locations in the Department of Quindío to enable a wider assessment of threatened species.

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Hibernation cell construction by the viviparous lizard *Zootoca vivipara*

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The viviparous lizard (*Zootoca vivipara*) has a Eurasian distribution that extends further north than any other lizard species, crossing the Arctic Circle in Scandinavia and Siberia, reaching to at least 71° N. To survive cold winter conditions, viviparous lizards hibernate in suitable shelters where they remain torpid until warmer weather prevails. The lizard has evolved some remarkable physiological adaptations to survive freezing winters and consequently can endure sub-zero temperatures in either a supercooled or a frozen state (Costanzol et al., 1995) but the lizard's choice of hibernaculum is also relevant to its survival. However, Beebee and Griffiths (2000) noted in relation to this species in Britain that, "We still know little about the hibernacula, but they are probably frost free refugia below ground or beneath large rocks or woodpiles." In a subsequent study of *Z. vivipara* hibernation in Siberia, Berman et al. (2016) report that all hibernating lizards were in various distinct cavities: burrows of the dung beetle *Anoplotrupes stercorosus*, tunnels left behind by decomposed roots, etc. No hibernating lizards were found in areas with loose soil. They concluded that the presence of small and stable cavities may be a necessary condition for hibernation. Furthermore, they suggest that relatively dry soil is required, as lizards survived hibernation at sub-zero temperatures in soil at 15 % moisture content (m.c.) but under experimental conditions all died at 70-80 % m.c.

This article relates incidental observations of hibernating *Z. vivipara* during a long-term study of reptile populations in a chalk grassland nature reserve in southern England (approx. 51° N, 0° E). Reptiles were detected in the open along a specific survey path (~6 km) and under artificial refuges placed along the path. These artificial refuges consisted of galvanised corrugated iron sheets (0.5 mm thick and 0.5 g/cm²) and roofing felt (2 mm thick and 0.3 g/cm²). They were both cut to the same dimensions (50 cm by 65 cm) and a total of 50 pairs, comprising one of each type, were placed along the path. From March to the end of October, 7-10 survey visits were made monthly giving about 70 visits each year since 2008. Normally, refuges were removed at the end of October but those felts with inactive lizards beneath them were retained – usually this affected only three or four of the fifty felts but in some years none. Soil moisture content beneath refuges, within 10 cm of hibernating lizards, was measured from time to time using an Extech M0750 moisture probe inserted to a depth of 10 cm and the measurements repeated three times. Adult viviparous lizards are sexually dimorphic and in dorsal view (the ventral view remained

hidden as this study did not involve handling) males can be distinguished from females by a penial swelling at the base of the tail, a broader head, and the absence of a continuous thin dark, dorsal stripe. Immature stages cannot be assigned to sex under these circumstances.

With the onset of colder weather in late September or October, the lizards begin entering hibernation and so are seen less frequently. When refuges were lifted in October, most lizards moved off quickly and gave no sign of being in hibernation. However, occasionally under felts, but never under corrugated iron, curled individuals were observed in an ovoid depression or cell (Fig. 1A). These individuals were torpid or nearly torpid as they failed to move or moved only slightly when disturbed. During October, the most common life stage to appear under the felts were neonates (Fig. 2) but surprisingly, nearly all individuals observed in cells were adult males with the exception of one sub-adult specimen (Fig. 1B) and only one neonate (Fig. 1C). Furthermore, it was common for at least two individuals to be present in the same cell (Fig. 1A & B).

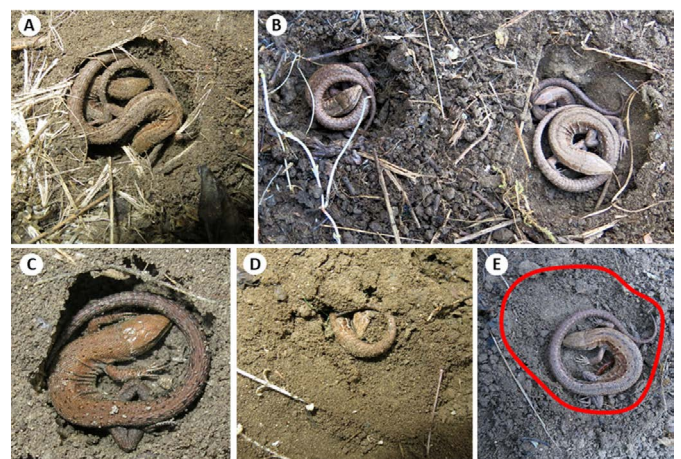


Figure 1. *Zootoca vivipara* in hibernation cells observed below roofing felt used as artificial refuges – **A.** Two adult males in a recently constructed cell in soft, relatively high moisture content soil, Oct 2021, **B.** Two cells, one with an adult male (left), the other containing an adult male and a sub-adult toward the end of hibernation Feb 2017, **C.** Neonate in a cell excavated on about 28 Oct 2021, **D.** Adult male excavating a cell in soft, relatively high moisture content soil, in Oct 2021, **E.** Adult male in cleared area (limits of the cell shown by red line) that has remained very shallow, the soil was hard and of relatively low moisture content, Oct 2021

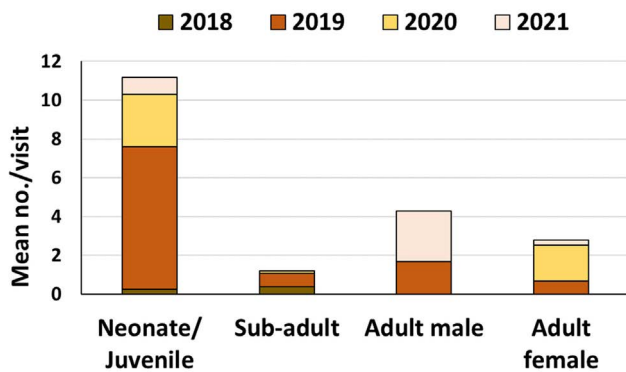


Figure 2. The frequency with which the life stages of *Zootoca vivipara* were seen in October under felt refuges placed on a chalk grassland nature reserve (2018 – 2021, 50 felt refuges visited a total of 29 times)

The cells in which the lizards were found appear to have been dug by the lizards themselves. As the cells are dug (Fig. 1D) the excess soil is used to fill the gap between felt refuge and the soil surface, consequently the cell is separated from the exterior with the felt forming the ‘roof’ to the cell. The depth of the cells varied and examination of deep cells (Fig. 1A & B) shows the soil to be soft and the mean moisture content (\pm s.d.) was relatively high ($15.6\% \pm 0.89$ and $17.1\% \pm 0.59$ respectively) while a shallow cell (Fig. 1E) was in drier, harder soil (mean of $7.9\% \pm 0.67$).

It is noticeable that the cells were a relatively tight fit for the lizards that had dug them (Fig. 1A, B, C). However, individuals do vacate cells before the end of hibernation and may not return, consequently in multi-occupancy cells one individual may be left in an unusually large cell. This was the case in Figure 3A and subsequently also happened in the case of the cell shown in Figure 1A. The cells themselves appear to be created quite rapidly. Refuges were visited as part of the routine monitoring programme every 3 or 4 days and cells would appear completely formed between visits. It was rare to actually find evidence of cells in the process of construction (such as in Fig. 1D). The tendency of some individuals to vacate their cells long before the end of hibernation may be due to disturbance as the felts are lifted, warmer weather, and/or even colder weather forcing them to move to more sheltered situations. In 2013/2014, we followed the fate of two individual males observed in a single cell in October 2013; we buried a temperature logger beneath the felt (Gemini TK-4014, TinyTag Talk 2, accuracy $\pm 0.4^\circ\text{C}$, recording at 3 h intervals) housed in an aluminium cylinder flush with the soil surface and 20 cm from the lizards’ cell. One of the males vacated the cell in November but the other (Fig. 3A) remained there until sometime between 2nd - 9th March 2014. Over the period the lowest temperature recorded was 1.4°C , well above the sub-zero temperatures at which the lizard can survive (Costanzol et al., 1995). It is clear that in some cases cells beneath felts end up being only temporary shelters but certainly not in all cases. Given that cells are constructed at a time when falling temperatures start to limit activity and that there is considerable effort involved in making them, it would seem unlikely that they are ‘intended’ to be temporary.

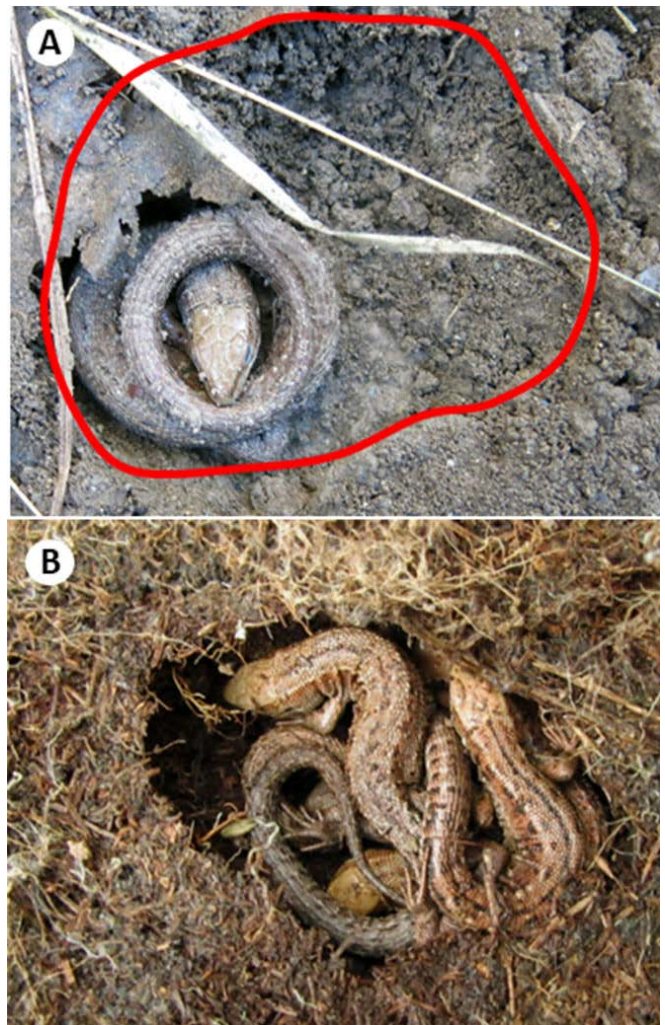


Figure 3. Viviparous lizards in hibernation cells – **A.** Adult male in a cell in Nov 2013 (limits of the cell shown by red line) where there is excess space that was originally occupied by a second male last seen in Oct 2013, **B.** Three common lizards (one adult female and probably two adult males) observed in a hibernation cell beneath a rock in County Antrim, N. Ireland on 19 Oct 2015 photographed by Philip McErlean

This is apparently the first written report of viviparous lizards actively creating a hibernation cell although there are photographs on the internet that look like the same thing. One in particular, by Philip McErlean (Flickr, <https://www.flickr.com/photos/64320477@N05/21689021213>), is of an adult female and two adult male *Z. vivipara* (Fig. 3B) in N. Ireland, that were discovered beneath a rock in a cell created in peaty soil suffused with many fine roots. The disposition of these three lizards, more or less filling an ovoid cell, is otherwise identical to that shown for the lizards in Figure 1. Furthermore, what appears to be a hibernation cell has been described in the northern taiga of Western Siberia where a lizard was found in an oval hibernaculum matching the size of the curled animal, positioned about 10 cm deep in a layer of humid peat with partially decomposed roots and wood debris (Bulakhova et al. 2011, quoted in Berman et al., 2016). The creation of a cell in soil with fine roots would be more

difficult than in the softer, looser soil beneath the felt refuges and is an indication of the physical abilities of the lizards. However, it would be difficult for the lizards to create such cells at greater depths unless there was already a cavity to receive the displaced soil. In Siberia, where all hibernacula were observed at depths of 5 to 13 cm, no obvious cell construction was reported (Berman et al., 2016). It may well be that cell construction is an activity that is generally confined to positions closer to the soil surface.

The creation of a distinct hibernation cell is an interesting feature of the viviparous lizard's behavioural repertoire. Such behaviour would be expected to have a distinct survival advantage, perhaps providing protection from predators, reducing water ingress (higher moisture conditions are associated with lower hibernation survival rates, Berman et al. 2016), and/or shielding the lizards from frost laden air thus slowing the rate at which the lizards are exposed to temperature drops. The predominance of adult males hibernating in cells is noteworthy. It suggests that adult males tolerate hibernation at shallower depths and this may be consistent with the observation that adult males emerge from hibernation one or two weeks before adult females in order to complete spermiogenesis (Beebee & Griffiths, 2000). It is therefore conceivable that cell formation close to the surface has a role in the reproductive biology of the viviparous lizard and furthermore that there could be differences between sexes and life stages in cold hardiness. The construction of hibernation cells in reasonably soft soil below felt refuges offers a convenient situation for further investigation of cell construction and its wider biological implications.

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An unusual record of ophiophagy and necrophagy in the common boa *Boa constrictor* in the Brazilian Atlantic Forest

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The common boa (*Boa constrictor*) is a large snake, reaching more than 3 metres long. It is widely distributed in South America and recognised as an opportunistic and generalist predator, feeding on invertebrates, fish, birds, mammals, amphibians and lizards, primarily using constriction to subdue its prey (Henderson et al., 1995; Quick et al., 2005; Pizzato et al., 2009; Marques et al., 2019). Herein, we present the first record of ophiophagy and necrophagy for a common boa that ingested a dead Patagonian green racer *Pseudablabes (Philodryas) patagoniensis* (Girard, 1858).

On 24th April 2021 at 1030 h, a juvenile female *Boa constrictor* (Fig. 1) was found in a coastal sand dune (restinga) environment of Área de Proteção Ambiental da Barra do Rio Mamanguape, Paraíba state, north-east Brazil (6° 46'40.96" N, 34° 55'20.90" W, WGS 84; altitude 6 m a.s.l.). It had a snout-vent length of 909 mm; tail length 93 mm and weighed 0.575 kg (measurements were taken using metric tape, digital caliper and pesola spring scales). The snake was captured during fieldwork (under collection permit SISBIO nº 74327-1), placed in a cloth bag, and then taken to the laboratory.

During handling to take measurements, it regurgitated a *P. patagoniensis* (snout-vent length 593 mm; tail length 255 mm) (Fig. 1).

The snake began to regurgitate its prey by its tail, indicating that it was consumed head first. During prey identification we verified the presence of several sarcosaprophagous dipteran larvae in the carcass (Fig. 1) indicating that the *P. patagoniensis* was already dead when consumed by the *B. constrictor*. Although snakes commonly accept carrion in captivity, necrophagy by snakes in the wild is uncommon, mainly because snakes prefer to consume live prey and because carcasses are quickly consumed by sarcosaprophagous fauna (Sazima & Strüssmann, 1990; Marques et al., 2017). However, when scavenging, the absence of injury risk and low energy expenditure may confer some benefits.

The common boa was sexed using a probe, marked with visible implant elastomer and then released at the place of capture.

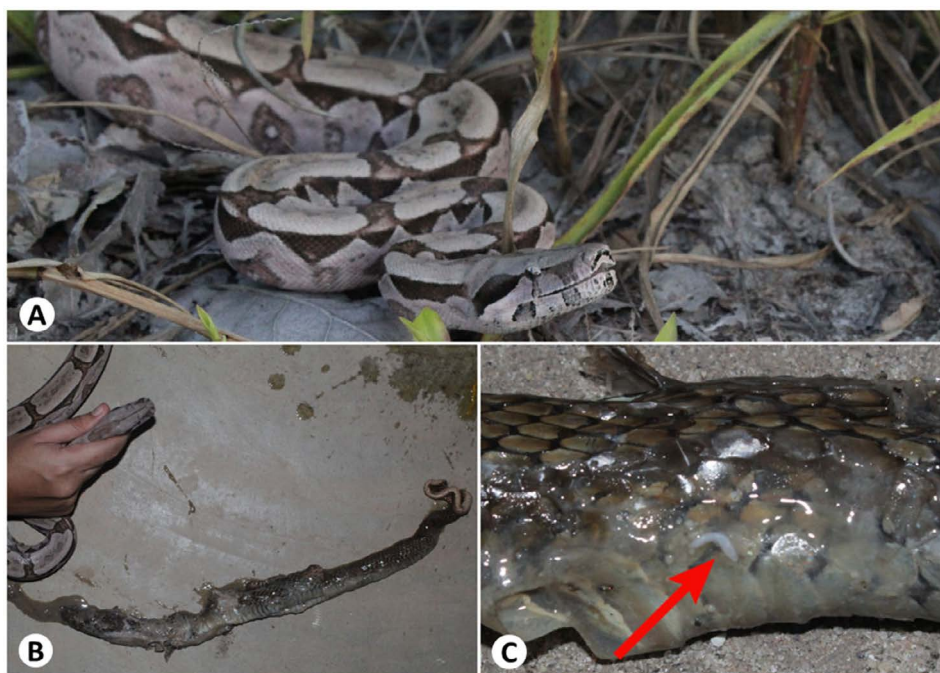


Figure 1. Ophiophagy of dead *Pseudablabes patagoniensis* by *Boa constrictor* - **A.** The juvenile female of *B. constrictor*, **B.** The regurgitated *P. patagoniensis*, **C.** Close up of the *P. patagoniensis* carcass showing a sarcosaprophagous dipteran larva (red arrow)

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A case of cannibalism in *Podarcis muralis* from Dorset, England

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The common wall lizard (*Podarcis muralis*) is the most widespread species of the genus *Podarcis* in Europe, ranging from Iberia to the Balkan peninsula. Moreover, it has been introduced into Britain, Belgium, the Netherlands, Germany, and even north America (Speybroeck et al., 2016). It is a small-sized lacertid, reaching a total of 22 cm in length, with a rather polymorphic brown to green pattern (Sacchi et al., 2013). Like most *Podarcis* spp., it feeds mainly on arthropods (Bombi & Bologna, 2002), but also on gastropods, annelids, small vertebrates, and plants (Barbault & Mou, 1986). It may also consume other lizards, when they are smaller than themselves, as evidenced by the predation of a *Zootoca vivipara* by an adult male *P. muralis* in Britain (Thomas et al., 2020). Here we report a case of cannibalism

that took place in Boscombe cliffs, Dorset (50° 43'15.7" N, 1° 49'29.6" W). According to Michaelides et al. (2015), this population results from a secondary introduction (from a naturalised non-native English population) of Venetian origin (Bologna, Italy).

On 23rd August 2020 at 13:09 h, an adult male was observed predating a juvenile conspecific. The juvenile was basking on a rock wall when, suddenly, a large male approached and seized it by the left hindleg and tail (Fig. 1A). The captured lizard, trying to defend itself, bit the left eye of the male (Fig. 1B&C) which responded by thrashing its head, left and right, causing the release from the juvenile bite (Fig. 1D). The adult male retreated to a more secluded spot where he finished ingesting the young lacertid backwards (tail first;

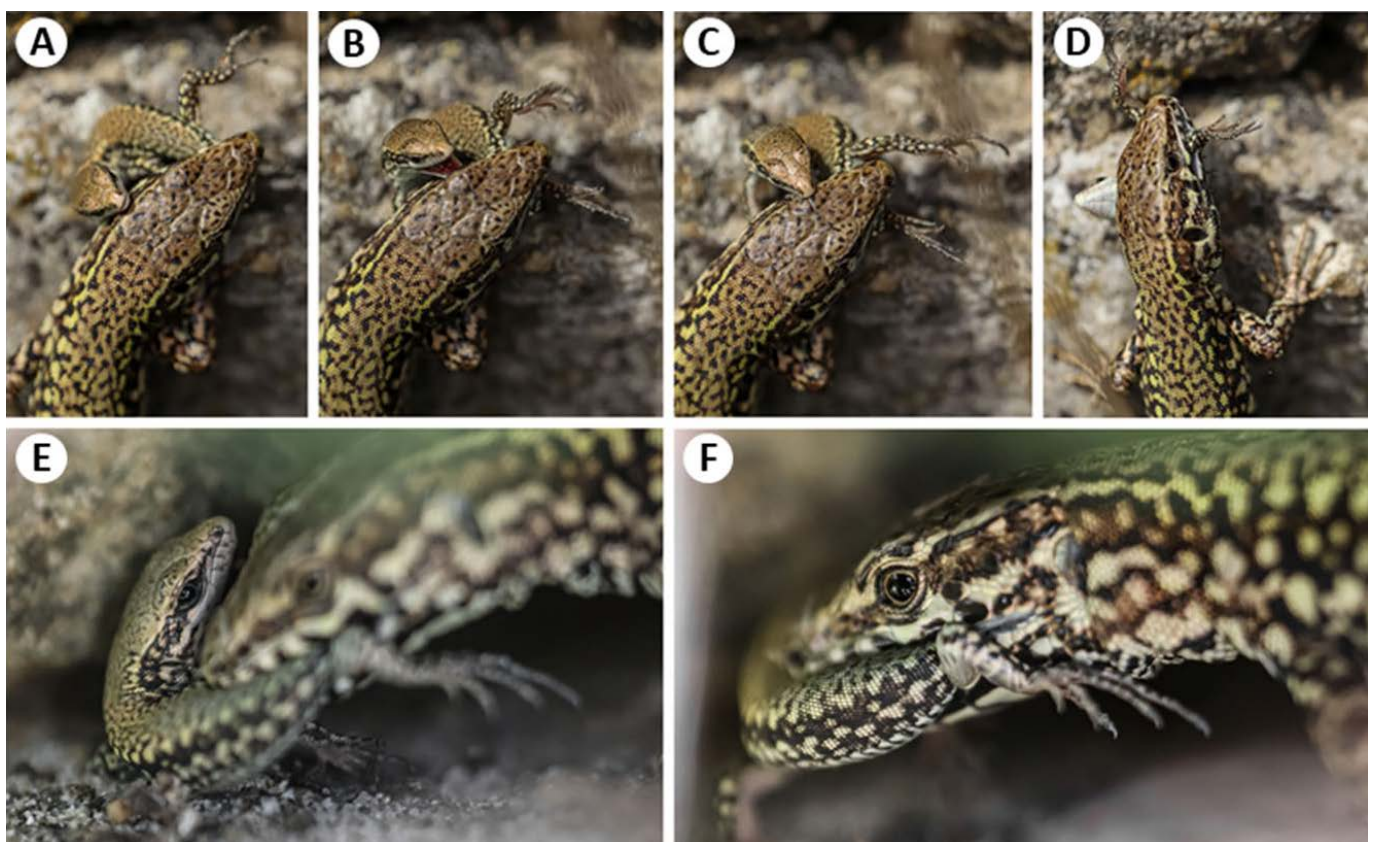


Figure 1. A large male *Podarcis muralis* capturing a juvenile conspecific- **A.** The male grabbing the juvenile by the left posterior leg and tail, **B.** & **C.** The juvenile trying to defend itself by biting the left eye of the male, which responded by thrashing its head, left and right, **D.** The juvenile has released its bite, **E.** & **F.** The male attempting to better position the juvenile for ingestion. The adult male subsequently, retreated to a hidden spot on the rock wall

Fig.1E-F). Several other individuals of the common wall lizard were seen in the area, including adult males and females, subadults and juveniles.

Cannibalism has been observed in several species of *Podarcis*, normally with adult individuals preying on juveniles, but also on adult tails, adult corpses and even eggs; this is the case for *Podarcis liolepis* (Castilla & van Damme, 1996), *Podarcis siculus* (Capula & Aloise, 2011; Grano & Cattaneo, 2011), *Podarcis virescens* (Dias et al., 2016) and *Podarcis erhardii* (Madden & Brock, 2018). It seems that cannibalism has only been observed infrequently in *P. muralis* (Žagar & Carretero, 2012; Simović & Marković, 2013).

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HERPETOLOGICAL BULLETIN REPORT

March 2022

At the end of December, Sarah Berry retired as Managing Editor of both The Herpetological Bulletin and The Herpetological Journal. Sarah joined us in 2014 and was The Bulletin's first ever professional, production manager. She has ensured that we have kept up to schedule and that our outputs have met high standards. Indeed, the very successful and attractive current format of the Bulletin is the work of Sarah. Sarah is based in France where she has a publishing business and leaves us to spend more time with her art work. In Sarah's place, we are very pleased to welcome Julie Tee to The Bulletin team.

In the last twelve months, all four issues of The Bulletin were published on time. However, we are now working to ensure that the electronic version of The Bulletin and the hardcopy, which subscribers received through the post, are released at the same time - roughly 1st March, 1st June, 1st September and 1st December.

There were 118 manuscripts submitted in 2021, a fall of 40 (-25%) from the previous year (Table 1). Most of this reduction was in the number of shorter articles whereas the number of full papers actually increased and over twice as many of them were accepted for publication. The overall acceptance rate for all types of article improved from 43% to 52% so that although the absolute number of articles submitted to The Bulletin decreased the amount of publishable material has continued to increase. Consequently, all four issues in 2021 have been a full 48 pages (indeed Issue 155 was exceptionally extended to 52 pages) but the proportion of articles published within 3 months of acceptance fell to 25% (56% in 2020) although the remainder was still published within 6 months.

Table 1. Submission and acceptance rates for manuscripts received in 2021 (2020)

	No. submitted	No. accepted	% accepted
Full Papers	21 (17)	15 (7)	71 (41)
Short Notes	9 (19)	6 (11)	67 (57)
Short Communications & Natural History Notes	88 (122)	40 (50)	46 (41)
Totals	118 (158)	61 (68)	52 (43)

The BHS is very grateful to the following people who gave their time and expertise reviewing manuscripts for The Bulletin in 2021: Roger Avery, Cesar Ayres, Karina Banci, Lee Brady, Richard Brown, Ashok Captain, Felipe Coelho, Carolyn Cray, Jenny Daltrey, Roger Downie, Andrew Durso, Xavier Glaudas, Richard Griffiths, Lee Grismer, Joanna Hedley, Anthony Herrel, Rick Hodges, Victor Jimenez, Jim Labisko, Steve Langham, James McCranie, Konrad Mebert, Roger Meek, Chris Michaels, Nitya Mohanty, Stephen Mohony, Anna Muir, John Murphy, Kanto Nishikawa, Cristiano Nogueira, Andrius Pasukonis, Anthony Plattenberg Laing, Max Ringler, Mauricio Rivera-Correa, Sean Rovito, Raul Sales, Brian Smith, Mario Solis, Ben Tapely, Christine Tilley, Luis Vera-Pérez, John Wilkinson, Mark Wilkinson, and Wolfgang Wüster.

Rick Hodges
Editor

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