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THE HERPETOLOGICAL BULLETIN

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Front Cover: A male smooth snake *Coronella austriaca* grasping the head of a female during mating in Larvik, Norway, photographed by Ståle Knutsen. See article on p.13.

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Advertisement call of Johnstone's whistling frog *Eleutherodactylus johnstonei* in Brazil

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ABSTRACT – Anuran advertisement calls play a role in attracting potential mates and can vary among individuals and populations. Here we describe the advertisement call of a non-native population of *Eleutherodactylus johnstonei* in São Paulo, Brazil and compare our data with other non-native populations in other countries. The advertisement calls of *E. johnstonei* show two-tonal frequency-modulated notes differing in spectral and temporal patterns. The first note is shorter and has lower frequencies than the second note. Our comparisons indicate that, except for the Montserrat population, the calls of the Brazilian population of *E. johnstonei* are similar to the other populations in terms of the call and note duration. Concerning spectral parameters, except for Bucaramanga (Colombia) and Caracas (Venezuela) populations, the dominant frequency of the first note is lower in the Brazilian population than in other localities. The advertisement call variations of *E. johnstonei* seem unrelated to morphological differentiation or the length of time since the population was introduced, but may reflect local female preferences or adaptation to environmental factors.

INTRODUCTION

Anurans exhibit acoustic, visual, chemical, and tactile modes of communication (Hödl & Amézquita, 2001; Wells, 2007; Belanger & Corkum, 2009). However, acoustic communication is the primary, most widespread, and most studied. Males of many species show a vast acoustic repertoire emitted in different social contexts, such as mate choice, sexual selection, kin recognition, and territoriality (Ryan, 2001). The advertisement call is the most conspicuous, highly studied, and has two main functions: i) to attract females to mate, and ii) to prevent agonistic interaction with other males (Littlejohn, 1977; Wells, 2007). Advertisement calls are species-specific and easy to record, making them helpful for species identification (Gerhardt & Davis, 1988).

Advertisement calls vary among individuals and conspecific populations (e.g., Castellano et al., 2002; Pettitt et al., 2013; Guerra et al., 2017). Individual call variations depend on intrinsic factors such as body size and conditions (Rodríguez et al., 2015; Ziegler et al., 2015). Also, extrinsic factors such as the presence of conspecific or heterospecific signals, background noise, and environmental temperature may influence call parameters at any given time (Lopez et al., 1988; Howard & Young, 1998; Wells, 2007; Schwartz & Bee, 2013). On the other hand, call variation among populations may follow a geographic cline resulting from genetic, morphological, and/or environmental factors (Castellano et al., 2000; Pröhl et al., 2007; Klymus et al., 2010). These variations directly impact sexual selection due to female mate choice (Gerhardt, 1991), leading to changes in communication systems and, ultimately, the evolution of

mating signals (Boul & Ryan, 2004; Wilkins et al., 2013).

Since non-native species are outside of their native habitat, they offer excellent opportunities to examine variation in call parameters among recently established populations. They may live without natural ecological pressure, such as competition in the acoustic niche between species in the community to minimise overlapping signals (Narins, 1995), and can show rapid differentiation in call parameters, as has been noted in the invasive cane-toad (Rhinella marina) and coqui frog (Eleutherodactylus coqui) (O'Neill & Beard, 2011; Yasumiba et al., 2016). The Australian populations of the invasive cane-toad have presented differences in dominant frequency and call duration (Yasumiba et al., 2016). On the other hand, the coqui frog exhibits differences in call frequencies, call rate, and call duration among populations at different elevations in its introduced range in Hawaii (O'Neill & Beard, 2011).

Eleutherodactylus johnstonei Barbour, 1914 (Anura: Eleutherodactylidae) is a nocturnal frog native to the Lesser Antilles (Lever, 2003) with successful populations in some other Caribbean islands, Venezuela, Colombia, the Guianas, Panama, and Costa Rica (Kaiser et al., 2002; Kraus, 2008). These countries were invaded by the frog over a period of 46 to 142 years (Hardy & Harris, 1979; Kaiser & Hardy, 1994). In Brazil, populations occur only in the urban megalopolis of São Paulo (Melo et al., 2014), where introduction occurred sometime before 1995 and appears to have been intentional (Toledo & Measey, 2018). *E. johnstonei* shows direct development (i.e., the fully metamorphosed froglet emerges from the egg), has a generalist diet and habitat (e.g., Kaiser et al., 2002; Ortega et al., 2005), and ecological modeling



Figure 1. Spectrogram (top) and oscillogram (bottom) of the advertisement call of *Eleutherodactylus johnstonei* (Air temperature = 25.3 °C, relative humidity = 64 %, voucher FNJV 50606). Spectrogram parameters: window size = 1.024, overlap = 90 %, window type = "Hann". Figure created with the R package 'Seewave' (Sueur et al., 2008)

predicts a high potential to occur in different biomes in Brazil (Forti et al., 2017; Brasileiro et al., 2021). Herein we describe the advertisement call of the Brazilian population of *E. johnstonei* and compare our data with other non-native populations.

MATERIALS & METHODS

Data collection

We recorded advertisement calls of six male *E. johnstonei* on 16 March 2020 between 20:00h and 22:00h, in the urban area of the municipality of São Paulo, São Paulo State, south-eastern Brazil (23° 38'02'' S, 46° 40'55'' W). We recorded each individual for 1–3 minutes using a Tascam DR-22WL digital record and measured the call intensity using a sound level meter, keeping a distance of 1 m from the recorded individual. We also measured the air temperature and relative humidity during each recording using a portable thermo-hygrometer (Icel, HT-200). All voucher specimens were anesthetised and euthanised with 5 % lidocaine, fixed in 10 % formalin, preserved in 70 % ethanol, and deposited in the Amphibian Collection (ZUEC), at the Museu de Diversidade Biológica, Universidade Estadualde Campinas, state of São Paulo, Brazil (ZUEC25183 - 25188).

Bioacoustics analysis

We digitalised the recordings at 44.1 kHz with a resolution of 16 bits and undertook acoustic analyses using the Raven Pro 1.6.1 software (Bioacoustics Research Program, 2019). For call selection, we used the waveform window. We adjusted a fast Fourier transformation of 1.024 points for spectral

measurements, with a window of 90 % overlap, temporal hope size of 102 samples, and grid spacing of 43.1 Hz. We followed the note-centered approach and the concepts of notes, pulses, and calls defined by Köhler et al. (2017). Five calls from each male's recordings were sampled at random to measure call parameters, totaling 30 calls. Estimates were made of call duration (ms), note duration (ms), intercall interval (ms), call rate (calls/min), dominant frequency of each note (kHz), low frequency of each note (kHz), and high frequency of each note (kHz). We obtained data for intercall interval (ms) and call rate (notes/min) by analysing the entire oscillogram (waveform) of the recording of each male (Charif et al., 2010) and constructed audio spectrograms in R using the Seewave package (Sueur et al., 2008). We have deposited our audio files of frog calls in the audio archive of Fonoteca Neotropical Jacques Vielliard (FNJV), Universidade Estadual de Campinas, State of São Paulo, Brazil (FNJV 50605-50610).

We compared the advertisement call of the Brazilian population of *E johnstonei* with descriptions of nine populations available in the literature (Watkins et al., 1970; Lemon, 1971; Hardy & Harris, 1979; Kaiser, 1992; Kaiser & Hardy, 1994; Kaiser et al., 1994; Tárano & Fuenmayor, 2008; Flechas et al., 2018). However, some of the descriptions did not include all the call parameters that we analysed, this prevented us making certain comparisons.

RESULTS

The advertisement call of the recorded individuals of E. johnstonei in Brazil shows two types of notes differing in spectral and temporal patterns (Table 1; Fig. 1). The advertisement call of E. johnstonei has harmonic structures, in which the lowest band (fundamental frequency) concentrates most of the energy. The first note has two harmonics, and the second note has three to five harmonics (Fig. 1). On average, the first note lasted 76 ms with a dominant frequency of 1.77 kHz, a low frequency of 1.61 kHz, and a high frequency of 1.96 kHz. The second note is longer than the first note and lasted 212 ms on average with a dominant frequency of 3.42 kHz, a low frequency of 2.87 kHz, and a high frequency of 3.54 kHz. The advertisement call (first and second notes combined) presents a duration of 288 ms. The average call rate was 36 calls/min with an average intercall interval of 989 ms. The air temperature during the recordings was 25.3 ± 0.6 °C, and the relative humidity was 64 ± 3 %. The call intensity was registered between 75 and 78.5 Db recorded a metre away from each male. All six recorded frogs were in ornamental vegetation in gardens less than 1 m from the ground.

The advertisement call of all populations of *E. johnstonei* is composed of two notes. Except for the Montserrat population, the Brazilian population's calls are similar to the other populations in terms of the call duration and the duration of the first and second note. In the Montserrat population, the call duration and second note duration are longer (Table 2). Concerning spectral parameters, the dominant frequency of the first note is lower in the present study than in other localities when considering average and variation range, except for in Bucaramanga (Colombia) and Caracas (Venezuela). The dominant frequency of the second

Table 1. Temporal and spectral characteristics of the advertisement call of *Eleutherodactylus johnstonei*. Calls were recorded from 6 males (totalising 30 calls) at Jardim Cordeiro, São Paulo, state of São Paulo, south-western Brazil. Each parameter includes mean \pm standard deviation, minimum and maximum values.

Call parameters	Mean ± std (min – max)
Call duration (ms)	288 ± 30 (236 – 340)
Intercall interval (ms)	989 ± 220 (581 – 1500)
Call rate (call/min)	36 ± 10 (25 – 49)
First note duration (ms)	76 ± 10 (49 – 100)
Second note duration (ms)	212 ± 20 (167.4 – 247.4)
Dominant frequency of the first note (kHz)	1.77 ± 0.03 (1.72 – 1.81)
Dominant frequency of the second note (kHz)	3.42 ± 0.13 (3.14 – 3.53)
Low frequency of the first note (kHz)	1.61 ± 0.04 (1.5 – 1.7)
Low frequency of the second note (kHz)	2.87 ± 0.12 (2.62 – 3.05)
High frequency of the first note (kHz)	1.96 ± 0.07 (1.88 – 2.08)
High frequency of the second note (kHz)	3.54 ± 0.13 (3.25 – 3.66)

note in the Brazilian population is similar to the populations of Caracas and Bermuda, but higher than the Bucaramanga population and lower than the Montserrat, Grenada, Georgetown (Guyana), Cumaná (Venezuela), and Barbados populations (Table 2).

DISCUSSION

The advertisement calls of the Brazilian population of *E. johnstonei* consist of two adjacent notes with distinct temporal and spectral parameters. Both notes are tonally frequency-modulated, with frequency modulation at the end of the first note and the beginning of the second note. Both notes present harmonics, however it can sometimes be challenging to visualise them in the spectrogram and therefore only the first harmonic of the first note is visible (Fig. 1). The harmonic structure of the call establishes a broad prevalent bandwidth (1.6-13.8 kHz).

While both notes play a role in male interactions, the function of the second note in the advertisement call of *E. johnstonei* is related to female attraction (Tárano & Fuenmayor, 2013). The harmonic structures recently identified in the advertisement call of *E. johnstonei* (Tárano & Fuenmayor, 2008, and this study) do not yet have a defined biological function. It is possible that harmonics were not detected in the older analysed recordings due to equipment limitations, as the first descriptions of these calls were documented in the 1970s (Watkins et al., 1970; Lemon, 1971; Hardy & Harris, 1979). The

function of harmonics has continued to be poorly investigated for anurans (Foratto et al., 2021).

The advertisement call structure of E. johnstonei in Brazil and the other nine non-native populations has revealed differences among them mainly in their dominant frequencies. Comparing the population of Brazil and Montserrat, differences occur both in temporal and spectral parameters. Usually, the dominant frequency among populations varies with the individual body size, while variations in temporal parameters occur due to temperature (Gerhardt & Huber, 2002; Ziegler et al., 2015; Tonini et al., 2020). Body size is positively associated with the mass of vocal cords that vibrate at lower fundamental frequencies as male size increases (Gerhardt, 1994). For E. johnstonei, the dominant frequency of the call can indicate the size of a male's body, but only in a limited way (Tárano & Fuenmayor, 2008). On average, males sampled in Montserrat are smaller than males in Brazil (Table 3), explaining the higher dominant frequencies in the Montserrat population.

Although the time since introduction varies among *E. johnstonei* populations, this factor does not seem to explain the variation in the advertisement call parameters (see Table 3). It was not possible to identify a pattern among newly established or long-established non-native populations. The cane-toad (*Rhinella marina*) in Australia also shows divergence in its advertisement call among invasive populations, and the variation has not been attributed to the time of invasion of each population, but to differences in adaptation to environmental, behavioral and/or ecological conditions (Yasumiba et al., 2016).

The variation in advertisement calls can be related to geographic distance gradients among populations (e.g. Smith et al., 2003; Smith & Hunter, 2005). For example, latitude and longitude largely explain the geographic variation in the advertisement call of *Litoria verreauxii* in the Australian Snowy Mountains (Smith et al., 2003). São Paulo, Brazil has the southernmost latitude (-23°) of the evaluated populations. Changes in environmental conditions associated with different latitudes may be responsible for call variations among *E. johnstonei* populations. Besides latitude, we should also consider the possible influence of social interactions (Gerhardt, 1994), sexual selection (Ryan et al., 1996), and the local acoustic environment (Ryan et al., 1990).

Unfortunately, variations both in sample size and the technology used in the analyses of previous studies has limited the comparisons that we could make with other introduced populations of *E. johnstonei*. For the future, we suggest detailed studies i) across the native distribution, including introduced populations of *E. johnstonei*, to focus on the ecological and behavioural characteristics of individuals to clarify the causes of call variation, and ii) far from the native distribution, to observe the effect of *E. johnstonei* introductions and consequences on established populations.

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We are thankful to all colleagues involved directly and indirectly with this study, especially to Wellington Palhares, Luana Moraes and Amanda D'Ambrosio for their help with fieldwork and laboratory activities. We also thank the residents of the **Table 2.** Comparison of the advertisement call of *Eleutherodactylus johnstonei* from several locations. Values are means ± standard deviation and/or variation ranges (in parenthesis).

Call Parameters						
Location	Call duration (ms)	First note duration (ms)	Second note duration (ms)	Dominant frequency of the first note (kHz)	Dominant frequency of the second note (kHz)	
São Paulo (Brazil, this study)	288 ± 30 (236 - 340)	76 ± 10 (49 - 100)	212 ± 20 (167.4 - 247.4)	1.77 ± 0.03 (1.72 - 1.8)	3.42 ± 0.13 (3.14 - 3.5)	
Bucaramanga (Colombia)	277 ± 23.1 (215.5 - 316.6)	77 ± 18.4 (1.5 - 107.5)	200.6 ± 17.5 (176 - 234)	1.723 ± 0.042 (1.6 - 1.9)	2.78 ± 0.146 (2.5 - 3.0)	
Caracas	314 (226 - 400)	86 (53 - 111)	223 (142 - 307)	1.8 (1.6 - 1.9)	3.3 (3 - 3.6)	
Montserrat	363	57	306	2.1	3.7	
Grenada	271	71	200	1.9	3.6	
Curaçao	271	71	200	1.85	3.5	
Georgetown (Guyana)	264	57	187	2	3.6	
Cumaná (Venezuela)	257	86	171	2	3.7	
Barbados	(240 - 300)	(70 - 90)	(180 - 220)	(2 - 2.2)	(3.6 - 4.1)	
Bermuda	-	85	180	2	3.5	

Bucaramanga- Flechas et al. (2018); Caracas-Tárano & Fuenmayor (2008); Montserrat & Grenada-Kaiser (1992); Kaiser et al. (1994)*.

Curaçao, Cumaná and Georgetown - Hardy & Harris (1979)*; Barbados-Lemon (1971); Bermuda-Watkins et al. (1970).

(*) values estimated from sonograms as they were not given in the text

Table 3. Comparison of the snout–vent length (SVL) of *Eleutherodactylus johnstonei*, local air temperature and invasion time from non-native populations. Values are means ± standard deviation.

Location	SVL (mm)	Temperature (°C)	Invasion time (years)	References
São Paulo (Brazil, this study)	21.8 ± 0.9	25.3 ± 0.6	26	Toledo & Measey (2018)
Bucaramanga (Colombia)	-	-	-	-
Caracas (Venezuela)	23.08 ± 0.75	-	63	Tárano & Fuenmayor (2008); Hardy & Harris (1979)
Montserrat	20.5 ± 3.1	24 ± 2	-	Kaiser (1992), Kaiser et al. (1994)
Grenada	-	24 ± 2	136	Kaiser (1992), Kaiser et al. (1994)
Curaçao	-	-	46	Hardy & Harris (1979)
Georgetown (Guyana)	-	-	98	Hardy & Harris (1979)
Cumaná (Venezuela)	-	-	54	Hardy & Harris (1979)
Barbados	(20-23)	26	142	Lemon (1971), Kaiser & Hardy (1994)
Bermuda	_	-	141	Kaiser & Hardy (1994)

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Description of the nesting sites of the critically endangered Jamaican iguana Cyclura collei

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ABSTRACT - The Jamaican iguana, *Cyclura collei*, is one of the world's rarest iguanas. It is restricted to the dry forest of the Hellshire Hills, where the availability of suitable soil for nesting limits population growth. Once a year, Jamaican iguanas move from the surrounding forest to two main nesting sites, the Upper Nesting Site (UNS) and the Lower Nesting Site (LNS), to excavate nests and deposit their eggs. There is little information on the soil condition at these two sites. Due to population growth, resulting from intensive conservation efforts, the availability of nesting sites is now limited. Consequently, qualitative and quantitative information is needed to improve existing sites and inform the creation of additional nesting sites. Here we investigate soil depth, nest openness, soil compaction and other soil characteristics at the Jamaican iguana's two main nesting sites and, from our observation, make recommendations for creating and/or improving nesting sites.

INTRODUCTION

The Jamaican iguana, *Cyclura collei* Gray 1845, is from a genus commonly known as the rock iguanas. It is among the 11 species of threatened rock iguanas found in the Caribbean (Buckley et al., 2016; IUCN, 2021) and is ranked as one of the world's rarest (Alberts, 1999). It was once common in southern Jamaica and the Goat Islands (Vogel & Kerr, 1992; Woodley, 1980, 1968). After the introduction of invasive predator species, the population declined and was thought to be extinct. However, it is now found only in a small, remote area in the Hellshire Hills (Vogel, 1994; Vogel & Kerr, 1992). Its population in 1991 was roughly estimated to be less than 100 mature individuals (Vogel et al., 1996).

The Jamaican iguana may nest in one of two ways depending on the nature of the substrate. When there is deep soil available, they will excavate tunnels that lead to an egg chamber (Vogel, 1994). Otherwise, they may nest in small rock crevices containing soil but with more or less no tunnel (Wilson, 2014). Few iguanas choose to nest in rock crevices compared to nesting in deep soil as there is limited soil to cover the eggs. When they nest in rock crevices, the females nest individually. Nests in rock crevices are more accessible to predators and are more heavily predated, mainly by the Indian mongoose, *Herpestes auropunctatus* (Wilson, 2014).

Nesting in deep soil is the primary form of nesting behaviour in the Jamaican iguana; however, such soils are rare in the limestone dominated Hellshire Hills and occur only in pockets. There are a few sites with deep soil that the iguanas nest in; however, there are two such sites they use yearly. These have been termed the Lower Nesting Site (LNS) and the Upper Nesting Site (UNS) (Vogel, 1994). Other sites have been used occasionally (van Veen & Wilson, 2017). Gravid females aggregate over a period of 3 months (June to August) at these two sites (Vogel, 1994) and, because nesting space is limited, there is often intense competition. Females are known to excavate existing nests containing clutches laid by other females and may protect their nests for up to 14 days by physical contact, fighting, and biting (Wilson et al., 2016; Vogel, 1994).

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The success of the conservation programme has led to an increase in the number of nesting females from 8 in 1991 (Vogel, 1994) to 53 in 2013, which resulted in increased competition between females at the two nesting sites (Wilson, 2014). For continued success, the programme will need to create artificial nesting sites. There are also plans to reintroduce the Jamaican iguana to the Goat Islands (Island Conservation, 2008; NEPA, 2019); however, this area has changed since the time that the iguanas became extinct on the island in the 1940s. Such changes resulted from occupation by the American Military, charcoal burning and goat farming. One of the most pressing questions linked to this species continued recovery is whether additional suitable nesting sites exist or whether there is a need for supplemental nesting sites. Answering these questions will require detailed knowledge of Jamaican iguana nesting preferences and habits. Hence, the main objective of the current study was to describe the physical characteristics of the two main nesting sites. This information can be used to i) advise on the construction of new nesting sites, and ii) assess the suitability of potential nesting sites on the Goat Islands. In addition, as there is little information on the nesting preferences of other Cyclura species, this information may also contribute to their conservation.

MATERIALS & METHODS

We conducted fieldwork under the purview of the National Environmental and Planning Agency (NEPA) of Jamaica. Permission is only granted to studies with limited disturbance to the nesting sites because the Jamaican iguana is listed as Critically Endangered (IUCN, 2021). This study was carried out during the dry season from December 2018 to February 2019, over a 14-day period that is outside of the nesting season although the nesting season itself is also within the dry season.

Study site

The Hellshire Hills are located in southern Jamaica. The area consists of limestone hills, rocky substrate, no surface water and the soil is restricted to rock crevices and small depressions. The Hellshire Hills region (60 km²) is also the largest remaining undisturbed dry limestone forest in Jamaica (Vogel, 1994) and has remained under-developed due to its inhospitable conditions. The vegetation types range from cactus scrub to dry evergreen thicket (Woodley, 1980). The patchy forest is also characterised by a thin canopy (IUCN, 2021). The location of both nesting sites was recorded using a Garmin eTrex 20 HikingGPS (Garmin Ltd., Schaffhausen, Switzerland). All measurements were taken in the middle of each site unless otherwise noted. The UNS is elliptical, while the LNS is roughly rectangular; the length and width of each were measured to calculate area.

Soil characteristics

Depth. We measured soil depth by hammering a 2 m metal rod into each nesting site until contact was made with the bedrock. Measurements were taken at the middle and two other random areas at each nesting site. When air pockets were encountered, the soil depth at the top and bottom of each pocket was measured.

Compaction (handheld penetrometer). To measure the soil compaction we used a handheld cone penetrometer (Eijkelkamp Soil & Water, Giesbeek, Netherlands). Due to soil dryness, the penetrometer was fitted with a 2 cm² cone. The soil compaction measurements were taken at 5 cm depth intervals until the probe could go no further. Most nesting occurs towards the centre of the sites. Thus, we took two samples in the middle and two other areas; these sample sites were at least 100 cm apart.Air pockets encountered while using the penetrometer were also recorded. We examined the relationship between soil depth and degree of soil compaction by plotting the data and estimating the correlation coefficient in RStudio by using the Rstatix package (Kassambara 2021).

Bulk density. We measured soil bulk density for only the upper 5 cm of soil, as going any deeper would have resulted in too much disturbance to the soil. One sample was taken in the centre of the nesting site, and the others at two random points. The sampling method used by Page-Dumroese et al. (1999)was adapted. A thin layer of soil was first cleared from the sample area and a metal coring cylinder, 50 mm in length and diameter, was then used to collect the samples (Page-Dumroese et al., 1999). The cylinder with soil was carefully removed and sealed with plastic caps. The samples were analysed by Soil Health, Plant Tissue and Water Laboratory, Agricultural Land Management Division, Ministry of Industry,

Commerce, Agriculture and Fisheries.

Soil profile. We extracted soil cores using a 53 mm split tube sampler (Eijkelkamp Soil & Water, Giesbeek, Netherlands). These cores provided data on soil profile, organic matter, pH and soil type (based on particle size classes; sand (50–2000 μ m), silt (2–49 μ m) and clay (<2 μ m). Prior to collecting the soil core sample, we drove a metal rod into the selected areas of the nesting site to ensure no rocks were present to damage or impede the auger. Cores were taken in two areas (middle and another random site) at each nesting site to a depth of 40 cm, as the likelihood of damage to the auger increased beyond this depth. Once the core was extracted, a photograph was taken of the soil profile. The samples were later analysed by the Soil Health, Plant Tissue and Water Laboratory, Agricultural Land Management Division, Ministry of Industry, Commerce, Agriculture and Fisheries.

Vegetation cover

The plants within the nesting sites, as well as within a 5 m perimeter around each nesting site, were identified directly in the field or photographed, and voucher specimens were taken for study at the herbarium at the Department of Life Sciences, University of the West Indies, Mona, Jamaica.

The fraction of the ground without tree cover (nest openness) was assessed using the CanopyApp (University of New Hampshire University, New Hampshire, USA) for the Android application installed on a Samsung S7 smartphone. The phone was held 0.5 m above the ground with the camera facing upward, and two pictures were taken in the middle of each nesting site and saved to the device for further analysis.

RESULTS

The LNS is the larger of the two sites, is rectangular, with an area of 47.5 m² (length 9.5 m x width 5.0 m); the UNS has an area of 28.0 m² (diameter = 6.0 m) (Fig. 1). The average soil depth (3 sample points) for both sites was 60 cm, the ranges being 41–77 cm and 46–76 cm for UNS and LNS, respectively. Soil depth decreased from the middle to the edge of the nesting sites, and the likelihood of encountering rocks increased. The general depth at the periphery of the nesting sites was < 20 cm.

The soil type at both nesting sites was silt loam, consisting of approximately 66 % silt, 26 % clay, and 4 % organic matter. The pH at the LNS was 7.6 and 6.7 at the UNS. The colour of the soil samples from the core is red, and it did not show any distinct soil horizons. Roots occurred mainly at the first 5 cm, and a few extended to depths of 10 cm. Iguana eggshells were found at various levels. Charcoal fragments were also observed in the soil at both nesting sites.

Using the cone penetrometer, we measured soil compaction to a depth of 25 cm. Compaction varied between 260 and 385 N/cm² in the UNS and 190 to 355 N/cm² in the LNS (Table 1). There was no significant correlation between soil compaction and soil depth in either the UNS (R = -0.057, p > 0.05) or the LNS (R= 0.42, p > 0.05).

The average bulk density for the first 5 cm was 1.2 g/cm³ at the UNS and 1.0 g/cm³ at the LNS. We encountered four air



Figure 1. The two nesting sites of *Cyclura collie* - **A**. The upper nesting site where a ring of stones was used to create an enclosure to capture the hatchlings for the Jamaica iguana headstart programme, **B**. The bucket in the forefront of the lower nesting site is a part of a trap used to catch feral cats in the invasive alien species IAS management programme

 Table 1. The soil compaction values, using the handheld penetrometer, at the two Cyclura collei nesting sites

Soil depth (cm)	Average soil compaction (N/cm2)	Shelter direction
	Upper Nesting Site	Lower Nesting Site
5	260	190
10	385	355
15	300	243
20	305	270
25	240	340

 Table 2. Depth and diameter of air pockets at the two Cyclura collei

 nesting sites

	Depth at which air pockets were encountered (cm)	Diameter of air pocket (cm)
	5	10
Lower Nesting Site	10	20
	10	15
	25	5
Upper Nesting	5	35
Site	40	10

Table 3. Plant species found in a 5 m perimeter around the upper (UNS) and lower (LNS) *Cyclura collei* nesting sites

Family	Scientific Name	Plant form	UNS	LNS
	Metopium brownei	Tree	х	x
Anacardiaceae	Commocladia pinnatifolia	Shrub	x	x
Apocynaceae	Plumeria obtuse	Shrub	x	-
	Thrinax parviflora	Tree	x	x
Arecaceae	Coccothrinax sp.	Tree	x	-
Bignoniaceae	Tabebuia riparia	Tree	x	-
Bromeliaceae	Bromelia pinguin	Shrub	-	х
Burseraceae	Bursera simaruba	Tree	x	-
Capparaceae	Capparis ferruginea	Shrub	-	x
Euphorbiaceae	Croton linearis Jacq.	Shrub	-	x
Myrtaceae	Eugenia maleolens Pers.	Tree	x	-
Deseres	Species 1	Grass	x	x
Роасеае	Species 2	Grass	-	x
Polygonaceae	Coccoloba jamaicensis Lindau	Tree	x	-

pockets in the UNS and two air pockets in the LNS, these are presumed to be old nest cavities. The air pockets occurred in areas with soil depth >20 cm and occurred between 5–50 cm (Table 2). The diameters of the air pockets varied from 5-35 cm.

The dry forest was well developed at the periphery of the nest sites (Fig. 1), with trees growing up to 4 m; however, there was no distinct canopy or shrub layer. The substrate was primarily rocky and the trees grew from pockets of soil/ leaf litter. A total of 14 species of plants (7 trees, 2 grasses and 5 shrubs/herbs) were recorded at the periphery of the nesting sites: the UNS had 9 of these while the LNS had 8 (Table 3).

The vegetation within the nest sites consisted of grasses, shrubs, and a few stunted trees no more than 50 cm in height. Consequently, the sites were very open. The average nest openness value of the centre of the nesting site was LNS 95 % and UNS 94 %. The openness value decreased from the centre of the nesting site to the periphery due to the forest shading the area. At the LNS, there was a section overgrown by *Bromelia pinguin*; there was a canopy overhanging this section, and an accumulation of leaf litter was observed.

DISCUSSION

Soil depth is one of the factors that could determine the suitability of a site for nesting. The average soil depth was 60 cm for both nesting sites. The depth was greatest in the middle of both nesting sites and decreased towards the perimeters. The air pockets assumed to be old nest cavities occurred in areas with soil depth >20 cm and occurred between 5–50 cm. The older and bigger females generally

occupy the central region, where more nest pockets are encountered while the younger females move closer to the edge (Jamaica iguana field team; Williams, pers. comm.). Further studies are needed to confirm if the central region is best for nesting.

The presence of rocks and roots may affect iguana nesting efforts by increasing the difficulty of digging, resulting in them abandoning their nest burrows (Morrison et al., 2009; Iverson et al., 2004). The number of rocks and roots decreases from the perimeter towards the middle for both nesting sites. In addition, no nest pockets were encountered in soil depth < 20 cm, which is the general depth at the periphery of the nesting sites. This suggests that nest construction is less successful in this area.

Soil cores from both nesting sites indicated the absence of the typical soil profile, i.e., topsoil (Horizon A) and subsoil (Horizon B) (Olson, 1984). This could be the result of the iguanas mixing the soil when digging test burrows throughout the site and also by actually building their nests. The volume of a hypothetical iguana nest (excluding the test digging test burrows) was calculated as 0.032 m³ (Vogel, 1990; Hope Zoo, unpublished). Twenty-five iguanas will potentially excavate 0.80 m³, representing approximately 3–4 % of the nesting sites annually. Consequently, the mixing of the soil by the iguanas contributes to the absence of a distinct soil profile.

Charcoal fragments found in the soil indicated previous use of the sites for charcoal kilns. The nesting sites are flat and have a large volume of soil, which are rare features in the Hellshire Hills, making them attractive to charcoal burners. The charcoal burners obtained hardwood from the forest and use areas similar to the nesting sites as they provide enough soil to cover the kilns. This activity contributes to the mixing of the soil. However, there have been no active charcoal kilns at these sites for the last 30 years, since the rediscovery of the iguana. Charcoal burning also has a negative impact on the iguana's habitat in terms of the loss of limited nesting sites and food resources (Wilson et al., 2004). In their study in the Hellshire Hills, Niñ et al. (2014) estimated that it would take approximately 45 years for regrowth in the clear-cut areas.

Soil compaction affects the suitability of the area for the iguana to nest and is generally affected by moisture, soil density, porosity and rock content of the soil (Kees, 2005). If the soil is too compacted, it increases the difficulty for an iguana to dig a tunnel; if the soil is too loose, the nest tunnel will collapse. Additionally, the area cannot be too compact if the hatchlings are to dig their way out of the nest cavity. Iverson et al. (2004) reported that iguanas took longer to dig their nest tunnels during drought conditions. The nest cavities occurred at compaction between 240 and 385 N/cm² (Table 1 and Table 2). The regular tunnelling by the iguana negates the trend of increasing compaction with depth seen in most soils. These values provide a guide for the construction of supplemental nests.

The soil compaction might partially explain the lack of trees in the nesting sites. Root growth begins to be inhibited at a compaction of approximately 150 N/cm² as measured using a handheld penetrometer (Kees, 2005), or a bulk density of 1.6 g/cm³ for silt loam soil (Arshad et al., 2015). The average bulk density of the upper 5 cm of the nesting sites were 1.2 g/cm³ at UNS and 1.0 g/cm³ at LNS, well within the limit for normal plant growth. Using the penetrometer, we obtained values of 260 & 190 N/cm² in UNS and LNS for the first 5 cm; these are just at the limits of root penetration.

Below 10 cm, the soil density ranged between 240 and 385 N/cm² (Table 1); this compaction is much too high for root growth. This explains why most of the plants observed growing in the nesting sites include *Bromelia pinguin* and small shrubs whose roots do not penetrate much below 5 cm. In the soil cores, few roots were observed up to a depth of 10 cm. The absence of roots in the nesting sites is ideal as roots do retard tunnelling by the iguana (Vogel, 1993). The absence of trees in the nesting sites also creates an open area.

The nest sites had very little canopy cover (openness of 94–95 %). This is much more open than the surrounding dry forest, with an openness of about 65 % (data from a similar dry forest on Goat Island). Rock iguanas (Cyclura cychlura inornata) usually nest in open areas, which provide conditions for optimal incubation temperatures (Iverson et al., 2004). It was found that tunnel length in rock iguanas was inversely correlated with canopy cover, and nests tend to be shallower in shaded areas and deeper in open areas (Iverson et al., 2004). No soil temperature readings were taken during the present study; however, Grant and Lemm (1996) reported surface temperature as high as 54.4 °C and at 0.9 m below in the egg chamber temperature remained at 26-30 °C. The depth of 0.9 m exceeded measurements in this study. It appears that Grant and Lemm (1996) assessed the depth of the egg chamber by measuring the length of the tunnel. However, tunnels do not go straight down (i.e., iguanas always dig their tunnels at an angle ranging from 50-60° to the ground), and so the final depth would be less than 0.9 m.

We calculated the soil depth for an effective artificial iguana nesting site. We encountered the uppermost air pocket nests at 5 cm (Table 2). The only record of the diameter of an egg tunnel was from a large female in captivity at the Hope Zoo, which was 26 cm (Hope Zoo, unpublished). Combining these two measurements, the floor of that tunnel would be 31 cm below the surface. Making allowance for variations, we estimate that a minimum soil depth of about 40 cm is necessary for the establishment of an iguana nest site. Although this leaves little or no room for changes in tunnel depth in response to the temperature variations from year to year. The top of one tunnel was encountered at 40 cm, in which case the floor of that tunnel could be over 60 cm below the surface. Therefore, ideally, if any new nesting areas are to be constructed they should be at least be 60–70 cm deep. However, it should be noted that the deep nests recorded here could only be constructed at the centre of both nest sites and in any case may not represent the deepest that the iguanas will burrow; they were probably limited by the depth of soil in the nesting sites (77 and 76 cm).

In Vogel's (1994) study of the nesting habits of *Cyclura collei* it is stated that the nest has a straight tunnel 60 cm in length burrowed to a depth of 50 cm, followed by a second section at right angles to the first, at the same depth and 30

cm long leading to the egg chamber. Given that the tunnel penetrated 50 cm below the surface, and if the straight tunnel of 60 cm is considered the hypotenuse of a right-angle triangle, the horizontal distance tunnelled was thus 33 cm. Making allowance for variation in size of the females and other factors, it might be safe to recommend at least a doubling of this distance to 66 cm. If females are allowed to tunnel from any direction, the site might be considered a square of 66 cm, therefore, the recommended minimum surface area for a nesting site would be 0.44 m²/individual; this will also accommodate egg chambers longer than 30 cm.

Recommendations for creating and/or improving nesting sites

i) In preparing potential nesting sites, obstacles to tunnelling should be removed. All plants, including the shallow root species such as *Bromelia pinguin*, should be removed as it is difficult for the iguanas or the hatchlings to tunnel through thick mats of roots. Large rocks and old tree stumps should also be removed.

ii) The trees overhanging the site should be cut or pruned, which would increase the amount of sunlight reaching the nesting site.

iii) The ideal nesting site should be of a depth >60 cm, and for determining the capacity of a site for usage by the iguana it may be assumed that each female would require at least 0.44 m^2 of nesting site surface.

iv) Soil added to the nesting area should be compacted to the levels found in this study. If clay-loam soils are being used, they should be compacted from approximately 190 N/ $\rm cm^2$ to 350 N/cm².

v) If other soils are used, there will be a need for further analysis. It should be noted that while these nests are constructed in clay-loam soils, this is not necessarily the only soil type or even the ideal soil type for nesting; they are the only soils available in the Hellshire Hills, but the iguana originally had a much wider distribution and may have used other soil types for nesting. However, as these are the only known major nesting sites for this species at present, we base our recommendations on their characteristics.

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Mating activity and parturition of the smooth snake Coronella austriaca in Norway

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INTRODUCTION

The smooth snake *Coronella austriaca* Laurenti, 1768, is distributed widely over the European mainland occurring as far south as Sicily and Greece and as far north as Sweden and Norway where it reaches 60° N (Artsobservasjoner, undated; Artportalen, undated). This is a slender snake with a total adult length of 70–90 cm that in Norway feeds mainly on slowworms *Anguis fragilis* and shrew species *Sorex* spp. The female is ovoviviparous, bearing live young mostly in August or September. The reproductive ecology of the smooth snake is poorly understood since mating and parturition have rarely been documented. Here we present the first records of smooth snake mating activity in both spring and autumn in Norway, and report how parturition date varies with summer temperature.

Mating activity

In Norway, smooth snake populations have been studied and monitored around the inner part of the Oslo Fjord since 1982 (Sørensen, 2014 & 2017) in Kristiansand, southern Norway, since 2014, and in Moss, south-east Norway, since 2019. Photography of unique snake head patterns and scalation has been used to identify individuals. Despite intense field work, the first observation of a spring copulation was recorded on 11 June 2018 (BHS video, 2022a), so highlighting the rarity of mating observations for this elusive species. During 2019, a citizen science project was launched (Johansen, 2019) asking the general public to submit photos of smooth snake observations (Johansen, 2021), so that there is now a total of seven observation of smooth snake copulation in both spring and autumn (Table 1) at various locations in southern Norway (Fig. 1).

Observations of smooth snake copulations in Norway reflect the known behaviour of males to bite the female's head during mating (Duguy, 1961; Braithwaite et al., 1989; Capula & Luiselli, 1997; Völkl & Käsewieter, 2003). Spring records of smooth snake copulation in Norway provide new evidence to support the belief that mating takes place in May and early June, after emerging from hibernation in April (Spellerberg & Phelps, 1977; Capula & Luiselli, 1997; Reading, 2012; Sørensen, 2017). The autumn copulation records from Norway reflect similar records from other European countries



Figure 1. Locations (red circles) where smooth snakes have been observed to copulate in southern Norway 2003–2021. The map also shows the three towns of Oslo, Moss and Kristiansand, which are the main regions from where smooth snake field data were obtained. Copyright: Open Street Map contributors. Map prepared in www. artsobservasjoner.no.

(Table 2). Rollinat (in Duguy, 1961) found live sperm in the female oviduct during hibernation, concluding that autumn mating must have occurred.

Sperm storage

The fact that male smooth snakes engage in both springtime and autumn mating is not surprising considering the normal pattern of spermatogenesis for colubrid snakes in northern latitudes (Duguy, 1961; Saint Girons, 1982; Feriche et al., 2008; Fahgiri et al., 2011). While the main mating period for northern colubrids is spring, autumn mating is also reported for other species (Lankes, 1928; Winkler, 2008; Friesen et al., 2014). In an aestival spermatogenetic cycle, sperm production starts in summer, with a peak in late summer. Sperm is present in the vas deferens in autumn, and springtime mating is performed with stored sperm produced in the foregoing season. The mating period often coincides with a peak in sex hormone levels in male snakes (Graham et al., 2008) but can also be displaced in time (Saint Girons et al., 1993). The annual cycle of sex hormones in male smooth snakes is not known. Fighting males in the autumn may indicate

Table 1. Observations of smooth snake copulations in the Oslo fjord region and the coast of south and south-eastern Norway, 2003 to 2021

Season	Date	Time	Photo/video reference	Recorder	Location	Behavioural notes
Spring	29/05/2003	11:50	Fig. 2	Trond Baugen	Larvik	Male bites females head (blood drawn)
Autumn	29/08/2014	14:00	-	Gro Pedersen	Sandnes, Rogaland	-
Spring	14/05/2018	19:40	-	Otto Munthe-Kaas	Rykkin, near Oslo	-
Spring	11/06/2018	15:20	BHS video (2022a)	Beate StrømJohansen	Kristiansand	-
Autumn	16/09/2020	14:12	-	Geir Hermansen	Moss	-
Spring	30/04/2021	15:30	-	Ståle Knutsen	Larvik	Male bites females head
Spring	01/05/2021	11:50	-	Beate StrømJohansen	Kristiansand	Male bites females head



Figure 2. Headbiting during mating attempt on 29 May 2003 at Mølen, Larvik. The female ended up quite bloody and escaped when disturbed by the photographer. This occurred during springtime which is the more common period for smooth snake mating.

greater sex hormone concentrations, which could then lead to autumn matings (Duguy, 1961). In Norway on 13 October 2020, a video was made of two male smooth snakes fighting at an overwintering site (BHS video, 2022b). Fighting males are also known from Germany and Italy (in Völkl & Käsewieter 2003; Capula & Luiselli, 1997), and Rollinat (in Duguy 1961) describes how males in France were observed fighting at the
 Table 2. Records of autumn mating of the smooth snake in other

 European countries

Country	Date	Reference
England	9 August 1987	Braithwaite et al., 1989
England	6 September 2009	Bull, 2010
England	27 September 2013	Limburn et al., 2013
Germany	23 September 2010	Malkmus & Sauer, 2016
Italy	17 October 1989	Capula & Luiselli, 1997
Italy	3 October 1995	Capula & Luiselli, 1997
The Netherlands	Latter half of August	Strijbosch & van Gelder, 1993
France	End August – beginning October	Duguy, 1961

beginning of every mating season, both spring and autumn. Both the behavioural interactions between male smooth snakes and that of males towards females in different stages of the reproductive cycle have been little studied. The sexual behaviour of females is most likely influenced by the status of sex hormones and Saint Girons et al. (1993) found that in female aspic vipers *Vipera aspis* levels of progesterone were low after birth and also in the following spring. Most likely, autumn matings in Norwegian smooth snakes occur in females at the end of an otherwise non-reproducing season. Whether these females also engage in mating the following spring is not known. However, female red-sided garter snakes *Thamnophis sirtalis parietalis* are known to participate in both autumn and springtime mating (Friesen et al., 2014).

It appears to be the rule that female snakes in the northern temperate regions ovulate in late spring or early summer (Duguy, 1961; Saint Girons, 1982). Capula & Luiselli (1997) state that spring mating, early summer oviposition and late summer hatching is the more typical phenological pattern in Italian colubrids. Ovulation in late spring or early summer is also seen in species for which the normal mating season is autumn (Brito, 2003; Schuett et al., 2005). Springtime mating often occurs weeks before ovulation. In most temperate snake species, male and female gametes mature at different times of the year. Sperm storage and delayed fertilisation represent normal reproductive strategies whether mating takes place in spring or autumn the year before. Snake species have shown the ability to store sperm for exceptionally long periods of time (Booth & Schuett, 2011). Strugariu (2007) reports successful reproduction in a smooth snake after a minimum of 475 days of isolation. In this particular case the litter contained neonates of both sexes. This excludes the possibility of parthenogenesis, which is known to occur in snakes (Booth & Schuett, 2011), since the progeny would all have been female. Long-term sperm storage could be considered a reasonable strategy for small populations of snakes with low probabilities of meeting a partner (Strugariu, 2007). Such sperm storage could also be a factor when the female needs a longer period to build up energy reserves to sustain gestation (Reading, 2004), and/or when sperm storage can assure fertilisation in biennial reproduction.

Authors offer different evidence regarding mating period for the smooth snake, although April and May are most frequently reported. However, they rarely report the number of observed copulations. Because mating is rarely observed, the true mating period may be incorrectly established based on a low number of observations. In Norway, the smooth snake shows an extended mating period compared to the sympatric grass snake Natrix natrix and northern viper Vipera berus. Grass snakes mate immediately after emerging from hibernation, whereas northern vipers copulate after the springtime molt of the male, normally in April in southern Norway. Like most ovoviviparous snakes in temperate regions, the smooth snake is generally regarded as a typical capital breeder (Bonnet et al., 1998). However, an extended mating period combined with a late ovulation may allow for a strategy defined as facultative income breeding (Lourdais et al., 2002). For southern England, Reading (2004) also concluded that breeding in the smooth snake is partly capital and partly income.

Parturition dates and temperature

Dates of parturition of smooth snakes in the Oslo fjord region and the coast of southern Norway have been established from a single observed birth in the wild, from 52 cases where females gave birth in captivity, 43 cases where neonates were observed at gestation sites, and 14 cases where females were observed post-partum close to gestation sites. Gestating females are highly sedentary and show a high site fidelity from one reproductive event to another. Fifty two gravid females from the Oslo region were brought into captivity at the end of their gestation period. Of these, 36 females (68 %) were collected after 10 August. The females were kept indoors in terraria at temperatures similar to those outdoors. Twenty three females (44 %) gave birth during the first 8 days. We believe that captive conditions did not significantly alter the date of parturition. The mothers and their offspring were then returned to the same place as they were found in the wild. The earliest recorded birth was 30 July, with the latest being 30 September (Fig. 3). This latest parturition date refers to an unusual case of a female that gave birth in two consecutive years. In the second year, vitellogenesis and ovulation were delayed and this would seem to account for the late parturition date. Most parturitions in Norway



Figure 3. Distribution of smooth snake parturition dates in Norway from 1984-2021, based on observations of a parturition observed in the wild, litters from captive females collected from the wild, litters found in the wild and postpartum females found in the wild

occurred in August (Fig. 3) and coincide with dates reported from France, Italy, Poland, Germany and The Netherlands (Duguy, 1961; Capula & Luiselli, 1997; Najbar, 2001; Käsewieter, 2002; Keijsers & Lenders, 2005) but occurs earlier than reported from England. Spellerberg & Phelps (1977) and Goddard & Spellerberg (1980) state that, in England, females give birth in September and October. A more continental climate around the inner Oslofjord and the south coast, with higher summer temperatures in smooth snake habitat compared to a more Atlantic climate in southern England may explain the difference. Mean temperature of the warmest month in Oslo is 17.6 °C compared to 16.1 °C at a study site in Dorset, England (Pernetta, 2009). Difference in duration of cloud cover may also result in an even larger difference in ground temperature. To examine the influence of summer temperature on the date of parturition in Norway we have subjected our data from the three regions to a statistical analysis that is reported in the next section.

Statistical analysis - parturition dates, sites and temperatures

The parturition dates collected herein, were from three regions in Norway (Fig. 2); Moss (1 site), Oslo (10 sites), and Kristiansand (9 sites). To study the influence of temperature on parturition times, we used air temperature data measured 2 m above ground, from the nearest Norwegian Meteorological Institute weather station in each region - Gullholmen (SN17280) 59.4352° N, 10.578° E; Oslo (SN18700) 59.9423° N, 10.72° E; and Kjevik (SN39040) 58.2° N, 8.0767° E.

We used a multiple linear regression in R (R Core Team, 2022), using the Im command: Im($t \sim x_1 + x_2 + x_3 + x_4$) where i) t = parturition's date (in days after 20 July) ii) x_1 = the parturition site (20 sites) iii) x_2 to x_4 are based on the mean temperature in month of the year in which parturition was observed, where x_2 = the deviation from the average June temperature in 1989–2021 (Oslo), 2014–2021 (Kristiansand) and 2019–2021 (Moss), x_3 = the deviation from the average July temperature and x_4 = the deviation from the average August temperature.

Table 3. Anova table, testing the statistical significance of 'site' and monthly temperature deviation from average in June, July and August on smooth snake parturition date within the linear model for the three regions of Oslo, Moss & Kristiansand combined. Site and temperature deviation in July are both statistically significant factors.

Country	Sum Sq	df	F value	Pr(>F) Exact probability
Site (x ₁)	4585.9	19	2.5531	0.002284
June (x ₂)	143.9	1	1.5219	0.221349
July (x ₃)	972.4	1	10.2858	0.002001
August (x ₄)	122.2	1	1.2928	0.259312
Residuals	6806.7	72		







QQ plot for July temperature + location model

Residual fit plot, July temperature + location



QQ plot for July temperature alone model

Residual fit plot, July temperature alone

Figure 4. Residual-fit plots and quantile plots (QQ-plots) for the parturition date models

The output for a full-country linear regression with all four variables (site plus the three temperature deviations) gave R² = 0.4926. R^2 being a measure of how much of the variability in parturition date (t) can be predicted from variation in x_1 , x_2 , x_3 and x_4 . Thus, approximately half the variability in the parturition date was explained by the four variables. We used residual-fit plots and quantile plots as quality controls to evaluate the fit of our model (Fig. 4, top pair). Although the results appear appreciable with all four variables, with only a slight and unproblematic right skew in the distribution, we elected to deploy the Anova command (in the R package car) (Fox & Weisberg, 2019) to explore which variables actually had a significant impact. The resulting Anova of the

Table 4. Regression table for parturition date and July temperature deviation alone (Im(t $\sim x_3$)) for all three regions combined

	Estimate Std. Error		t value Pr(> t)	Significance
(Intercept)	36.1670	1.1593	31.197 < 2e-16	p<0.0001
July (x ₃)	-3.0455	0.8735	-3.487 0.000749	p<0.0001

Residual standard error = 11.29. df = 93

Multiple R² = 0.1156, Adjusted R² = 0.1061

F-statistic = 12.16, df = 1, 93, p = 0.0007489

linear model informed us that only the variables 'July' and 'site' were statistically significant (i.e., p <0.05) (see Table 3). In this case, significance was determined by how much of the variability (sum of squares) was explained by each variable, compared to its degrees of freedom (df). This is the number Pr(>F) in Table 3.

Since the June and August temperatures were not statistically significant (Table 3), we revised the regression model to exclude those predictor variables, giving $Im (t \sim x_1 + t)$ \mathbf{x}_{a}). The result was that the appreciable fit of the model was maintained (middle row of Fig. 4).

Our results indicate that 'site' was an important factor for parturition dates. However, there were no historical records of the local temperatures at the sites themselves, so instead we assigned the historical temperature data from the nearest regional meteorological station. This introduces a potential inaccuracy, since the temperature profiles of the sites may differ from that of their corresponding meteorological station. This means that much of the difference attributed to the site variable could simply reflect how the temperatures differ between the different sites and the meteorological stations, rather than any intrinsic influence from the site itself. We also note that the site variable could have become a significant factor solely due to the low number of observations per site. We do not know. Nonetheless, 'not knowing' is not the same as 'knowing that not', so we suggest that further studies be aware of the potential importance of the site variable as a factor in such a linear model. To improve future analysis, dataloggers should record the ground temperatures at the relevant sites themselves.

At least for this pioneering analysis, we do not believe that the site variable matters as much as our results would suggest, since when we excluded the site variable from the model and ran the final regression for all sites combined for the July temperature deviations, the residual-fit and quantile plots (bottom row of Fig. 4) were still appreciable. This conclusion holds even though this reduced model accounted for less of the observed variability, as $R^2 = 0.1156$ (Table 4).

The mean parturition day, given July temperature deviation (x_{3}) , is given by the formula t = 36.1670 - 3.0455 x₃. This implies that for every 1 °C rise in mean July temperature, the mean parturition date will be 3.0455 days earlier.

We also tested each of the three regions separately. For Oslo, using all four factors $R^2 = 0.4097$. Using temperatures only (all three months) resulted in $R^2 = 0.1609$, with only the July temperature being statistically significant. The same held true for Moss. For Kristiansand, however, we obtained R² =

0.867, p = 0.0004749 when using all four factors. Even when looking at temperatures only, we obtained R² = 0.6756, p = 0.00002336 and all three months were significant, with June and August p<0.01 and July p<0.001. The equation for the mean parturition date in the Kristiansand region is t = 44.106 - 6.021 x₂ - 5.359 x₃ - 11.381 x₄.

Our results indicate that deviation from average July temperature significantly influences the parturition date. Looking at the Kristiansand data, we revealed indications that the temperature deviations in June and August also made a significant contribution.

Although Norwegian smooth snake populations are among the northernmost populations of the species, the warm and dry summer climate along the southern coast helps speed up the gestation period of gravid females, so parturitions are mainly in August, as seen in continental Europe. It seems that England differs from the rest of Europe by having later parturition dates, probably because summers there are more cloudy and/or have lower mean temperatures. However, there are local variations, and we hope that researchers will look deeper into this in the future.

Gravid overwintering

Atkins (2011) reports hibernation of a gravid smooth snake in England, which showed clear signs of advanced gestation when found in April 2011. The specimen also had a substantially higher body mass/length ratio than 12 other female snakes. The pregnant snake was followed through spring and summer showing high site fidelity, a characteristic behaviour of gestating female smooth snakes (Sørensen, 2014, 2017; Stribosch & Van Gelder, 1993). Atkins comments on two more females showing signs of being gravid in that same spring. Spellerberg & Phelps (1977) also report from England that pregnant females were occasionally found in the spring. Gravid overwintering has later been confirmed by ultrasound scanning of three gravid female smooth snakes on 14 May 2017 in England (Atkins pers. comm.). In Norway, the smooth snake is at its northern edge of the species' range, but gravid overwintering has never been observed or reported. Nor has gravid overwintering been observed in an intensely monitored Alpine cool climate population (Capula & Luiselli, 1997). However, we would be interested in performing ultrasound scanning of potentially gravid female smooth snakes in spring in Norway. As parturition normally occurs later in the season in England compared to Norway, overwintering in a gravid state could be more common in England. We encourage researchers throughout the range of the smooth snake to perform ultrasound scanning of potentially gravid smooth snakes in the spring in order to add to the growing body of evidence that will advance our understanding of mating and parturition in this species.

To conclude, the parturition dates of smooth snakes in Norway depend on summer temperature, especially in July. Warm summers speed up the gestation period, resulting in parturition in August, which gives the neonates one to two months to forage before hibernation in October. Although Norway is on the northern range of the species, these higher latitudes, with colder winters compared to England and central Europe, seem to have no negative effect on the reproduction of the smooth snake in southern Norway. A relatively warm and dry summer climate in the distributional range in Norway normally assures successful reproduction for the smooth snake in Norway.

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Aggregation and movements of male ocellated lizards *Timon lepidus* during hibernation in mainland France observed with an endoscope

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INTRODUCTION

Due to environmental constraints, winter aggregations of squamates otherwise considered solitary are becoming increasingly common worldwide (Gardner et al., 2015). Community aggregations in lizards are little known and probably underestimated (Gardner et al., 2015) although certain European species are known to aggregate for hibernation, for example *Agama stellio* (Loumbourdis, 1983), *Chalcides chalcides* (Capula et al., 2003), *Anguis fragilis* (Karch, 2022), and *Zootoca vivipara* (Hodges & Seabrook, 2022).

Ocellated lizards generally hibernate from November to March depending on temperature (Matéo, 2017) but for reptiles suitable hibernation sites are scarce in the wild (Whiting & Wile, 2017) and crucial for their survival (Bonnet et al., 2009). Ocellated lizards occupy a network of refuges both to avoid predation in the reptile active period and to act as a hibernacula (Tatin et al., 2013), but only two or three of these refuges are used regularly (Grillet et al., 2010). It would appear that in continental Europe the ocellated lizard is a territorial and solitary species (Vincente, 1989) but, in contrast, island populations have developed pronounced social behaviour due to environmental constraints. Consequently, in France, on the island of Oléron, communal refuges are known (Doré et al., 2015) and in Portugal, on the island of Berlenga, a communal hibernaculum has been reported (Paulo, 1988).

Given that suitable hibernation sites are essential for the survival of ocellated lizards, a knowledge of their physical characteristics and how they are used is crucial for effective conservation. We set out to characterise a hibernaculum in a small population of ocellated lizards in a Mediterranean almond grove. After the discovery and confirmation of the existence of a communal hibernaculum, we filmed the occupants during hibernation and made several associated temperature measurements. The research reported here was undertaken in the framework of the French PNA (National Action Plan) on the ecology of this threatened species (Thienpont, 2020).

MATERIALS & METHODS

Ocellated lizards are known to frequent old almond groves in the French Mediterranean region. In May 2016, a suitable population for our study was found in a former almond grove at the foot of the Luberon massif in the commune of Mérindol (43° 45' N, 5° 15' E, 166 m a.s.l.) (AHPAM, 2016; Thienpont, 2020) where, during the reptile active season, there was high occupancy of tree roosts by ocellated lizards (AHPAM, 2016). In order not to disturb resting animals, we searched for hibernacula in the middle of winter (from the beginning of January) when the animals were in 'deep' hibernation. We located a hibernaculum that was in the remains of an old tree stump, below ground level (Fig. 1A). This contained two males that could be differentiated by their morphology and colour pattern as well as by the presence of a patch (of undetermined origin) on the back of male #1 and the same on the head of male #2. In order to avoid contact and disturbance, we observed the lizards with an endoscope (Ancel WF100 equipped with a lamp and relayed by Wifi). This allowed us to view and film the two lizards in the hibernaculum with a mobile phone (Fig. 1B). We made ten insertions of the endoscope with the first on 4 January and the last on 6 February. The endoscope probe is semi-rigid, which allowed us to work around obstacles. In order to be able to record potential movements during the resting period, the hibernaculum was photographed and filmed each time the endoscope was inserted. On seven occasions, we collected temperature data from the hibernaculum by inserting the probe of a Thlevel Mini LCD digital thermometer (Fig. 1A) to depths of 15 cm and 37.5 cm; the latter depth was level with the resting lizards. We obtained data on air temperature outside the hibernaculum from a weather station (43° 45'08" N, 5° 11'34" E) that is 1.5 km from the site.

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Figure 1. Old tree stump used as a hibernaculum by two male ocellated lizards - **A**. The tree stump and equipment used to measure temperatures within the hibernaculum (Thlevel Mini LCD digital thermometer), **B**. Insertion of the Ancel WF100 endoscope into the hibernaculum

Table 1. Temperatures (°C) at a depth of 15 cm and 37 cm (alongside the hibernating male ocellated lizards) within the hibernaculum and maximum and minimum external air temperatures from a local weather station

Date	Hibern tempe	aculum erature	Air temperature		
but	15 cm deep	37.5 cm deep	Maximum	Minimum	
15/01/2022	2.3	3.2	11.7	-4.2	
19/01/2022	4.3	3.7	9.9	-0.4	
23/01/2022	1.1	3.1	12.1	-4.4	
23/01/2022	4.5	4.2	12.4	-2.7	
28/01/2022	2.8	3.4	12.8	-4.4	
31/01/2022	6.4	4.5	12.8	-1.8	
06/02/2022	7.3	6.4	13	0.4	

RESULTS

The films and photographs taken during the insertion of the endoscope show the movements of the individuals throughout the hibernation period (BHS video, 2022). Although the

hibernaculum provided sufficient space for the two lizards to sleep at a distance from each other, the males were lying one on top of the other. The sleeping position adopted by male #2 on male #1 shows an almost total overlap with its body lines following very closely those of the male below (Fig. 2). The overlap of male #1 on male #2 was brief and mainly observed in early January, male #1 remained under the slightly smaller male #2 for a longer period. During the coldest periods of the year, from 4 January to 6 February 2022 (Table 1), they swapped their top and bottom positions. On two occasions, the individual on top completely covered the one below, potentially insulating it from the cold exterior. While male #1 was observed to make only one movement during the coldest period, male #2 was awake on 4 January and thereafter had several repositioning movements including one filmed live on 23 January.

Even though the minimum air temperatures fell well below freezing during the study period, on all seven occasions when temperatures were measured by us within the hibernaculum they were well above freezing; the lowest observed temperature adjacent to the lizards was $3.1 \degree$ C (Table 1) and closer to the surface at a depth of 15 cm the lowest observed was $2.3 \degree$ C (Table 1). At the time of our observations, the temperatures at the two depths differed little as at 15 cm and



Figure 2. Two male ocellated lizards hibernating in a tree stump, below ground, observed and filmed using an endoscope and mobile phone. The four images demonstrate movements of the two lizards during hibernation

37.5 cm the mean (±sd) temperatures were 4.10±2.22 °C and 4.07±1.14 °C respectively.

DISCUSSION

Refuges must provide effective protection from predation but also from extreme thermal conditions (Mohanty, 2021). However, a refuge chosen as a hibernaculum should also have good exposure to sun light so that it will warm rapidly (Pringle et al., 2003; Webb et al., 2005). Such ideal hibernacula are a limited resource for ocellated lizards (Grillet et al., 2010) and furthermore they need to be clearly visible as these lizards search for them by sight (Tatin & Renet, 2016). Given that the occupancy rate of tree roosts by the lizards during the reptile active season was high (AHPAM, 2016), it can be inferred that there is likely considerable competition for the ground refuges that will serve as hibernacula. Such scarcity of suitable refuges is expected to lead to lizard aggregations at available hibernacula (Gregory, 1984). Sharing of overnight refugia between adult males of similar build is known in other European lacertids (Aubret et al., 2014) but also for hibernation (Hodges & Seabrook, 2022) and given that external air temperatures fell below 0 °C it is understandable that the lizards would choose to hibernate at considerable depth to avoid freezing. On the island of Oleron, an artificial hibernaculum located at 40 cm depth had very similar thermal conditions, with a minimum of 3.5 °C at the coldest time of the year (Grillet et al., 2008).

Once reptiles have taken up residence below ground for hibernation they may then move about within the hibernaculum as an adjustment to thermal conditions (Zuffi et al., 1999; Cobb & Peterson, 2008). The movements observed in this study confirm this mobility, which is interspersed with waking. The longer period of time spent by male #1 under the shelter of male #2 may indicate that male #1 may be the dominant individual. The position adopted by male #2, in which it is shielding individual #1 from cold air, potentially put it at a physical disadvantage. Nevertheless, there are a significant number of benefits conferred by an aggregation, including - a reduced risk of being located by a predator (Meddis, 1975; Shine et al., 2003; Bors et al., 2020), the prevention of heat loss (Boersma, 1982; Lanham, 2001; Aubret & Shine, 2009), and a reduction in water loss by evaporation (Lancaster et al., 2006). While aggregations increase the risk of parasitic cross-infection (Leu et al., 2010; Sih et al., 2018), the stability of squamate aggregations may increase immune control capabilities (Gardner et al., 2015).

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Food supplementation of the Montpellier snake *Malpolon monspessulanus* in the wild

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nder natural settings, a variety of snake species are occasional scavengers, especially piscivorous snakes and pit vipers (DeVault & Krochmal, 2002). In the case of the Montpellier snake Malpolon monspessulanus the species has been observed both eating bait intended for other animals and roadkill (Valverde, 1974; Ventura, 2012). Several researchers have taken advantage of the propensity of wild snakes to accept carrion (e.g. mammals, fish) offered by humans to address a variety of ecological questions (Sazima & Strüssmann, 1990; Margues & Sazima, 1997; Wasko & Sasa, 2012; Glaudas & Alexander, 2017). Further investigation of the foraging response of wild snakes to carrion offered by researchers is of interest because it has the potential to improve animal welfare, for example to place electronic devices without the need for force-feeding (Shine, 1987). It could also be used to supplement the food intake of wild snakes, thereby promoting reproduction and recruitment in fragile populations (Taylor et al., 2005). Weitzmann & Pretus (2018) made an unsuccessful (prey not consumed despite snake interest) attempt at food supplementation with the Montpellier snake and this led us to undertake a feeding test in 2021 in Bédarrides, Vaucluse district, France, with two large free-ranging male Montpellier snakes both in the range of 1.7-1.8 m long and 1.5 to 1.6 kg body mass.

We offered different food items (approximately 20-110 g). The mass of the meals was estimated to be in the range of



Figure 1. Setting food supplementation tests for Montpellier snakes - **A**. A camera trap (Num'axes) was positioned by food deposited in a place frequently used by snakes, **B**. A successful test, the snake swallowed the chicken leg (BHS video, 2022)

the prey commonly consumed by large Montpellier snakes (e.g., small to large rodents). We placed the food items in locations where snakes were frequently observed during the study and used a video trap to monitor the snakes' behaviour (Num'Axes Trail Camera PIE 1023) (Fig. 1). We tested three types of easily available food items: chicken legs, quail eggs, and chipolata (pork) sausages (Table 1). Chicken legs were presented alone, eggs and sausage together. Tests where the bait was quickly covered by ants were discarded.

Chicken legs were tested five times with the first snake and once with the second snake (Table 1). The first snake found

			Time of various activities				
			Swallowing				
Snake #	Food type	Date	Food deposited	Snake detects food	Begins	Ends	Test outcome
1	Chicken leg	18 Jun	11:44	12: 22	13:15	13: 36	Successful
1	Chicken leg	29 Aug	10:44	-	13:42	14: 00	Successful
1	Chicken leg	5 Sept	14:00	-	17:00	NA	Successful
1	Chicken leg	16 Jul	15:00	-	-	-	Unsuccessful
1	Chicken leg	13 Sept	13:10	-	-	-	Unsuccessful
1	Quail egg + chipolata sausage	30 Jun	17:00	-	-	-	Unsuccessful
2	Chicken leg	1 Sept	11:35	14:10	-	-	Unsuccessful
2	Quail egg + chipolata sausage	24 Jun	10:10	11:05	-	-	Unsuccessful

Table 1. Details of dietary supplementation tests with Montpellier snakes (#1 and #2) using different food items

and ate chicken legs on three occasions (BHS video, 2022) but rejected it twice while the second snake twice showed interest in the chicken leg but did not eat it on either occasion (Table 1). Both snakes were tested once with the egg and sausage combination but neither consumed the food.

Video recording is an essential means for describing behaviours that are difficult to observe and quantify in the field (Ballouard et al., 2016; Clark, 2006; Glaudas et al., 2017) and on this occasion has been used to reveal the first successful food supplementation for a wild Montpellier snake. We recommend using video monitoring to assess potential benefits and disadvantages of food supplementation. This process, which allows remote monitoring from a mobile phone, is a technology that is not yet widely used for behavioural monitoring of snakes. However, it is particularly appropriate because i) most wild snakes are scared by humans and are reluctant to eat in their presence and ii) it is essential to determine which animal has eaten the food supplement (e.g., the targeted snake species, scavengers, or potential snake predators).

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Observations on the climbing behaviour of the smooth newt Lissotriton vulgaris and great crested newt Triturus cristatus in south-east England

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he smooth newt (Lissotriton vulgaris) is a widespread amphibian species found throughout western Europe, and is the most common newt species encountered in the British Isles (Speybroeck et al., 2016). Smooth newts can grow up to 11 cm long and can be found inhabiting a variety of aquatic and terrestrial habitats. The great crested newt (Triturus cristatus) is found throughout northern Europe, and is the largest newt species found in Britain, measuring up to 16 cm long (Beebee & Griffiths, 2000; Speybroeck et al., 2016). Most individuals of both species leave aquatic habitats in June, and the adults then return to water in February for the breeding season (Beebee & Griffiths, 2000). It is during this post-breeding dispersal that newts are most likely to be encountered in terrestrial habitats.

At 21:30 h on 7th October 2021, during an amphibian survey at Grosvenor and Hilbert Park, Tunbridge Wells, Kent (51° 08'29" N, 000° 16'15" E), 15 smooth newts (12 adults and 3 juveniles) were seen positioned on the foliage of several ferns at between 15 cm and 50 cm off the ground (Fig. 1). Two of these dropped out of the ferns, before they could be photographed in-situ. The air temperature was 15 °C, the humidity was 96 %, and the wind speed was 3 mph S. Within a 100 m radius of the location of these ferns, more than 90 smooth newts were observed actively foraging on the ground and in the crevices of a nearby stone wall. All individual newts remained still when initially observed on the foliage, but many began moving when exposed to torchlight. Newts were seen on the following fern species: lady fern (Athyrium filix-femina), male fern (Dryopteris filixmas), and Japanese lace fern (Polystichum polyblepharum). The latter species is an ornamental fern, not native to Britain, often planted in rock gardens.

On the mild and still evening of our observation, numerous gastropod species, such as the leopard slug (Limax maximus) and white-lipped snail (Cepaea hortensis) and insects, including the black clock beetle (Pterostichus madidus), were also active, and it is likely that the newts were feeding upon them.

Two other observations of newt climbing-behaviour have been recorded previously by the second author. The first in March 2014, during a routine amphibian survey in central Cambridge (52° 12'55" N, 000° 08'37" E) involved a female L. vulgaris found climbing in cow parsley (Anthriscus



Figure 1. Four smooth newts Lissotriton vulgaris (indicated by arrows) observed climbing on ferns in south-east England

sylvestris) (Fig. 2). The second in April 2015, during a routine amphibian survey in Peterborough (52° 32'25" N, 000° 16' 55" W) when a female great crested newt (T. cristatus) was found climbing a stinging nettle (Urtica dioica) (Fig. 3). Both of these observations were made in the evening, during favourable conditions for amphibians, i.e. damp weather with air temperature above 10 °C. On both occasions, these



Figure 2. A female smooth newt (Lissotriton vulgaris) scaling cow parsley (Anthriscus sylvestris) in south-east England



Figure 3. A female great crested newt (*Triturus cristatus*) found climbing a stinging nettle (*Urtica dioica*) in south-east England

individuals were the only newts found to be climbing among vegetation. Previous records of climbing behaviour in *L. vulgaris* and *T. cristatus* have been confined to Denmark and Germany, where it is suggested that the behaviour is likely linked to foraging, rather than having resulted from parasitic infection that might alter newt behaviour, but the latter cannot be ruled out (Bringsøe, 2013). Climbing behaviour seems not to have been observed in other Palaearctic newts, although it has been recorded in other families, especially plethodontid salamanders (Jaeger, 1978).

This is the first report from Britain of climbing behaviour by *L. vulgaris* and *T. cristatus*. Neither observation was of true arboreality, as the newts were found among non-woody plants and polypodiophytes. Nonetheless, our observations suggest that climbing behaviour in newts is potentially an important consideration when undertaking ecological surveys, as active newts may be missed if terrestrial habitats are not searched thoroughly.

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Reproductive behaviour of the threatened rusted frog *Telmatobius rubigo*

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he genus *Telmatobius* Wiegmann, 1834 (Anura: Telmatobiidae) includes 63 species of aquatic and semiaquatic frogs associated with high Andean ecosystems and distributed from Ecuador to Chile and Argentina (Frost, 2021). The high-altitude distribution and the aquatic habits of Telmatobius are its most distinctive characteristics and pose a series of physiological challenges for the genus (Lavilla & De la Riva, 2005; Barrionuevo, 2017). Telmatobius frogs were used by the Inca culture as symbols to propitiate rainfall and fertility (Elías et al., 2019; Otero et al., 2020), possibly due to frogs' conspicuous reproductive biology (Wells, 2007). Despite this, there is little information about the reproduction of these Andean water frogs. The genus appears to have low fecundity (Barrionuevo & Mangione, 2006), with clutch size varying from 80 eggs (Pisanó, 1955) to about 500 eggs per clutch for Telmatobius culeus and Telmatobius laticeps (Pérez, 1998; Barrionuevo & Mangione, 2006). Some earlier evidence suggests that male game togenesis in T. laticeps and Telmatobius pisanoi occurs twice yearly (Montero & Pisanó, 1990) but is continuous and asynchronous in females of Telmatobius arequipensis (reviewed Lavilla & Barrionuevo, 2005). T. culeus probably reproduces continuously as amplexus was recorded mainly during winter months and females with mature ovules were recorded in the summer (Pérez, 1998). Telmatobius jelskii probably also reproduces throughout the year as eggs, breeding adults, and cohorts of tadpoles at several developmental stages have been observed in both wet and dry seasons (Catenazzi et al., 2013).

In Argentina, the 15 species of the genus are threatened (Vaira et al., 2017) due to habitat alteration, the introduction of exotic predatory fishes, chytrid fungus infection and the indirect consequences of extreme climate events (Barrionuevo & Mangione, 2006; Barrionuevo & Ponssa, 2008; Vaira et al., 2012; IUCN, 2021). However, there is limited knowledge of the natural history of Andean water frogs (Lavilla & Barrionuevo, 2005; Barrionuevo & Abdala, 2018; Acosta et al., 2020; Araos et al., 2022). The Rusted Frog *Telmatobius rubigo* Barrionuevo & Baldo 2009 is a fully aquatic frog endemic to the endorheic basin of the Laguna de Los Pozuelos in the Central Andean Puna ecoregion of Jujuy province in Argentina (Barrionuevo & Baldo, 2009), at 3,500 - 4,300 m a.s.l. This frog has a unique feeding mechanism among Neobatrachia, using inertial suction to capture their

prey (Barrionuevo, 2016), mainly aquatic coleopterans, insects, and crustaceans (Akmentins & Gastón, 2020). It appears that the larval period in *T. rubigo* lasts more than a year, given the simultaneous occurrence of different larval stages in the same site and the low temperatures of the high mountain permanent streams inhabited by the species (Barrionuevo, 2018). Other aspects of their natural history, such as breeding behaviour and reproductive ecology, have not been reported. Here, I present novel data on the reproductive behaviour of *T. rubigo* related to amplexus, clutch characteristics, comments on developmental stages of embryos, and the reproductive mode of the species.

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I carried out frog surveys in 2018, 2020 and 2021 at Laguna de Los Pozuelos basin and the surrounding area in the Central Andean Puna ecoregion, north-west Argentina. Here, the altitude and widening of the Andes produce specific climatic conditions being a semi-arid region, with high-temperature fluctuations, intense solar radiation, and high evaporation (Murray et al., 2019). Data from the meteorological station in Natural Monument Laguna de Los Pozuelos (22° 28'24.00" S, 65° 59'35.00" W; 3,675 m a.s.l) indicates an annual mean air temperature of 8 °C (range -19° to 27 °C), annual mean relative air humidity of 44 % and annual mean precipitation of 317 mm for the 2018 and 2019 period (data provided by Administración de Parques Nacionales of Argentina). Precipitation occurs mainly in the austral summer between December and March (wet season), and the hydric deficit increases from April to November (dry season) (Murray et al., 2019). The watercourses of the Pozuelo's basin are typical of the high-altitude grassland being mostly alkaline (pH values > 7) and highly oxygenated (dissolved oxygen generally range from 8-10.6 mg/L), the conductivity increases from the headwaters of the tributary rivers to the centre of the basin with values from 140 to 448 μ S/cm (Murray et al., 2019).

On 23 January 2018, I observed one axillary amplexus of *T. rubigo*, with male clasping female behind the front legs (Fig. 1A), at Cusi Cusi locality (22° 22'8.9" S, 66° 13'29.7" W; 4333 m a.s.l.). A total of ten egg clutches of *T. rubigo* were recorded in three localities, five clutches in Austral summer (wet season) and five clutches in Austral winter (dry season) (Table 1). The sites of the clutches were wells or backwater in the beds of permanent streams (Fig. 1 B–D). Across both seasons in Santa Catalina, five clutch sites were measured with a wooden ruler and the mean (±sd) depth and width

were 10.27 \pm 3.15 cm and 179 \pm 64.65 cm respectively. All eggs were included in a gelatinous mass attached to the underside of flat rocks and some subaquatic vegetation in permanent streams (Fig. 2 A & B), in agreement with previous descriptions of *Telmatobius* spp. in Argentina (Lavilla & Barrionuevo, 2005). Nine clutches were photographed next to a photo scale using a digital camera, and images were processed using ImageJ 1.53K software to estimate the clutch and egg sizes. These clutches had between 34 and 390 eggs. The eggs showed a black pigmented animal pole and a white vegetative pole (Fig. 1 B & C) and had a mean (±sd) diameter of 0.17 ± 0.02 cm (range 0.11-0.26 cm), similar to egg sizes mentioned for T. culeus (Pérez, 1998) and T. hauthali (reviewed in Lavilla & Barrionuevo, 2005). Some embryological characteristics of T. rubigo were observed and are described according to the terminology of Gilbert (2000) and Gosner (1960). The grey crescent is present in fertilised eggs, the small, pigmented, furrowed animal pole and the large vegetative pole were observed in cleaved eggs, the yolk plug and blastopore lips indicate the gastrulation, the neural plate and neural tube formation are present at neurulation, and protuberances of the forebrain, tail bud, muscular responses, and body pigmentation were present in the pre-hatching tadpole (Fig.2 A-F). In some cases, these embryological stages were present in the same egg clutch (Fig. 2 D & F).

In Santa Catalina, four clutches associated with different males of *T. rubigo* (one male per clutch) where observed in March 2021 and September 2021. Each male was resting under the rock that had the clutch. Adult frogs and tadpoles at all development stages were observed in all surveys, as previously reported by Barrionuevo (2018).

The present observations suggest that *T. rubigo* breeds during the wet and dry seasons, laying eggs in clutches in



Figure 1. *Telmatobius rubigo* and its habitats in Argentina- **A.** A pair in axillary amplexus, **B.** & **C.** Permanent streams at Casa Colorada and Santa Catalina localities where egg clutches and individual *T. rubigo* were observed, **D.** Clutch sites of *T. rubigo* (arrows indicate groundwater rocks where the clutches were found)

permanent shallow and highly oxygenated waters. Since eggs, adults of both sexes and different stages of tadpole development were observed in both wet and dry seasons, it is suggested that *T. rubigo* reproduces continuously. According to the reproductive mode (RM) classification of Nunes-de-Almeida et al. (2021) the species seems to conform to RM 16, in that it lays eggs on the bottom of fast moving fresh water and has free-living tadpoles that feed on nutrients in their environment without parental support (i.e. do not rely on trophic eggs). Axillary amplexus has been observed in *T. culeus* and has been suggested typical of the *Telmatobiidae* (Mantilla Mendoza, 2018; Carvajal-Castro et al., 2020) and is now confirmed for *T. rubigo*. For *T. culeus*, the amplexus lasts up to three days with lengthy oviposition in bouts

Locality	Co-ordinates	Date	Seasons	N° of clutches	N° of eggs	Developmental stage
Casa Colorada	22° 22'8.9" S, 66° 13'29.7" W	23 Jan 2018	Wet/Summer	1	140*	С, Р
	4,333 m a.s.l.	12 Jan 2020	Wet/Summer	1	np	np
Cusi Cusi	22° 24'15.7" S, 66° 32'25.4" W 3,803 m a.s.l.	24 Jan 2018	Wet/Summer	1	88	F
Santa Catalina	21° 56'58.2″ S,	9 March 2021	Wet/Summer	2	349	F, C, N
	3,802 m a.s.l.				159	F
		24 August 2021	Dry/Winter	1	295	G
		13 Sept 2021	Dry/Winter	4	63	F, C, N, P
					390	Р
					34	С
					296	С, Р

Table 1. Egg clutches of Telmatobius rubigo observed during frog surveys in the Central Andean Puna ecoregion, Argentina

Abbreviations: C- cleavage; F- fertilisation; G- gastrulation; N- neurulation; np-, not photographed; P- pre-hatching. *Minimum number counted because there were dirt sediments in the gelatinous mass.



Figure 2. Clutch characteristics of *T. rubigo* - A. & B. A clutch of eggs attached to the underside of a rock and subaquatic vegetation. The eggs are in the fertilisation stage by the appearance of the grey crescent. C. Eggs in gastrulation, visualising yolk plug and blastopore lip. D., E. & F. Clutches with different development stages of the embryo. Light blue arrows indicate eggs or embryos in the early stages of development (fertilisation-gastrulation), red arrows indicate embryos at more advanced development stages (neurulation -pre-hatching).

releasing a small proportion of the eggs throughout these days (Ramos, 2000; Pérez, 2005; Mantilla Mendoza, 2018). *T. rubigo* appears to have a similar oviposition behaviour. The difference in clutch sizes reported for the species suggests consecutive laying events of small egg masses (~ 30 eggs) that could eventually result in an egg mass of ~ 400 eggs. If spawning occurs at the same place, then embryos at different development stages would be found in a clutch consistent with the data presented here for *T. rubigo*. Nevertheless, the possibility that there are instead community clutches, as suggested for other species (Mantilla Mendoza, 2018), cannot be dismissed. Further studies are needed to unveil this behaviour and other reproductive aspects of *T. rubigo*, such as parental care behaviour that has been suggested by the association of males with the clutches.

T. rubigo is threatened by direct and indirect consequences of human activities (Vaira et al., 2012; IUCN, 2021). To the threats observed during fieldwork by Akmentins & Gastón (2020) I would now add climate change. Increases in extreme precipitation events were reported for the region (Alabar et al., 2020) and occurred in Santa Catalina during the weeks before my 2021 fieldwork. An intense hailstorm and a historic stream flood during February and March of 2021 damaged crops and livestock (local news and residents comm.) and modified the stream's banks, removing stones and vegetation of frog shelters (pers. obs.). Unusual climate coupled with an increase in erosive processes and debris flowing events in mountain streams were associated with population declines of other *Telmatobius* from Argentina (Barrionuevo & Ponssa, 2008). Thus, the effect that increasingly frequent extreme climatic events have on the biology of the threatened frog *T. rubigo* needs to be thoroughly studied. Knowing how reproductive behaviour can be affected by climate change and other human-related threats is crucial for developing conservation strategies for the species.

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First recorded birth of suspected non-identical twins in the Cuban boa *Chilabothrus angulifer*

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he occurrence of identical (monozygotic) or non-identical (dizygotic) twins in snakes is a rare phenomenon, reported in less than 1% of more than 3,970 currently recognised species (Smith, 1999; Wallach, 2018; Uetz et al., 2022). Even rarer are twins in viviparous snakes, being recorded in only seven species belonging to seven families: Boa constrictor (Boidae), Coronella austriaca (Colubridae), Diadophis punctatus (Dipsadidae), Erpeton tentaculatum (Homalopsidae), Pseudechis colletti (Elapidae), Thamnophis sirtalis (Natricidae) and Vipera berus (Viperidae) (for reviews see Smith, 1999; Wallach, 2018). Smith (1999) reported the only known twins in the family Boidae (B. constrictor), accounting for one case in approximately 600 litters obtained in captivity by that author, evidencing its very low frequency. Herein we report the first case of twins, suspected to be non-identical, in the Cuban boa (Chilabothrus angulifer), which represents also the second case of twinning in the family Boidae and the eighth species of viviparous snake in which this phenomenon has been recorded.

On 30 June 2021, we rescued a gravid female *C. angulifer* (1,800 mm SVL, 200 mm tail length) that had been collected by local people about three weeks earlier at Cuatro Caminos town (22° 53'34" W, 82° 22'44" N; 75 m a.s.l.; WGS 84), Bejucal Municipality, Mayabeque Province, Cuba. According to the collectors, the snake was in a tree hole by day when first seen. Its mouth was severely damaged, apparently because of the poor conditions in which it had been kept for three weeks, and also showed clear signs of pregnancy. We kept it to treat the mouth injury with antibiotics and to collect data related to the birth and the neonates. We placed it in a plastic, wellventilated container (700 x 350 x 350 mm) with newspaper and chopped dry banana leaves as substrate, and offered water ad libitum. Temperature during the time it spent in that container varied from 24.7-35.3 °C. It only accepted food once during the time from its collection to birth: it ate two freshly euthanised adult lab house mice (Mus musculus) about one month before it gave birth. On 18 July it shed its skin (53 days before birth).

We took measurements of the neonate snakes to the nearest millimeter from photographs using the application Snake Measurements v1.0 for Android (https://apkpure. com/snake-measurement/com.theultimatelabs.snake), which proved to be highly accurate when compared to the string method (Rivas et al., 2008). Body mass was recorded to the nearest gram with a WeiHeng[®] digital scale in the case of the mother and the normal siblings, and to the nearest 0.1 g with a TANITA[®] digital scale in the case of the twins. We recorded the body measurements and masses of the neonates twice: one day after birth (with the yolk inside) and after the neonatal shed. We made all comparisons taking into account the measurements one day after birth, but for masses we waited until after the neonatal shed, in order to avoid any possible bias because of the absorbed yolk. Using metallic probes, we recorded the sex based on the distance of penetration (females: < 6 subcaudals; males: > 7 subcaudals; Frynta et al., 2016).

After the neonatal shed, we placed the baby boas in separate containers. As a first and second meal we offered each several food options at the same time, differing in size, texture and other features to test for food preferences. The prey options were: lab house mice (pinky: <25 mm body length, <3.0 g; hopper: 40–50 mm body length, 7.0–9.0 g; and weaned: 60–80 mm body length, 14.0–15.0 g), fuzzy lab brown rats (*Rattus norvegicus*: 60 mm body length, 9.0 g), adult anoles (male *Anolis porcatus*: 60 mm SVL, 4.0 g; female *A. porcatus* 55 mm SVL, 3.0 g; or male *A. sagrei*: 50 mm SVL, 4.0 g), and Cuban treefrogs (*Osteopilus septentrionalis*: 40 mm SVL, 3.0 g). During the subsequent months we continued offering food to the four siblings on a weekly basis, each time offering mice as the first option and then anoles if the mice were refused.

During the afternoon of 9 September (71 days after rescue) the female gave birth to two normal babies (within the range of sizes reported for neonates of this species, see references below), a pair of twins and five infertile ova (data on neonates in Table 1) (Fig. 1). The twins were encapsulated in the same fetal membranes (Fig. 1) and were connected to a single yolk sac by separate umbilical cords (Fig. 2). We left the four neonates, as well as the infertile ova, with their mother overnight. The next day we moved them to a smaller container with paper towel as substrate and a water bowl.

The twins were the most delayed of the four siblings in both umbilical cord detachment and first shed, and thus in the taking of their first meal. The two larger babies absorbed their yolks completely within the 24 h following birth (Fig. 3), which was not the case with the twins. One twin absorbed a small amount of yolk (as suggested by its slightly swollen abdomen) and the other one showed no visible signs of having absorbed



Figure 1. Litter of *Chilabothrus angulifer* with its mother shortly after birth. Notice the infertile ova and some of the babies still encapsulated in the fetal membranes. The arrow indicates a single fetal membrane encapsulating the twins.



Figure 3. A normal sibling of *Chilabothrus angulifer* one day after birth (No. 2; Table 1). Notice the umbilical cord ending in the empty yolk sac and the swollen abdomen due to the absorbed yolk.



Figure 2. Twins of *Chilabothrus angulifer* one day after birth: left No. 3, right No. 4 (Table 1). Notice the common yolk sac (still not absorbed) and the separate umbilical cords.

yolk; their common yolk sac remained almost as large as the day before (Fig. 2). After that time and in order to avoid any infections, we decided to tie off the twins' umbilical cords close to the yolk sac and cut them off. The umbilical cords dried and detached from the neonates 3–5 days later. During the period prior to the neonatal shed, the four babies did not ingest food, some of them struck at us when disturbed, and some occasionally spent time in the water bowl when their

skins turned dull. The neonatal shed of the two normal siblings occurred between 17-18 days after birth, but that of the twins occurred between 57–58 days after birth (Table 1). Indeed, the two normal siblings shed twice during the period in which the twins shed only once (Table 1). The period between the first and second shed of the twins was also considerably longer (>3 times) than that of the normal siblings (Table 1). Of the two normal siblings, one kept its body mass and the other lost 15 g between birth and the neonatal shed (Table 1). By the time the twins had completed their neonatal shed, they had lost 11 and 14 g respectively when compared to their masses one day after birth. Nonetheless, the twins showed good vitality and behaved similarly to their normal siblings. The normal siblings accepted their first meals between 1-16 days after the neonatal shed, which consisted of hopper and weaned mice and a Cuban treefrog (Table 1). The larger twin accepted its first meal five days after the neonatal shed (63 days after birth), eating a female A. porcatus (No. 3, Table 1) and 17 days later it accepted a hopper mouse. The smaller twin accepted its first meal (a frog) 46 days after the neonatal shed and 103 days after birth (No. 4, Table 1) and it took its second meal (a male A. sagrei) five days later.

The four babies continued feeding on a weekly basis after

Table 1. Data on the four neonates of *Chilabothrus angulifer*, two of which were twins (Nos. 3 and 4), born in captivity on 9 September 2021. For each variable we present two values: one day after birth/after the neonatal shed. Abbreviations: SVL = snout-vent length, TL = tail length, HL = head length. The number of days since birth (1st shed) or since the previous shed (2nd shed) are in parenthesis. All dates refer to 2021 except the second sheds of the pair of twins that occurred in 2022.

No.	Sex	SVL (mm)	TL (mm)	HL (mm)	Mass (g)	1st shed (days)	2nd shed (days)	1st and 2nd meals (prey type)
1	М	552/580	48/50	27/28	165/150	27 Sept (18)	25 Oct (28)	13 Oct (hopper mouse and frog) 20 Oct (weaned mouse)
2	F	534/561	46/49	27/28	140/140	26 Sept (17)	1 Nov (37)	27 Sept (hopper mouse) 13 Oct (hopper mouse)
3	F	402/457	38/43	25/26	54.8/40.9	6 Nov (58)	27 Feb (113)	11 Nov (female Anolis porcatus) 28 Nov (hopper mouse)
4	F	384/430	36/40	23/24	42.5/31.6	5 Nov (57)	2 Mar (119)	21 Dec (frog) 26 Dec (male Anolis sagrei)

their neonatal shed, until we measured them again on 1 April 2022 (ca. 7 months after birth). During this period the twins always refused mice as their first food option but readily took anoles instead, whereas the normal siblings accepted mice as their first food options most of the time (accepted anoles a couple of times only). The smaller twin increased 16 mm in length and 10.5 g in mass with respect to the previous measurement; the larger twin increased 20 mm and 15.5 g. In contrast, the normal siblings increased from 80–106 mm in length and from 80–90 g in mass with respect to the previous measurement.

The mother did not consume any of the infertile ova during the first night or thereafter. It weighed 3,330 g on 23 August (17 days before birth) and 2,130 g a few hours after birth (the neonates, infertile ova and fetal membranes summed up together ca. 1,200 g). It accepted a young Guinea pig (*Cavia porcellus*; 150 g) as food two days after birth, and four days later it accepted an adult lab brown rat (200 g). After that, it started to show signs of being close to shed and we stopped offering food.

The twins reported here resembled each other closely and were both females, but they showed strong phenotypic discordance in colour pattern, and to a lesser degree in size, suggesting they were dizygotic twins (Figs. 4, 5 & 6). Phenotypic discordance in twin snakes has been largely attributed to cases of non-identical twins. Dizygotic twins are encapsulated in the same fetal membranes in the oviduct and are initially attached to separate yolk sacs with their own umbilical cords, but the yolk masses and even the umbilical cords may eventually fuse together forming a single yolk mass (Marion & Nowak, 1980; Wallach, 2007, 2018). Fusion of non-identical twins is thought to go even further, since conjoined dizygotic twins (even of separate sexes) have been reported in Crotalus durisus, V. berus, V. ursinii and T. sirtalis (Dorner, 1873; Vanzolini, 1947; Tóth et al., 2005; Wallach, 2007). The set of twins of B. constrictor reported by Smith (1999) were attached to the same yolk sac by separate umbilical cords, similar to the twins of C. angulifer, but they showed high pattern concordance, which led Smith to assume that they were monozygotic. Thus, if our and Smith's (1999) hypothesis are correct, the twins of *C. angulifer* reported here would be the first dizygotic twins documented in the family Boidae.

Twin snakes can be up to nearly 50 % shorter and weigh up to nearly 70 % less than their normal siblings (Wallach, 2007), probably because of resource sharing. Smith (1999) commented that the twins of B. constrictor apparently had no birth defects other than a much smaller size than their normal siblings, but they were poor feeders and died some months later. The twins of *C. angulifer* were also smaller than their normal siblings between 24-30 % in length and between 71-79 % in mass (Fig. 5, Table 1) and apparently had difficulties in absorbing the yolk during the time span in which their normal siblings did. We suspect that the lack of that first boost provided by the absorbed yolk in the twins of C. angulifer apparently affected their further performance and development, hence the delay in the neonatal shed. The longest period before the neonatal shed previously reported for the species was 37 days (20 days less than the minimum time in the twins reported here), in a neonate from an apparently normal litter, where



Figure 4. Detail of the anterior part of the body of the twins of *Chilabothrus angulifer* in dorsolateral view. Notice the similar but not identical spot pattern.



Figure 5. The four neonates of *Chilabothrus angulifer* four days after birth. Notice the considerable difference in size between the twins (centre, right) and the normal siblings (lower left, upper centre).

the total lengths ranged between 600–610 mm (Sheplan & Schwartz, 1974); neonates of *C. angulifer* frequently exceed 600 mm SVL and 146.6 g (see Rodríguez-Cabrera et al., 2015 and references therein). The twins of *C. angulifer* represent the smallest viable individuals of this species ever reported (Fig. 6), being 103 mm (20 %) and 121 mm (24 %) shorter, and weighed 39 g (49 %) and 48 g (60 %) less than the previous minimum size record for the species, respectively (505 mm SVL, 80 g; Rodríguez-Cabrera et al., 2015).

Considering that the twins absorbed a minimal amount of yolk, as evidenced by the slight or no visible swelling of the abdomens and the almost intact yolk mass one day after birth (Fig. 2), it is unsurprising that they lost mass during the unusually prolonged period before their neonatal shed (Fig. 6). During this period one of the normal siblings remained at the same mass while the other lost 15 g only (Table 1),



Figure 6. Twins of *Chilabothrus angulifer* on 7 November 2021 (shortly after the neonatal shed, nearly two months after birth): left No. 3, right No. 4 (Table 1). Notice the skinny appearance, suggestive of having lost mass. They both represent minimum size records for the species with the smaller twin (No. 4) being the smallest individual of *C. angulifer* ever reported.

suggesting that yolk was converted to tissue but also there was some weight loss; the two normal siblings increased 30 mm in total length during this period. The twins increased 50–60 mm in total length from birth to the neonatal shed, but during a much longer period (57–58 days vs. 17–18 days in the normal siblings). Even after several months with regular food intake, the increase in total length and mass of the twins was considerably less than that of the normal siblings (4–6 times in total length and 5–9 times in mass).

The two normal siblings took both endothermic and/or ectothermic prey as their first and second meals (Table 1) and continued to do so in subsequent meals (not recorded here), but they took ectothermic prey only sporadically. This is consistent with Rodríguez-Cabrera et al. (2015, 2020) who presented compelling evidence for neonates of *C. angulifer* being large enough and equipped with specialised structures (heat-sensing labial pits) to consume mammals. Despite their very small size, heat sensing labial pits are present and presumably functional, indeed at least the largest twin accepted both ectothermic and endothermic prey as its first two meals (Table 1). Nevertheless both twins showed strong preferences for ectothermic prey during the subsequent months, a behaviour possibly related to their small size.

Chilobothrus angulifer is a Cuban endemic species widely distributed in the archipelago (Rodríguez et al., 2013). The apparent absence of substantially smaller neonates (potential twins) of C. angulifer in the wild suggests that twinning might represent a disadvantage with respect to normal neonates, possibly decreasing their fitness. To the best of our knowledge, no data on longevity have been recorded for twin snakes, but it has been documented that some anomalous snakes (e.g., dicephalous) may live for up to more than 22 years in captivity (Wallach, 2007). Nonetheless, it seems unlikely that the twins reported here would have survived for very long in the wild given their small size. Free-ranging neonates of C. angulifer from the same litter tend to stay together during the period between birth and the first shed, sometimes not as well hidden as expected for an individual of this species (T.M. Rodríguez-Cabrera, unpublished data). During this period, they do not eat or drink water, living off their yolk reserves only. They seem

highly vulnerable to potential predators during this time and consequently any elongation of this period, as observed in the twins reported here, would likely be detrimental to their survival.

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First live stranding of a leatherback sea turtle Dermochelys coriacea in Alagoas, north-east Brazil

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Since 2009, the NGO Biota (Instituto Biota de Conservação) has developed several activities focused on the management, research and conservation of marine animals along the coast of Alagoas (Brazil). This has included a periodic beach monitoring programme to record sea turtle egg laying, hatchling emergence, and stranded marine animals (turtles, mammals and birds). In addition, Biota involves local communities in this monitoring where they can participate by sending records and information of stranded animals (including photos for species identification and animal state, GPS of locations, date and hour, etc.).

The leatherback sea turtle Dermochelys coriacea (Vandelli, 1761) (Testudines: Dermochelyidae) is a pelagic species with a circumglobal distribution, with feeding areas in temperate and subarctic zones and breeding areas in tropical zones (Eckert et al., 2012). Its distribution in Brazil is discontinuous, with scattered records from the north-east, south-east and south of the country (Barata et al., 2004). The species is categorised as Vulnerable in the IUCN's Red List of Threatened Species (Wallace et al., 2013) and Critically Endangered in the Brazilian List of Fauna Threatened with Extinction (BRASIL, 2014).

In Brazil, several factors put the leatherback under particular conservation threat: the low estimated number of females breeding in each reproductive season (between 1 and 18) in a restricted geographical range for nesting (Espírito Santo); the apparent isolation of the population (high philopatry) (Thomé et al., 2007, Colman et al., 2019) and exposure to pollution, coastal development, climate change and especially industrial fishing activities (Colman et al., 2019; Sales et al., 2008). In this context, every individual is important for the recovery and maintenance of the regional population. Here, we describe the first recorded stranding of D. coriacea in the state of Alagoas (north-east of Brazil) including information about its rescue, management and release.

At 19:00 h on 22 April 2013, fishermen made a report to Biota of a female leatherback sea turtle found alive on the beach Lagoa do Pau in Coruripe, Alagoas (10.106247° S, 36.098132° W) (Fig. 1). The Biota team promptly activated its protocol for attending a stranding and the rescue team arrived at 19:20 h. The female turtle had a curved carapace length of 146 cm and curved carapace width of 107 cm, suggesting that the turtle was of reproductive age (Zug & Parham, 1996; Avens



Figure 1. Map of the state of Alagoas, Brazil, indicating the stranding and release sites of the female leatherback turtle

et al., 2009). With the help of fishermen and local residents several attempts were made to return the animal to the ocean, but every time the waves brought it back to shore. The turtle was lethargic and prostrate and, after confirming that it was not able to return to the sea by itself, a decision was made to intervene. At 21:30 h, six adult men carried her (~20 m), with the help of a tarpaulin placed under the plastron, to a pickup truck (Chevrolet S10) in which it was transported to the rehabilitation facility. The turtle was placed at the back of the pickup truck and the careful transport took ~2:25 hours (92 km). During transportation and subsequent treatment, the animal was constantly monitored and watered to prevent drying of the carapace and ocular region.

At the rehabilitation facility, the leatherback was carried by eight adult men and accommodated in a partially filled 2000 litre pool (Fig. 2a); a lower water level was used to facilitate breathing and lubrication of the eyes. Following a clinical evaluation conducted at the pool and a visual-assessment of its body condition (plastron shape and tissue bulk around the neck and shoulders, Thomson et al., 2009), the animal was found to have a good nutritional status. Nonetheless, it had multifocal erosive lesions with caseous deposits on the



Figure 2. Adult female leatherback turtle with anterior right flipper amputated - **A.** In rehabilitation after the rescue, and **B.** During release back into to the sea

carapace and plastron. Moreover, the anterior right flipper had been amputated and was healed. Since only a shallow pool was used for examination, it was not possible to assess the specimen's buoyancy. The animal received subcutaneous fluid therapy (15 ml/kg/day, Ringer's lactate, glucose 5 %, sodium chloride 0.9 %, multivitamin), antibiotic therapy (Enrofloxacin 5 mg/kg), liver protector using the allometric extrapolation method (Pachaly, 2007; 0.01 ml/kg, antitoxic multi vitamin and multi aminoacid: acetylmethionine, choline (chloride), riboflavin (phosphate), pyridoxine (hydrochloride), nicotinamide, dextrose) and cleaning/ treatment for the carapace lesions (debridement, povidone iodine and antibacterial ointment). These clinical procedures were performed every 48 h during the rehabilitation period, respecting the recommended dosage interval of the antibiotic (Carpenter, 2005) and to reduce animal handling and stress. It was not possible to conduct additional tests due to the lack of appropriate equipment for collecting blood from an animal of this size and the impracticality of radiographic examination.

Although various fresh cnidaria (jellyfish and caravels) were offered, we did not observe the turtle spontaneously feeding. To avoid stress and potential weakening of the animal, a decision was made to release it back into the sea as soon as possible. It was released at 12:12 h on Riacho Doce beach, Alagoas (9.576012 S, 35.655377 W) (Fig. 1), five days after the stranding and shortly after receiving another dose of fluid and antibiotic therapy. The animal was placed on a raft that was transported beyond the wave zone and there released (Fig. 2b). It was observed to float for a few minutes, drifting close to a sandstone reef; after approximately one hour, visual contact was lost. The night of the release and on the following mornings the Biota team monitored the adjacent beaches in case of a repeated stranding. Furthermore, partners around the state were alerted in case of a possible stranding. The animal was not sighted again.

In Brazil, the main leatherback sea turtle breeding area (of the south-west Atlantic Ocean subpopulation) is located in the southern state of Espírito Santo (Thomé et al., 2007), ~1,100 km, in a straight line, from the stranding site here described. However, other occasional breeding sites have been recorded in the north-east (Prado, southern state of Bahia: Barata & Fabiano, 2002; Luís Correia, state of Piauí: Loebmann et al., 2008; Cajueiro da Praia, Luís Correia and Parnaíba, state of Piauí: Silva et al., 2010; Entre Rios, northern state of Bahia: Gandu et al., 2014). Typically, the nesting season for this species occurs between October and February (Thomé et al., 2007). These highly migratory animals move great distances between nesting and foraging sites (Almeida et al., 2011) and D. coriacea turtles from other subpopulations are recorded elsewhere along the Brazilian coast (Colman et al., 2019). Since the stranded individual did not have an identification tag, there is insufficient information to surmise the subpopulation to which it belongs.

A compilation of sightings, strandings and incidental captures of leatherback turtles on the Brazilian coast from 1969 to 2001 (Barata et al., 2004) indicates that the majority of these events involved either dead animals that were washed up or incidental captures in fishing tackle. Live D. coriacea stranding records in Brazil are rare; only one previous live stranding had been recorded on the north-east coast, near Salvador, Bahia in February 1999 (Barata et al., 2004). The right flipper of the female of the present report had been amputated by an unknown cause, however the lesion's characteristics were similar to those reported for specimens of Caretta that had been caught in plastic/nylon cords of fishing nets (Barreiros & Raykov, 2014). The absence of the right flipper is unlikely to be the cause of the stranding but it may have had some influence given the turtle's size and weight. The absence of the flipper could have resulted in some difficulty swimming, diving or even in movements to avoid accidents (i.e., collisions and fishing nets). Moreover, the extra effort of swimming with only three flippers can result in an increase in energy expenditure (Franchini et al., 2020).

Although there is some information about health parameters of free-living leatherbacks (e.g. Innis et al., 2010), there is no established and standardised protocol for maintaining leatherbacks in captivity, especially for adult individuals. For this reason, a decision was made to return the stranded individual to the sea quickly. It is hoped that this description of a stranding event and the procedures applied (management and veterinary care) will contribute to the improvement of care in any future incidents of this kind.

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Rehydration of mummified salamander tissues using fabric conditioner

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Dead amphibians are prone to rapid desiccation and mummification, which may hamper opportunistic specimen collection of rarely encountered species, for example from roadkill or escaped captive animals. Many identification characters require observation of normal tissues and mummified remains may, therefore, be difficult to identify based on morphology. A protocol for rehydrating mummified human tissues, restoring them to the point where fingerprints can be successfully obtained, using an isotonic salt solution combined with Comfort fabric softener, has been developed for human tissues (Turner & Holtom, 1981). I trialled this easily recreated approach to a desiccated amphibian specimen, to trial the extension of this method to amphibians.

An adult (six-year-old) *Echinotriton andersoni* escaped from its vivarium in December 2021 and completely desiccated before being discovered. The body was kept in a cool, dry place until January 2022 and was completely mummified (Fig. 1A). In order to rehydrate the tissues prior to preservation, the protocol described by Turner & Holtom (1981) was trialled. The specimen was submerged for 24 h in a 0.9 % isotonic saline solution with 0.2 % Comfort fabric conditioner ('Comfort', Unilever) at 2 °C, this temperature intended to prevent decomposition of tissues. After 18 h tissues had rehydrated substantially but the solution was failing to penetrate into the core of the specimen so a 15 mm incision was made along the ventral surface of the newt before further submersion for 6 h.

After 24 h, substantial yellow staining had leached into the solution, likely fat, and the specimen had substantially rehydrated, increasing mass from 4.7 g dried to 8 g rehydrated. In life, the animal weighed approximately 10 g. Colours that had faded (red-orange costal wart tips, toe tips and tail margins) were recovered, fleshy morphological characters assumed relatively normal appearance and the specimen was no longer rigid (Figs. 1B & C). However, it had not fully rehydrated and was not fully flexible; eyes remained sunken (Fig. 1C). There was no visible damage to tissues, which is often caused by soaking dried amphibians in water alone due to over-absorption of water in surface tissues. Further submersion was not possible as decomposition would begin to degrade the specimen. The specimen was rinsed, patted dry with paper towel and then preserved in 90 % ethanol.

The rehydration protocol appears to be reasonably



Figure 1. *Echinotrition andersoni* male mummy - **A.** Before, **B.** & **C.** After attempted rehydration with isotonic saline solution with 0.2 % Comfort fabric conditioner

successful in amphibian tissue and may be a useful tool to improve the quality of opportunistically recovered dry specimens. Longer term immersion in the rehydration solution may improve results if some decomposition is acceptable.

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First record of ultraviolet fluorescence in the geckos *Hemidactylus turcicus* and *Tarentola mauritanica*

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iofluorescence occurs in living organisms when they f Dabsorb short wavelengths of light and then re-emit longer wavelengths (Lamb & Davis, 2020). This phenomenon is known in many different species and has been observed recently in some frogs and salamanders (Taboada et al., 2017; Lamb & Davis, 2020) and reptiles such as sea turtles (Gruber & Sparks, 2015), chameleons (Prötzel et al., 2018), sea snakes (Seiko & Terai, 2019) and to date in six gecko species; Chondrodactylus bibronii (Sloggett, 2018), Cyrtodactylus baluensis (Jeng, 2019), Cyrtodactylus quadrivirgatus (Top et al., 2020), Hemidactylus parvimaculatus (Mendyk, 2021), Kolekanus plumicaudus (Pinto et al., 2021) and Pachydactylus rangei (Prötzel et al., 2021). The latter is an interesting case as the flourescence arises from iridophores. Here we report a study of biofluorescence in two further gecko species Hemidactylus turcicus (L., 1758) and Tarentola mauritanica (L., 1758).

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The study was undertaken in Sassari province, northern Sardinia, Italy (approx. 40.711002° N, 8.568792° E) in August and September 2021. We located geckos at night (21:30 -24:00 h) in a variety of natural and anthropogenic habitats. They were illuminated with UV torches (Alonefire SV13, 15W, 365nm) and photographed with a Nikon D4 camera with a 60 mm Nikkor lens, a Nikon D850 camera with a 105 mm lens and iPhone 11 mobile phone camera.

Both gecko species fluoresced when exposed to UV light but unlike exposure to common white lights, UV light did not seem to induce escape behaviour in either species. In UV light their eyes were black, the skin generally had an overall blue hue, while some bones flouresced. These included the skull (anterior processes of maxilla, ascending nasal processes of premaxilla, premaxillae, nasals, frontals, parietals, lower jaw), vertebral column, and all the bones of the front and hind legs. With respect to variation between the two species, juveniles showed more brilliant and evident bones than adults (the latter mainly had just skulls visible); furthermore, H. turcicus showed more contrasting colours than T. mauritanica. Some juvenile H. turcicus had a slightly greenish hue, which was not detected in other conspecifics. Skull bones were clearly visible in all specimens, as in other species (e.g. Sloggett, 2018), in particular on the anterior part of the head and lower jaw, thanks to their position just below the epidermis. Adult T. mauritanica glowed less than H. turcicus, probably due to more sclerotised skin, that reduces the UV radiation reaching the skeleton. Other bones, such as vertebrae and skeletal parts in both front and



Figure 1. Bone biofluorescence in two gecko species (both adults) under UV light- A. *Hemidactylus turcicus* where skull, vertebral and limb bones are fluorescing, B. *Tarentola mauritanica* where only the skull can be seen to fluoresce

hind legs, were clearly visible mainly in juvenile specimens, most probably due to a more translucent skin than adults. Fluorescence of these bones corresponds to what has been observed in other species (Sloggett, 2018; Pinto et al., 2021), and in particular the appearance of *H. turcicus* under UV light clearly matches that of the conspecific *H. parvimaculatus* (Mendyk, 2021). After discovering this case of biofluorescence in the field, a video of *H. turcicus* under UV light was also found on YouTube (2019). The pattern of biofluorescence reported here is not necessarily shown by all geckos, for example in *C. quadrivirgatus* it was restricted to the top and lower margins of the head and toe articulations (Top et al., 2020).

The results of this study raise the number of gecko species known to fluoresce from six to eight. However, a further as yet unidentified species is shown fluorescing under UV light in a video (YouTube, 2020). Bone fluorescence in geckos may well be purely coincidental, resulting simply from the visibility through translucent skin of bones that happen to fluoresce (Prötzel et al., 2021). However, there may be some functional significance of biofluorescence in reptiles. In chameleons, it has been demonstrated to play an important role in intraspecific communication and mate signalling (Prötzel et al., 2018). In *H. turcicus* there are UV-sensitive pigments in the eyes (less than 5 % of overall pigments) that have been suggested as involved in "a sensitive indication of spectral conditions of illumination (direct sun/moon, sky, green light reflected from plants, etc.)" (Loew et al., 1996).

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Rapid colour change in the agile frog *Rana dalmatina* in north-west Italy

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The agile frog, *Rana dalmatina* Fitzinger 1838, is widely distributed in western, central and south-eastern Europe (Speybroeck et al., 2016). It lives mainly in deciduous and mixed forests and adjacent meadows. The colouration of this slender and long-legged ranid is generally uniform brown, light brown or beige above, often reddish or yellowish, with a few darker markings.

On 27 July 2021, during a visit in a forest near Lago di Viverone, Piedmont, in north-western Italy (45° 24'46" N, 8° 1'11" E, 235 m a.s.l.) the author captured three adult R. dalmatina with blackish brown markings. These were placed temporarily in plastic bags. After about 30-45 minutes, I came to photograph and release them and to my surprise they had turned much lighter; basically to a colouration that I consider normal throughout their distribution. On 28 July, I found yet another adult (SVL 46 mm) that was also very dark. Hence, I photographed it approx. 30 seconds after capture (10:33 h), as it still had its conspicuous dark colouration (Fig. 1). The dorsum and body sides were dark brown with blackish brown blotches and two cream-coloured dorsolateral ridges with black markings anteriorly. The individual was placed in a transparent plastic bag, then in my backpack. At 54 minutes later (11:27 h) it was photographed again when it appeared much lighter (Fig. 2); much the same light colour as the three individuals the day before. The dorsum and upper body sides were light brown and nearly uniform, except for the two dorsolateral ridges which were basically the same as when the frog was found initially. The brown blotches on the lower body sides had turned lighter. In both Figures 1 and 2 there are small, nearly black remains of dead leaves on the frog. All four frogs were released after I had photographed them.

The habitat consisted of dense deciduous forest with a moist substrate of nearly black soil. Over decades, I have observed *R. dalmatina* in most of its European distribution (though by far most in Denmark), but I have never observed such dark individuals. However, once the frogs had been removed from their habitat and put in plastic bags for half an hour to an hour, their colouration corresponded well with my general experience with *R. dalmatina*.

Kang et al. (2016) observed rapid colour change, i.e., occurring within an hour, in *Hyla japonica*, apparently with the purpose of resembling natural backgrounds. Similar results were achieved in *Dryophytes cinereus* and *Pseudacris crucifer* concluding that colour change might enhance crypticity and function in predator avoidance, but it was also linked to changing temperatures (Kats & Van Dragt,



Figure 1. Very dark Italian *Rana dalmatina* photographed on 28 July 2021 at 10:33 h, immediately after capture



Figure 2. The same *Rana dalmatina* as in Fig. 1, but photographed 54 minutes later after having been kept in a plastic bag in the dark of a backpack

1986; King et al., 1994). The potential role of stress as the agile frogs were handled and subsequently spent 30–55 minutes in a plastic bag should also be considered. However, the general effect of stress on colouration is that frogs turn darker due to the darkening agent α -melanocyte stimulating hormone (α -MSH) (Isoldi et al., 2010; Bringsøe, 2020). As the four *R. dalmatina* in this case turned markedly lighter, I find it likely that the change was not caused by stress. An unusual colour change in a close relative was observed as *R. graeca* in

Albania turned bright yellow at night whereas the frogs had their normal brown colouration during the day (Bringsøe, 2011). To my knowledge, this is the first report of rapid colour change in *R. dalmatina*.

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Birth and neonate colouration of *Ahaetulla prasina* in north-east India

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The oriental vine snake, *Ahaetulla prasina* Boie, 1827, is a wide ranging Indo-Malayan species (Uetz et al., 2021) exhibiting polymorphic colouration, typically green but greyish, brownish or orange-yellowish (Amber et al., 2017; Das, 2018). It is an ovoviviparous species known to produce 4–10 neonates with total lengths of 240–490 mm (Das, 2018). In the past the colour of neonates has very rarely been commented upon although the only reference we are aware of states that they are light brown (Lim & Tat-Mong, 1989).

On 14 May 2020, an adult female snake measuring 1,320 mm in total length (snout-vent length 845 mm) was rescued from a cultivated field, Kawrthah, Mamit District,



Figure 1. A female *Ahaetulla prasina* giving birth in captivity and the colouration of the neonates - **A–E**. A neonate emerging from the female, **F.** A neonate still within the amniotic sac, **G.** Four greyish neonates before they were released back into the wild, **H.** The female parent *A. prasina* prior to parturition

Mizoram, north-east India (23.956772° N, 92.353856° E; 296 m a.s.l.). The gravid snake was monitored in captivity in a glass terrarium (60 cm length; 43 cm height; 23 cm width) at Mizoram University. On 24 Oct 2021, the female bearing a total of four neonates commenced parturition at around ca. 14:30 h and finished at ca. 18:30 h with the birth of each individual taking about 3–5 min. During birth, in each case the mid-body loop of the neonate was extruded first following gradual contraction of the female's belly (Fig. 1A-E). Soon after delivery to the ground, the neonates slithered out from the thin, gelatinous and translucent amniotic sac (Fig. 1F). After taking necessary biometric data (total length 408-450 mm; snout-vent length 270-287 mm; weight 3.40-3.71 g), the four neonates (Fig. 1G) and the adult female (Fig. 1H) were released back into the nearest forest from their collection site. Despite the female being a greenish morph, it gave birth to four greyish neonates; a similar condition was also observed earlier in another clutch (N=7) born on 25 September 2010 to a captive greenish adult male and female. These neonates were released after 151 days (23 February 2011) and by this time they had attained the greenish colour of the parents (Romalsawma, pers. obs.).

In some species of snake, it is not uncommon for neonates and juveniles to be a different colour from older life stages (e.g. green tree pythons, green cat snakes and king cobras). In some cases this has been correlated with differences in habitat preferences between the life stages. Further study would be of interest, especially to determine for *A. prasina* what the ecological correlates of this change might be.

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Remains of a female Kemp's ridley sea turtle *Lepidochelys kempii* found on the north Somerset coast of Britain

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Kemp's ridley sea turtles *Lepidochelys kempii* are the world's rarest and most endangered marine chelonian (Wibbels & Bevan, 2018). They are found primarily in the Gulf of Mexico, Caribbean, and in warmer Atlantic waters but have long been known to visit British and European waters as vagrants (Ernst & Barbour, 1989).

On 17 December 2021, the remains of a Kemp's ridley were recovered by the author from the strandline of Kilve



Figure 1. Carapace of a Kemp's ridley sea turtle washed up on the north Somerset coast **A.** Anterior view showing strong keel **B.** Dorsal view.



Figure 2. Ventral view of a Kemp's ridley sea turtle washed up on the north Somerset coast- **A.** Plastron, **B.** Cloaca and short tail (indicating a female specimen)

beach, Somerset, on the southern side of the Bristol Channel (51° 11'26" N, 3° 14'21" W). The specimen weighed 371 g and had a carapace with a distinct keel (Fig. 1A). The carapace was 215 mm long and 195 mm wide (Fig. 1B); adults have a carapace length of at least 550 mm, making this a young specimen. The plastron was 160 mm long with a left and right row of four inframarginal scutes, each with a pore (Fig. 2A). Some soft tissue remained including the tail and cloaca, indicating relatively recent mortality. The tail did not extend past the posterior carapace margin indicating that the turtle was female (Fig. 2B).

Storm Arwen and associated weather patterns appear to have been responsible for the arrival of this species in British waters; in December 2021 a live specimen was also washed up on a beach in north Wales (Baynes, 2021). The current record was submitted to Somerset Amphibian and Reptile Group on the day of discovery. It has also been submitted to the Marine Conservation Society stranding database. Fewer than 75 specimens have been found since records began in 1748. The closest previous Kemp's ridley record was on the northern side of the Bristol Channel and a little further west at Tresilian Bay in 2012, making the current record the deepest known penetration of the Bristol Channel (NBN, 2021).

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Partial xanthism in the mesoamerican cane toad Rhinella horribilis

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Pigmentary abnormalities occur when there is an absence or predominance of certain pigment cells (chromatophores) or variations in the production of pigments within them (Duellman & Trueb, 1994). In amphibians, the most frequent cases of abnormal pigmentation are albinism and leucism, which result from deficient production of melanin (Lunghi et al., 2017). Albinism is a genetic disorder characterised by partial or complete lack of skin and eye pigmentation (Bechtel, 1995) and xanthism, the subject of this report, is a type of albinism in which individuals produce predominantly yellow pigments (Stephenson & Drace, 2014).

The toad *Rhinella horribilis* Wiegmann, 1833, is distributed from the south of Texas (USA) to the north of Peru, with a western distribution to the Andes mountains (Acevedo et al., 2016). Dorsal coloration varies from light to dark brown with or without yellowish, orange or reddish tones. The toads may have scattered black or dark brown spots on their backs and on the tips of their tubercles and on large red warts (Ron, 2018). This note documents the first record of partial xanthism for *R. horribilis* in Colombia.

During a local fauna observation walk, at 18:56 h on 18 August 2021, in the municipality of Ituango, Department of Antioquia, Colombia (7.16666° N, 75.76186° W; 1534 m a.s.l.), an adult *R. horribilis* was observed, which presented an unusual coloration. From the parotoid glands to the middle region of the back, colouration was largely orange, consistent with partial xanthism (Fig. 1). In the same area, 23 other adults were observed but these all had colouration that is typical of this species.

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Figure 1. Partially xanthic adult *Rhinella horribilis* at Ituango, Department of Antioquia, Colombia

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Predation on the common house gecko Hemidactylus frenatus by an Asian hermit spider Nephilengys malabarensis in Sri Lanka

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he common house gecko *Hemidactylus frenatus* is a relatively small (SVL 51 mm) arboreal lizard. It is native to south-east Asia with a widespread distribution in the Pacific islands; it has also been introduced widely elsewhere by anthropogenic means (Rodda, 2020). There are numerous records of various species of spider predating lizards (Bauer, 1990; Koch & Arida, 2017; van Blerk et al., 2020; Tripathy & Priyadarshini, 2022), including Hemidactylus sp. (Purkayastha & Sourav, 2011; Lanschi & Ferreira, 2012; Turner, 2018). However, these have not included the Asian hermit spider Nephilengys malabarensis (Araneae: Nephilidae).

We report the first observation of *H. frenatus* predation by N. malabarensis (Fig. 1A). The observation was made in a house at Peradeniya (Kandy District), Sri Lanka (7.26704° N, 80.58407° E). There was no vegetation close to the web of the spider which was built around 60 cm from the floor on a staircase open to the natural surrounding. At 10:34 h on 26 July 2018, we observed an adult female N. malabarensis feeding on a dead H. frenatus, without its tail, that was entangled in her web (Fig. 1B).

Diniz (2011) has already reported nephilid spiders as predators of Hemidactylus sp. in Brazil. The frequency of this in Sri Lanka remains unrecorded.

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Figure 1. A female Asian hermit spider Nephilengys malabarensis feeding on a common house gecko Hemidactylus frenatus, at Peradeniya, Kandy District, Sri Lanka

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