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Front Cover: The lovely leaf frog *Phyllomedusa venusta* from Colombia photographed by José Pérez-González, see article on p. 14.

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Effects of different heat and light sources on the behaviour of captive reptiles

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ABSTRACT – Reptiles are sensitive to electromagnetic radiation at wavelengths between the ultra-violet and infrared bands. Husbandry practitioners designing artificial lighting arrays should aim to provide spectra that approximate those to which a given species has become adapted in nature. This would satisfy biological needs ranging from thermoregulation and vitamin D_3 photobiosynthesis to vision and communication. We evaluated the spectral outputs of two artificial lighting arrays (A & B) in their ability to provide this. Array A was designed to replicate those used as standard over the last decade in many controlled environments, while the Array B was intended to provide a more solar-like output. We measured behaviour and calculated a modified Spread of Participation Index (mSPI) for three species of reptile (turtle, lizard and snake) as the arrays were alternated in an A-B-A design (in observation periods 1, 2 and 3). Data were analysed using randomisation to deal with small sample size and repeated measures. Although in theory Array B provided a better spectrum than Array A, there were no clear patterns where behaviour and mSPI shifted going from Array A to B then reverted when going from Array B to A. However, the lizard and turtle both showed unexplained behavioural shifts, and the lizard an associated mSPI shift, when going from Array B to A. Our results demonstrate the power of combination lighting arrays to better approximate solar spectra, but also that behavioural observations may not reflect theoretical implications. We suggest reasons for this and directions for future research.

INTRODUCTION

Reptiles kept under controlled environmental conditions, such as in zoos and private collections, are typically maintained using artificial heating and lighting as they are held outside of their natural habitats but are dependent on conditions found therein (Baines et al., 2016). Being ectotherms, reptiles are particularly sensitive to the electromagnetic spectrum, and especially wavelengths from and between ultra-violet B (UVB) and infrared C, to which they are exposed. Under controlled conditions, current herpetocultural thinking holds that artificial lighting should replicate the solar spectra to which a species is exposed in the wild (Silvestre, 2014). Benefits of this may include, inter alia, the ability to reach and maintain a suitable body temperature (Tosini & Avery, 1996), to maintain metabolic processes such as vitamin D_3 photobiosynthesis (Baines et al., 2016), as well as appropriate vision and circadian rhythms. Vision is, in many species, sensitive to light outside the spectrum visible to humans (Vergneau-Grosset & Péron, 2020).

Thermo- and photo-sensitive behaviours are intrinsically coupled such that the two often occur in tandem. That is, the exposure to different intensities of light via behavioural means often dictates, to a large extent, the body temperature of an ectothermic animal. During the day, warmth and brightness are frequently correlated so that in nature direct sunlight is the primary creator of basking zones. Thermoregulation can be achieved via behavioural or physiological means (Seebacher & Franklin, 2005; Tattersall et al., 2006). Moreover, all solar wavelengths are important for warming tissues, although some more so than others (Barolet et al., 2016). In many diurnally active reptile species this is often most clearly exhibited by basking in direct sunlight to increase body temperature or retreating to cooler or less intensely irradiated microhabitats to decrease body temperature (Carrière et al., 2008), as well as retreating to dark areas for sleep or rest.

Wavelengths of light of 780–4,000 nm are in the infrared spectrum and occur naturally in sunlight. Infrared-A (IR-A), from 780–1,400 nm, is relatively short wavelength radiation that provides a larger amount of energy when absorbed compared to longer wavelengths of infrared. Along with visible light, it is the primary source of energy for basking species. Red light and IR-A radiation are transmitted through the skin and directly into the deeper subcutaneous tissues, giving more rapid bodily warming than solarinfrared-B (IR-B) (Barolet et al., 2016). The epidermis is heated most intensely by visible light and IR-B radiation, with 60–70 % of incident sunlight being absorbed at this level, compared to 10–20 % for the underlying muscle (Porter, 1967). With these wavelengths, much of the inner heating of the deeper tissues occurs through conduction of heat from the skin. Lower energy wavelengths, in the infrared-C (IR-C) (3–1,000 μm) range, which are not components of natural sunlight, are less able to penetrate the core of objects but do constitute a relevant source of heat as they are naturally radiated from the surface of sun-warmed objects in the environment. Warmed objects may also transmit heat energy by conduction

(Thomas et al., 2019), which enables thigmothermic species to warm themselves by direct contact with objects that are warmer than themselves (Garrick, 2008).

Radiation at wavelengths of 380–780 nm are classed as human-visible light (Kasajima, 2019), however biologically relevant wavelengths of light are not confined within this range. The time a lizard spends basking has been shown to depend on the wavelengths supplied, with basking duration increasing when wavelengths <540 nm are removed (Tosini & Avery, 1996). It is possible that all wavelengths from ultraviolet to infrared may be important to any given reptile species, and specific requirements may be linked to activity patterns and microhabitat preferences.

In the case of UV radiation, terrestrial wavelengths of UVB range approximately 290–320 nm, while UVA wavelengths are somewhat longer at 320–400 nm (Baines et al., 2016). Of the terrestrial UV wavelengths, those in the shorter ranges are more closely associated with vitamin D_3 synthesis in the skin (Lindgren et al., 2008). Furthermore, important physiological feedback mechanisms require in addition short wavelength UVA ranging from 320–335 nm. A review of the relevance of UV light to reptiles is provided by Baines et al. (2016). UVA, from around 350 nm, and visible light are important for most reptile species to enable visual processing of information, vital for essential intra-specific communication and identification of food items (Govardovskii & Zueva, 1974; Honkavaara et al., 2002; Fleishman et al., 2011). In recent years an increasing number of species have been shown to utilise markings visible in the UV spectrum, with most hypotheses for function favouring selective communication (Prötzel et al., 2018; Thompson et al., 2019; Badiane & Font, 2021; Mendyk, 2021).

A wide array of potential heating and lighting technologies are available commercially and each is associated with different emission spectra, none of which in isolation resembles the spectrum of natural sunlight. Available technologies include mercury vapour (MV), fluorescent, incandescent, light-emitting diode (LED), and metal halide lamps (MH), designed specifically for reptile husbandry and/or adapted from domestic or industrial use. All these technologies have often radically different output spectra.

Self-ballasted MV lamps have traditionally been used to provide basking zones for reptiles in controlled conditions (Baines et al., 2016; Thomas et al., 2019). These lamps emit a mixture of UVA and UVB as well as visible light and IR-A and -B. Fluorescent T5 tubes are also commonly used to provide UV as well as a relatively small amount of visible light. However, most of their visible light is distributed between discontinuous peaks rather than over a continuous and broad range, as is found in unfiltered sunlight (Baines et al., 2016). Therefore, these lamps alone cannot be relied upon to provide light similar to unfiltered sunlight. Incandescent lamps, which include halogen lamps, emit predominantly short-wavelength infrared (IR-A) with a very small proportion of UVA, moderate levels of visible light (predominantly red) and some IR-B and IR-C, in a continuous spectrum (MacIsaac et al., 1999). These are therefore well-suited as basking lamps but are lacking in UV and provide only low intensity visible light. LED lamps, which are typically designed to emit light that appears white to humans, currently provide no safe UV or infrared but can provide a broad spectrum of high illuminance in the human-visible range. Metal halide lamps (unless of the type designed for use with reptiles) produce no significant UVB but do often produce moderate UVA and visible light distributed continuously up to around 750 nm. Details of the spectra of specific lamps are often available for download from the manufacturers' websites and may be of particular interest to herpetoculturalists.

Combining different lighting technologies in composite lighting arrays is being increasingly considered best practice for reptile husbandry (Griffiths & Kane, pers. obs.). These composite arrays can provide overlapping spectra that better replicate natural sunlight. Each lighting component is chosen to complement the spectra of the others to provide wavelengths across as much of the spectrum of natural sunlight as practical, providing increased physiological and behavioural potential for reptiles. However, little peer-reviewed published evidence exists to demonstrate the efficacy of combining lighting arrays to replicate solar spectra.

It is important to underpin husbandry practice with evidence, but there have been only a few studies looking at the impact of theoretical best practice on reptiles in captivity (Ferguson et al., 2010; Schmidt et al., 2010; Ferguson et al., 2021). Without evidence, heating and lighting strategies risk falling into the compass of 'folklore husbandry' (Arbuckle, 2013). Physiological evidence (Ferguson et al., 2010; 2021) may provide insight into the impact of lighting strategies on specific biological processes, such as vitamin D_3 production, but is not available for the majority of captive collections of reptiles, and for most taxa. Behavioural observations are readily collected and may provide a broad brush insight into the impact of lighting strategies, and therefore can be more easily applied to a range of species. In the present work we seek to assess the efficacy of two different lighting arrays at replicating a solar spectrum and to evaluate any behavioural effects these arrays may have on three phylogenetically and ecologically disparate reptile species. Our experimental hypothesis was that there would be observable changes in reptile behaviour when a heating/lighting array providing a relatively poor sunlight spectrum was switched to an array with better sunlight replication and vice versa.

MATERIALS & METHODS

Data were collected from January to April 2021 during which time London Zoo, a ZSL conservation zoo, was closed to the public due to the COVID-19 pandemic. An ethical review of the project was deemed unnecessary since it fell within the normal bounds of husbandry and the project was approved on this basis (internally registered as ZDZ149). Data were collected and recorded on an ethogram, adapted from Thomas et al. (2019) and provided in Table 1. Observations were made on three species of reptiles from three separate suborders: a spotted turtle *Geoclemys hamiltonii* (Testudines; Cryptodira), a blue tree monitor *Varanus macraei* (Squamata: Anguimorpha) and a pair of inland taipans *Oxyuranus microlepidotus* (Squamata: Serpentes) (Fig. 1A, C & E). These species were chosen as they can all be

Table 1. Ethogram of categorised behaviours and areas of the enclosures used in the present study

	Behaviour/Activity	**Definition**
Behavioural state	Locomotion	Animal moving anywhere within the field of view
	Basking	Animal stationary in the bask zone
	Resting	Animal stationary outside the bask zone
	Out of sight	Animal not visible in field of view
Area of enclosure	Basking site	Area of highest temperature and UVI, directly beneath bask lamps
	Elevated wall/branches	Area of enclosure above the floor, created by branches or vertical walls
	Floor	Terrestrial ground area of enclosure
	Out of sight	The area outside the field of view of the camera(s)

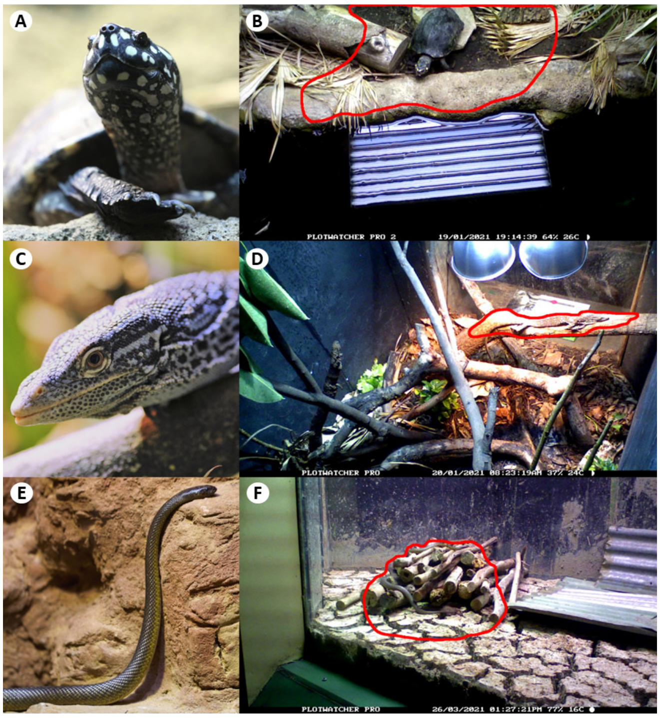

Figure 1. Portraits of the focal species and their enclosures - **A.** & **B.** *Geoclemys hamiltonii*, **C.** & **D**. *Varanus macraei*, **E.** & **F.** *Oxyuranus microlepidotus*. Basking zones for each enclosure are outlined in red.

generally categorised as diurnal species which, in captivity, seek out basking zones in order to thermoregulate and are known to be active throughout the year. This allowed for a representative view across phylogenetically distant taxa to investigate the effect different heating and lighting has on each species' behaviour.

We compared two primary lighting arrays (A & B) for the three species. Lighting Array A represented current standard practice in many reptile keeping institutions (e.g. Harding et al., 2017; Kane et al., 2021) while Array B represented a lighting approach aimed at better sunlight replication. The lighting arrays varied between species in the nature of the lamps used (Supplementary material Table S1) but in principle lighting Array A was self-ballasted MV lamps combined with T5-HO UVB fluorescent lamps, while Array B was incandescent lamps combined with T5-HO UVB fluorescent lamps and metal halide lamps. Spectrographs showing the different spectral qualities of these lighting arrays are shown in Figure 2. The lighting arrays were designed, as far as possible, to maintain identical surface and air temperatures and UV index (UVI) provision as well as thermoregulatory resource areas (basking zones) across treatments (see Table S1 in Supplementary material). Basking zone surface temperatures were measured with a non-contact infrared thermometer (GM320; Zanmax, USA) and UVI was measured with a Solarmeter 6.5 (Solar Light Company, LLC., USA).

The two lighting arrays (A & B) were applied alternately in an A-B-A design, each with a 10-day observation period, termed observation period 1, 2 and 3. In almost all cases, MV lamps and incandescent lamps were exchanged within the same fittings so that the position of the devices did not change. T5-HO lighting units also remained unmoved and metal halide lamps were installed prior to the study, but not turned on until observation period 2 of the study.

Spectrograms (Fig. 2) were obtained from individual enclosures under lighting Arrays A and B for each species using an Ocean Optics USB2000 + spectral radiometer with a UVB-compatible fibre-optic probe with cosine adaptor and a FLAME UV-Vis spectral radiometer with a UVB-compatible fibre-optic probe with cosine adapter (both Ocean Optics, Inc., Dunedin, Florida USA). Recordings of the arrays were taken in-situ with the fibre-optic probe perpendicular to the axis of the lamp at the approximate height of the different species whilst basking.

To record reptile behaviour, time-lapse video cameras (Plotwatcher Pro, Day6 Outdoors, USA) were placed in, but out of reach, or outside of each enclosure to have as full a view as was practical of the inside of each enclosure. Cameras were set to take a photograph every 30 seconds, but recording in a video format, during pre-set observation windows of 09:30–10:00 h, 13:00–13:30 h and 17:30–18:00 h. These observation windows were designed to capture diel behavioural changes, as reptiles usually display predictable changes in behaviour across the day (Tosini et al., 2001). Cameras were light activated so that when natural light penetrated the enclosures and enclosure lighting turned on, cameras automatically began recording. Natural light was always indirect and diffuse, though actual levels were not measured and likely some difference existed between

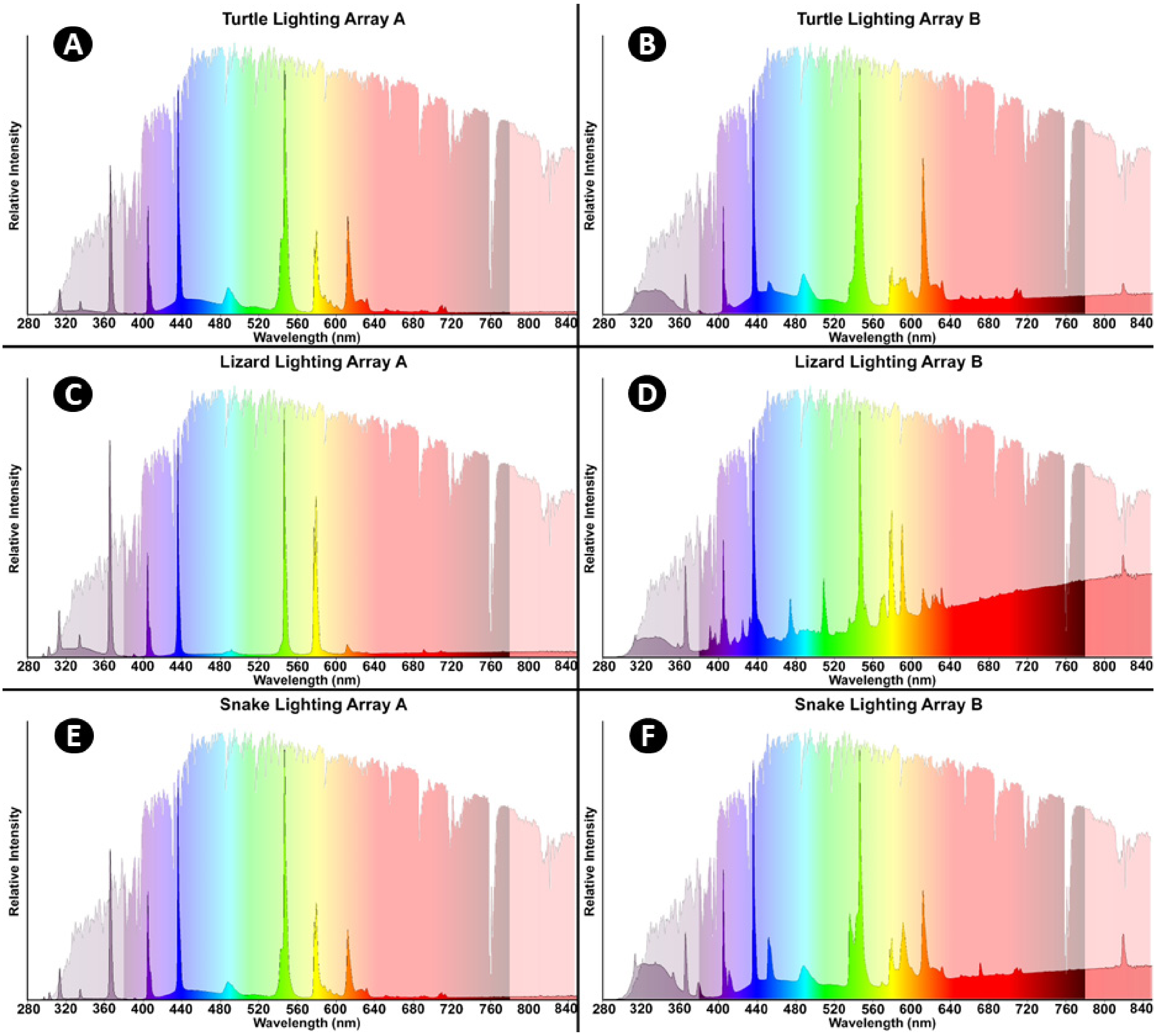

Figure 2. Spectral measurements of lighting arrays over basking areas during observation period 1 (lighting Array A) and observation period 2 (lighting Array B, intended to be a better simulation of the solar spectrum), for **A.** & **B.** the turtle, **C.** & **D.** the lizard and **E.** & **F.** the snakes. The solar spectrum ASTM standard provides a useful point of reference when visually evaluating the wavelengths generated by each lighting array. Relative intensity is presented with no units to aid visibility of the peak wavelengths of the corresponding lighting array used in this study, though all lamps show equal relative intensity on the scale. The spectral plot of the lighting array and the solar spectrum ASTM standard (ISO 98451) are not to scale.

enclosures. Cameras were installed one day prior to data collection to allow the reptiles to habituate to their presence, and the following ten days of footage was recorded for each of the three observation periods. Ten days was viewed as an acceptable time period to capture relevant behaviours while not extending data collection across a large amount of environmental change, which could influence results. This yielded a total of thirty days of data per species. Media files were analysed in GameFinder Module software (V.1.7.2, Day6 Outdoors, USA) as this software is able to display the file in a time-lapse video format. Each reptile enclosure was divided into functional resource areas following Thomas et al. (2019) and Plowman (2003), allowing different behaviours, such as basking and resting, to be effectively defined and recorded (Fig. 1B; 1D; 1F). Functional resource areas for all three species included a basking zone. In addition, for the lizard, non-basking zone (arboreal) and non-basking zone (terrestrial); for the snake, non-basking zone (terrestrial); and for the turtle, non-basking zone (terrestrial) and water. Only those observation windows when the reptile(s) were within the field of view of the cameras were included in analysis; this affected only the snakes, as the turtle and lizard were always visible for at least part of each observation period.

Reptiles were recorded as occupying a given resource when the largest proportion of their body was contained within that resource. If the reptile's body appeared to be positioned equally between multiple resources, then the resource that included the reptile's head was recorded; if the

Table 2. Results of primary one-way ANOVA-like randomisation analyses. Significant results (Bonferroni-adjusted P values) are indicated in bold

		Bonferroni-adjusted P value			
Species	**Analysis**	**mSPI**	**Locomotion**	**Basking**	**Resting**
Turtle	Primary (one-tailed)	0.057	**0.021**	**0.019**	>0.999
	Post hoc (two-tailed)	N/A	1 vs 2: 0.391 2 vs 3: **0.001** 1 vs 3: **0.001**	1 vs 2: 0.958 2 vs 3: **0.006** 1 vs 3: 0.248	N/A
Lizard	Primary (one-tailed)	**0.016**	**0.038**	**0.028**	>0.999
	Post hoc (two-tailed)	1 vs 2: >0.999 2 vs 3: **0.054** 1 vs 3: **0.007**	1 vs 2: >0.999 2 vs 3: **0.036** 1 vs 3: 0.688	1 vs 2: >0.999 2 vs 3: **0.002** 1 vs 3: **0.002**	N/A
Snake	Primary (one-tailed)	>0.999	0.065	>0.999	0.076
	Post hoc (two-tailed)	N/A	N/A	N/A	N/A

reptile was in line with the boundary between two opposing resources and equally occupied both, then the resource to the right of the reptile's head, from the perspective of the animal, was chosen. In almost every instance a resource zone occupied could be confidently assigned. Data were entered into Microsoft Excel for 365 (version 2111) and 10 % of the time lapse video footage spread across all three species was re-scored, and Spearman's rank correlation coefficients were used to assess reliability of observations.

The modified Spread of Participation Index (mSPI - Plowman, 2003) is an index derived from the proportions of time an animal spends in each resource-based zone of its enclosure. The index varies between 0 (even enclosure use, all zones used equally) and 1 (completely uneven use, only one zone used). This is a simple descriptor of how evenly the available space is used by a captive animal. Even enclosure usage is often sought-after, but ultimately interpretation is dependent on the specific context, especially with regards to the intended use of resource zones, and animals' behaviour and ecology (Plowman, 2003). We analysed behavioural counts and standardised for time spent in view by dividing counts of locomotion, basking and resting behaviour by the sum of counts of those three behaviours. mSPI was calculated using only in-view observations with a reasonable assumption that out of sight (OOS) reptiles could be in any part of the resource zones not in the field of view.

Data from individuals represent repeated measures, this violates assumptions around independence of the observations so that standard parametric and non-parametric analysis techniques were not appropriate. Although Generalised Linear Mixed Models (GLMMs) could have accounted for Poisson distributed behavioural count data, they could not control for repeated measures as with only three repeated units (species) showing different overall patterns, random effects could not be reliably estimated (Oberpriller et al., 2021). The most appropriate analytical approach was to use randomisation analysis, which makes no assumptions of independence or data distribution as it relies on internally generated probability distributions (Dugard et al., 2012). Initial analyses represented randomisation equivalents of one-way ANOVAs. Using the mosaic package (Pruim et al., 2017), experimental observation period labels (1, 2 and 3) associated with each data point were shuffled in order to randomly assign each observation to an experimental observation period. An F-statistic, which summarises the ratio of variation between and within groups, was then calculated for the shuffled data comparing the now randomised observation period labels to define groups, using the ANOVA function of the stats package (R Core Team, 2022). This was repeated for 9,999 replicates and used to create a null distribution, i.e. the distribution of F-statistics expected if the data were random rather than influenced by experimental observation period. The true observed F-statistic was then calculated from original data (making the 10,000th observation in the sample) and compared with the null distribution to calculate a p-value. (see Dugard et al. (2012) for further details). This process was conducted for each species and for each behaviour; Bonferroni corrections were applied to the resulting p values to correct for multiple comparisons. For species-behaviour combinations that yielded a significant corrected p-value, we then conducted post-hoc pair-wise randomisation comparisons with the same method, but using the difference between treatment means as the test statistic instead of an F ratio, which is appropriate when only two groups are compared (Dugard et al., 2012); these comparisons were also subject to Bonferroni corrections. All analyses were performed using R version 4.2.0 in RStudio 2022.02.02.

RESULTS

Spectrograms were taken of the basking zones in each enclosure (Fig. 1) under lighting array A. Discrete peaks of irradiance were emitted by lighting array A in all three enclosures, and are visible in the spectrographs (Figs. 2A, C & E) whereas the use of lighting array B provided more continuous spectra (Figs. 2B, D & F). The relatively high intensity of continuously distributed light shown in the lizard enclosure under lighting array B (Fig. 2D) is likely due to the physically close positioning of the two incandescent

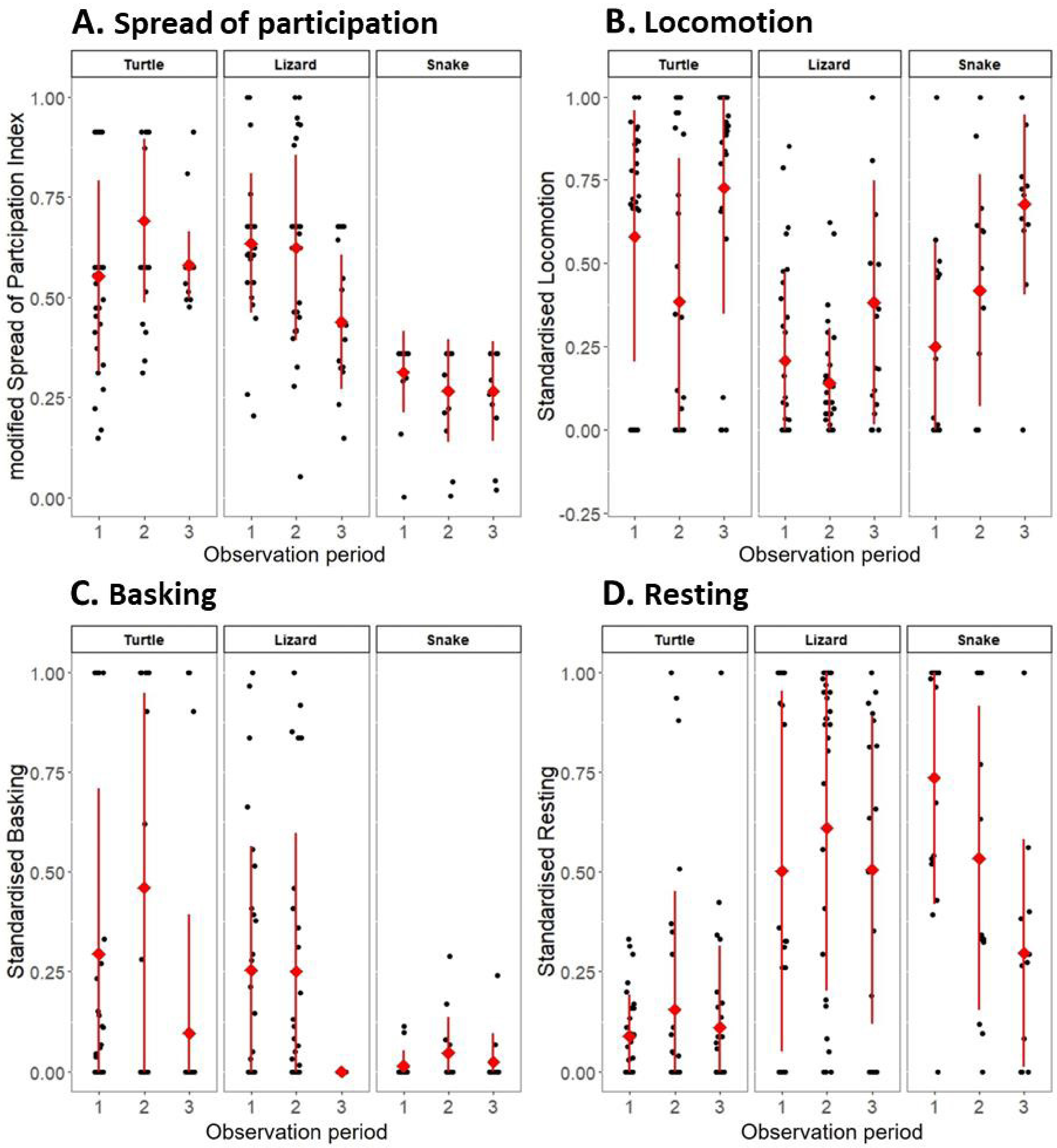

Figure 3. Behavioural responses of the three focal reptile species across three observation periods 1–3. Standardised square root counts of – **A.** modified Spread of Participation Index, **B.** Locomotion, **C.** Basking and **D.** Resting. Black points represent actual data, red diamonds indicate means and red bars show standard deviations.

lamps to the basking zone. This resulted in a relatively larger proportion of the light emitted by these lamps being recorded by the spectrometer compared to that emitted by lighting array B in the other enclosures, where they were positioned physically more distant from the basking zones. Spearman's rank correlation coefficients were greater than 0.9 for all four response variables comparing original and re-scored data, indicating good reliability in data collection.

For the turtle, analyses detected significant effects of observation period on locomotion and basking behaviour, for the lizard significant effects on mSPI, locomotion and basking behaviour, but for the snakes there were no significant effects of observation period on any of the four parameters (Table 2, Fig. 3A–D). Post-hoc pairwise tests for both the turtle and the lizard demonstrated a significant increase in the proportion of time spent in locomotion, and a significant decrease in the proportion of time spent basking. A significant decrease in mSPI (i.e. more even enclosure usage) was observed in the lizard, during observation period 3 (Table 2). Mean and standard deviation values are presented in Supplementary material Table S2.

DISCUSSION

The enhanced lighting (Array B) achieved the aim of improving spectral coverage and intensity of represented wavelengths across more of the solar spectrum, with associated increased brightness. However, as demonstrated in Fig. 2, these wavelengths were not fully representative of those found in unfiltered sunlight expected in the natural range of the focal reptile species. Array B did provide a measured improvement over Array A, but there is a need for more technological development if relative amounts of the wavelengths found in natural, unfiltered, sunlight are to be better replicated. The addition of full spectrum LED lights could help achieve this, as well as raise the overall brightness of enclosures. We encourage future work to incorporate this principle, as well as encourage more field study of the light environment in the natural habitat of these three species and other captive species of interest. While no two solar spectra will be identical, in general, far more continuous provision of light when compared to artificial lighting arrays is expected. Due to equipment shortage there was an unavoidable decrease in measured basking zone UVB under the lizard Array B lighting (Supplementary material Table S1) however the overall spectrum still showed fuller coverage of key wavelengths compared to Array A (Fig. 2C & D).

Our results show that the turtle and the lizard spent a greater proportion of visible time in locomotion during the third observation period at the expense of basking behaviour (Fig. 3B & C). For the lizard this was associated with more even use of the enclosures (Fig. 3A).

Our experimental hypothesis was that a change in behaviour may be induced by the more complete solar-like spectrum of lighting Array B compared with Array A. The interpretation of behavioural changes in response to lighting changes is challenging, due to the complex interactions of physiology, physics, social and species-specific behaviour, as well as external factors. At least theoretically, each species should be able to reach an optimal core body temperature more quickly when exposed to lighting Array B. This is due to the shorter wavelengths of light more effectively heating the reptiles' core, thus increasing time available for non-basking behaviours. Alternatively, attraction to basking sites with richer spectra might cause an increase in basking behaviour. In previous work, reduced spectra in a basking zone, containing only wavelengths > 480 nm, was shown to increase basking duration and lower heating rate of lizards (Tosini & Avery, 1996). Additionally, research on captive *V. macraei*, including the individual in the present work, showed that lighting arrays with a greater reliance on infrared B radiation also affected the durations of basking and other behaviours (Thomas et al., 2019). In the present study, our results were unexpected; with no changes in mSPI when going from array A to array B for all three species. However, changes were noticed when going from array B to array A for the turtle and lizard, with no changes at all for the snakes. This is hard to interpret and highlights the difficulty of using behaviour to evaluate artificial lighting arrays for reptiles.

The snakes present the simplest data for interpretation as no behavioural effects were detected across the experiment. Correcting for the higher proportion of observations that were spent out of sight, the overall proportion of visible time spent basking showed that the snakes spent less of their visible time basking than did the other two species. Being observed to be more cryptic than the other two species, for thermoregulation the snakes may have relied more on conducted rather than on radiated heat, or had preferred body temperatures closer to the ambient temperature than did the turtle or the lizard and so were less sensitive to the quality of the basking lamps. Additionally, the snakes were housed together whereas the turtle and lizard were housed individually, so it is possible that social interactions may have influenced the observed behaviours.

For both the turtle and the lizard, reductions of time spent engaged in basking behaviour were detected on return to Array A with corresponding increases in locomotion. This pattern does not fit that expected under either our null (no difference) or alternative hypothesis (B is better than A) and is difficult to explain. Also, we are unable to determine whether changes in locomotion are a result of changes in basking behaviour, or vice versa. The reduction in basking and increase in locomotion is particularly striking for the lizard and is suggestive of an unidentified process also influencing behaviour. By collecting data from the same three time periods and restricting data collection to one season, the timing and experimental design controlled for, as far as was feasible, external disturbance (as in Carter et al., 2021), and seasonal and diel changes in thermobiology (Secor & Nagy, 1994; Díaz & Cabezas-Díaz, 2004; Arribas, 2010; Millar et al., 2012; Zamora-Camacho et al., 2013; Amadi et al., 2020) but other potential uncontrollable changes intrinsic or extrinsic to individual reptiles are diverse. For example, social behavioural effects were not controlled for and there is no literature available assessing the relative impact of social compared to solitary behaviour for the focal reptile species. Therefore, given that we do not understand the drivers behind the pattern of behavioural changes observed, it is difficult, too, to interpret the change in mSPI seen in the lizard. Although usually used to imply better welfare (Michaels et al., 2020), in this case mSPI changes were likely driven by the same forces that drove increased locomotion and should probably not be used to infer better welfare.

The phylogenetic and ecological differences between our chosen study species, along with the desire to collect data that were comparable between species, meant that the ethogram used to record data may not have been fine scale enough to capture all behaviours relevant to each species. For example, the blue tree monitor spent much time climbing whereas the turtle did not. However, the turtle did spend much time swimming whereas the snakes spent much time beneath cover. It may also be expected that, for example, reptiles which spend much time in trees would have a higher daily exposure to light than would a reptile which spends much time below ground. Intrinsic behavioural differences such as these may present an unavoidable bias during data collection. This is because the amount of time spent in each resource zone, due to factors not experimentally manipulated, may differ due to certain life history traits. By generalising, for example, any movement as 'activity' we were not able to differentiate between locomotory behaviours as different as climbing, swimming, mating or feeding. Future more focused data collection with a greater sample size containing more closely related species would likely reveal any such more subtle effects. Additionally, there was a potential bias towards the basking zones as the cameras were set up so that 100 % of this zone was in view, at the expense of other zones. In reality, this bias existed across all treatments so should not be a dramatically limiting factor, though there may have been a slight, unavoidable, inflation of the proportion of time spent basking. This is because the data analysed was the proportion of visible time spent performing the behaviours, and as all of the bask zone for each species was in the field of view, this behaviour could not have occurred out of view, whereas other behaviours could have.

Overall, our data demonstrate that incorporation of a wider range of different light sources in lighting arrays allows an improvement in the ability to replicate expected solar spectra under artificial conditions and highlights the importance of multi-parameter assessment of lighting arrays, and of viewing heating and lighting as two intertwined parts of one aspect of captive care. However, our behavioural data showed that animal responses to theoretically improved conditions may not be as predicted, and that substantially more research is required to better link theoretical ideas underpinning modern herpetoculture. It is essential to understand the differing methods, context and requirements for artificial provision of heat and light for reptiles. However, we suggest that an important starting point in this endeavour is the ecologically appropriate provision of heat and light. As with any change in methodology, it is important to continually monitor physiological and behavioural measures of health of reptiles maintained under novel conditions to assess any change. Reporting results such as these is important to avoid positive publication bias which may go on to influence further study. However, despite the absence of fully interpretable data, we suggest that reptile keepers should aim to create lighting conditions that are as close as possible to natural sunlight.

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SHORT NOTE

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Helminths associated with the threatened Alcatrazes snouted treefrog *Ololygon alcatraz*

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ABSTRACT - *Ololygon alcatraz* is a threatened treefrog endemic to a single small island in Brazil. Research on ecological interactions for this species is scarce, and nothing is known about parasitism. Thus, we investigated the presence of macroparasites of the gastrointestinal system of *O. alcatraz*. We found only two types of helminth, nematodes and acanthocephalans, both in the larval stages. Our results suggest that individuals of *O. alcatraz* are part of these parasites' life-cycle, using them as paratenic or intermediate hosts.

INTRODUCTION

Amphibians are hosts to various parasites, with helminths being the most studied among them (Herczeg et al., 2021). Despite recent progress in understanding helminth communities in neotropical amphibians, knowledge is still scarce when considering the remarkable diversity of amphibians in this region, which is the highest in the world (Campião et al., 2015).

Amphibians have been recognised as definitive, intermediate or paratenic hosts of helminths (Herczeg et al., 2021). Depending on several factors, such as parasite prevalence, immune condition (Clayton, 2005) or life stage of the amphibian (Holland et al., 2007), some helminth species have the potential to cause diseases (Poynton & Whitaker, 2001). Amphibians are the most threatened group of vertebrates, with diseases being one of the major contributing factors alongside habitat losses and other synergetic effects (Hof et al., 2001; Becker et al., 2007; Fisher & Garner, 2020). Consequently, it is crucial to investigate the diversity of parasites and their interactions with amphibians, especially in the case of threatened species.

This study has documented the occurrence of helminths in *Ololygon alcatraz*, an insular Brazilian anuran that completes its life cycle exclusively within bromeliads (Brasileiro, 2008). Currently, *O. alcatraz* is categorised as Vulnerable in the national Red List (MMA, 2022), and there has been a captive breeding program for it since 2009 (Lisboa et al., 2021).

Ololygon alcatraz is endemic to Alcatrazes Island, a single small island covering 135 hectares located on the northern coast of the State of São Paulo, Brazil (24° 06' S, 45° 41' W). Despite its restricted range, the population is abundant and occurs throughout the entire island (Brasileiro, 2008). In 2016 we caught 15 individuals on the island that were used here to investigate the diversity of parasites in comparison to 15 captive-born individuals (F1 generation).

MATERIALS AND METHODS

The frogs were euthanised with isoflurane inhalation anesthetic (CFMV, 2013). We used scalpel and tweezers to assess the body cavity via a longitudinal ventral incision. We examined the stomach and intestines using a stereomicroscope (Nikon Smz 460); then we counted the parasites and recorded the site of infection. We fixed parasites in 70 % EtOH solution and placed them in Petri dishes for sorting. We applied chloridric carmine to stain acanthocephalans and clove oil (eugenol) to diaphanise them. For nematodes, we used lactophenol to clear them (adapted from Amato et al., 1991). We analysed morphometric, morphological and photomicrograph data of the helminths under an optical microscope (Leica DM-2500) with phase contrast system equipped with computerised image analysis V4 LAS (Leica Application Suite). We identified the helminths based on morphological traits (Amin, 1987; Anderson et al., 2009; Gibbons, 2010). We deposited voucher specimens of helminths in the Coleção Microbiológica de Genética de Biomas Brasileiros, São Paulo State, Brazil (SCP 294 - SCP 295).

We calculated ecological descriptors of parasitism: prevalence (% of the *O. alcatraz* population parasitised), mean abundance (MA) - total number of parasites divided by the total number of anurans examined, and mean intensity of infection (MII) - total number of parasites divided by the total number of anurans parasitised (according to Bush et al., 1997). We estimated MA and MII with bias-corrected and accelerated bootstrap intervals with 2000 replications, using the Quantitative Parasitology on the Web (Reiczigel et al., 2019). Mean values were expressed with standard deviation and 95 % confidence intervals.

OBSERVATIONS AND DISCUSSION

We observed two types of parasites infesting the gastrointestinal system of *O. alcatraz*: nematodes

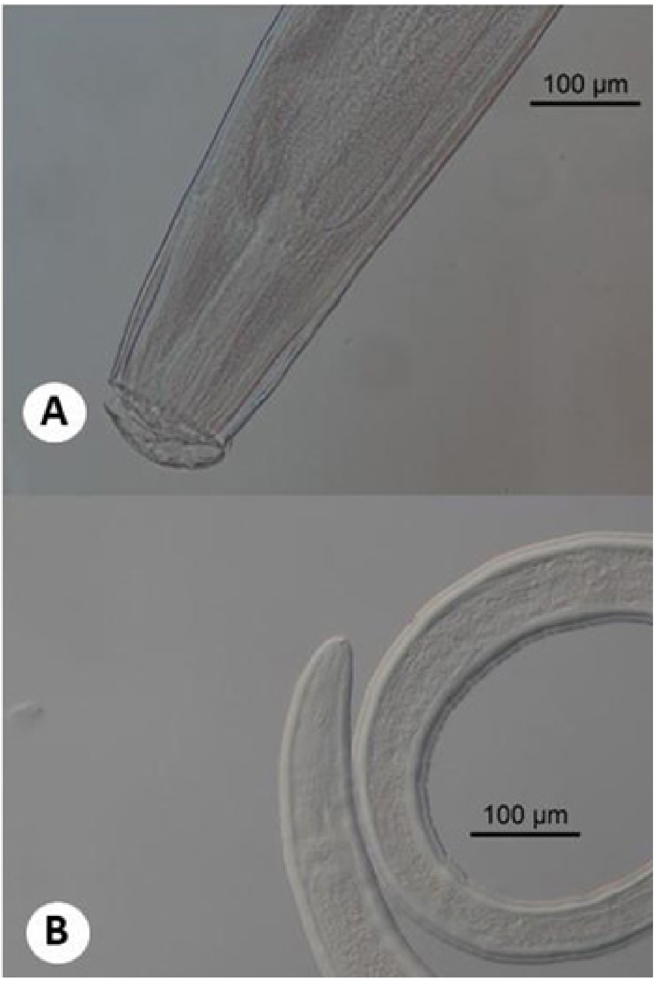

Figure 1. Encysted larva of an acuariid nematode – **A.** anterior end, **B.** caudal region

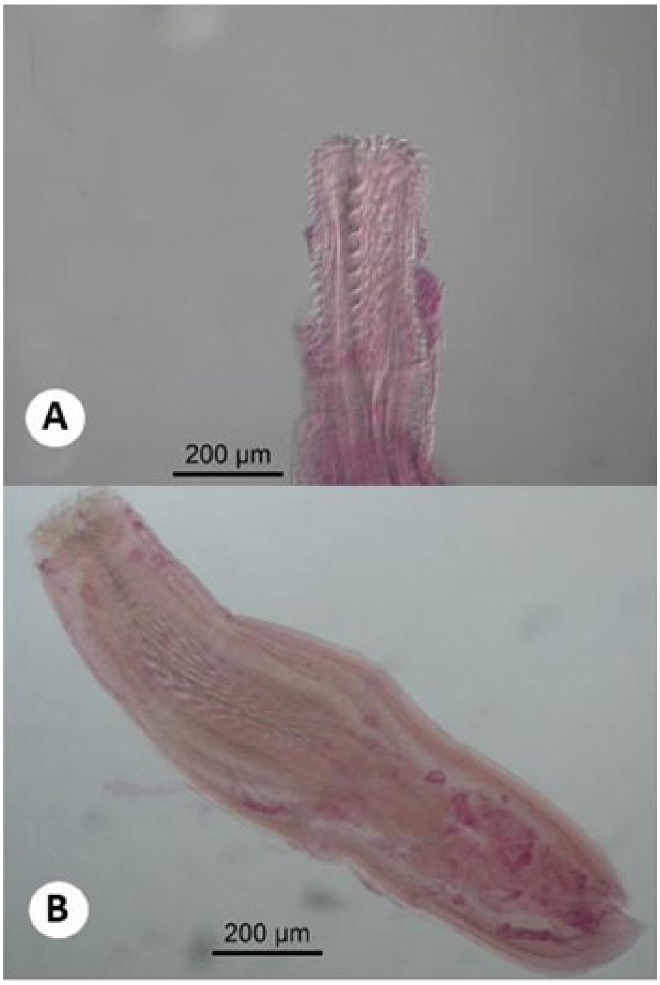

Figure 2. Cystacanth of the echinorhynchid acanthocephalan – **A.** proboscis detail, **B.** total view

(Acuariidae) (Fig. 1) and acanthocephalans (Echinorhynchidae) (Fig. 2), both in larval stages. Captive individuals, however, did not present any parasites. From the 15 wild-caught individuals of *O. alcatraz*, ten were infested with at least one type of parasite, resulting in an overall prevalence of 66.7 %, and three had both parasite species (prevalence of 30 %). Nematodes were exclusively found inside the stomach, while acanthocephalans were collected from the stomach attached to its external wall, and also found in contact with the body cavity (Fig. 3). The prevalence of nematodes was 46.7 %, the MA was 1 ± 1.41 (0.4 – 1.8) and the MII was 2.14 ± 1.35 (1.29 – 3.14). In comparison, the prevalence of acanthocephalans was 40 %, the MA was 0.47 ± 0.64 (0.13 – 0.73) and the MII was 1.17 ± 0.41 (1 – 1.5).

Our results show that wild individuals of *O. alcatraz* are prone to gastrointestinal parasitism with a high prevalence of both nematodes and acanthocephalans. On the contrary, captive-born individuals were free of parasites. Both nematodes and acanthocephalans are common in amphibians, especially nematodes that normally have a higher prevalence than any other helminths (Poynton & Whitaker, 2001; Aguiar et al., 2021), as observed in this study for *O. alcatraz* as well. In this study the species richness of helminths was low, this may be a reflection of the small body size of *O. alcatraz* (Campião et al., 2015) and/or by the fact that the species inhabits an isolated island habitat (Poulin, 2004), although we cannot rule out the fact that our results are based on only a small sample. The overall prevalence of helminths in the population of *O. alcatraz* was high but the abundance and intensity of

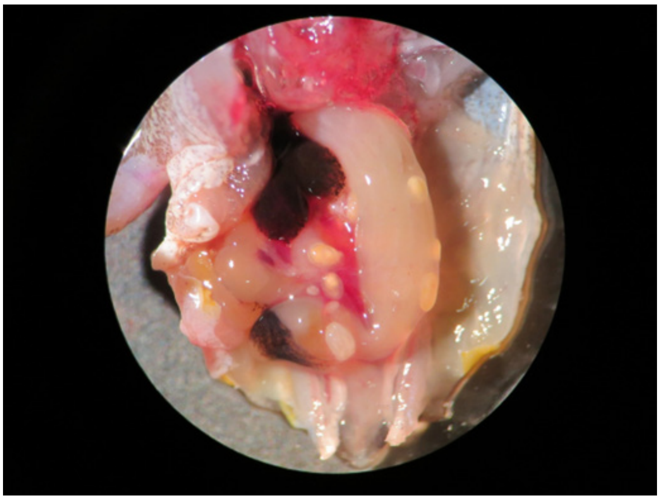

Figure 3. The gastrointestinal system of a necropsied *Ololygon alcatraz* with nematode larvae and acanthocephalan larvae encysted *in situ*

infection of each parasite species were low. Insular effects can also influence some of these parameters by increasing (Aguiar et al., 2020) or decreasing them (Roca et al., 1999); however, as this species is endemic to one very small island there are no populations of this species elsewhere to which they could be compared.

As the helminths were observed only in immature stages it was not possible to identify them to genus and species (Aguiar et al., 2021). Amphibians frequently serve as intermediate or even paratenic host for larval helminths life-stages (Campião et al., 2015), as these animals eat arthropods that carry infective larvae and are prey for several vertebrates where the life-cycle of the parasite can be completed (Poynton & Whitaker, 2001). Acanthocephalans of the Echinorhynchidae family always need at least one intermediate host (heteroxenic life-cycles), and the definitive hosts are usually fish and, sporadically, amphibians and reptiles (García-Varela & Andrade-Gomez, 2021). The nematodes of the Acuariidae family also have heteroxenic life-cycles but their definitive hosts are mainly aquatic birds (Schramm et al., 2018).

The types of parasites we found in *O. alcatraz* may be explained by the vertebrate diversity of Alcatrazes Island which consists of amphibians, reptiles (snakes and lizards) and seabirds, while mammals are absent (ICMBio, 2017). Some seabirds and the snake *Bothrops alcatraz* are potential predators of *O. alcatraz*. Thus, these anurans could be part of the parasites' strategy to complete the life-cycle in an ecosystem with an abundance of birds and snakes. The absence of these parasites in captive individuals suggest that the cycles of these two parasites have been interrupted, however, we emphasise the need for further studies to confirm the dynamics of the parasites' life-cycles both in nature and in captivity.

Our study presents the first record of helminths infecting *O. alcatraz*. Our findings suggest that individuals of *O. alcatraz* serve as an intermediate or paratenic host of these helminths (Loiseau et al., 2017). Overall, this study highlights the significance of basic science in describing the interactions between organisms and underscores the necessity for further research to understand the consequences of these interactions for both organisms. The knowledge gained from this research could help support conservation management practices for *O. alcatraz* and other threatened species.

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SHORT NOTE

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New records of the lovely leaf frog *Phyllomedusa venusta* from Sierra Nevada de Santa Marta, Colombia with a description of the female release call

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ABSTRACT - *Phyllomedusa venusta* (Anura: Hylidae) is known to have a distribution in lowland areas of the Caribbean region, Magdalena Valley and northern Pacific coast of Colombia. We have discovered new records of *P. venusta* from the Sierra Nevada de Santa Marta that expand its known distribution. Additionally, we describe the release call of a female *P. venusta*, which consists of two series of notes of low intensity. The first has three consecutive notes and the second has five notes. The duration between the notes was measured to be 0.36 seconds ± 0.31 and there was a time interval of 8.12 seconds between the two series.

INTRODUCTION

The genus *Phyllomedusa* Wagler, 1830, currently has 16 species (Frost, 2023) that are distributed from southern Mexico, extending throughout Central America, through the Andes, the Amazon rainforest of Brazil and as far south as Uruguay (Faivovich et al., 2009). *Phyllomedusa* species are also found on the Caribbean Island of Trinidad and Tobago (Downie et al., 2013). In Colombia, there are currently five recognised species of *Phyllomedusa*: *P. bicolor* (Boddaert, 1772), *P. coelestis* (Cope, 1874), *P. tarsius* (Cope, 1868), *P. vaillantii* (Boulenger, 1882) and *P. venusta* (Duellman & Trueb, 1967). *Phyllomedusa venusta* was described based on morphological differences and has been assigned to the *P. tarsius* group (Barrio-Amorós, 2006; 2009).

Phyllomedusa venusta is distributed in western Venezuela (Infante et al., 2006), Colombia (Ruíz Carranza et al., 1996) and Panama in the Darién (Duellman & Trueb, 1967), at 0 to 1250 m a.s.l. (Acosta-Galvis, 2023). It is an arboreal species associated with tropical dry forest, although it can also be found in humid forest and degraded forest patches (Renjifo et al., 2004). Individuals are usually found roosting at heights between 1–3 m, similar to other species of the Phyllomedusinae (Dias et al., 2017). During the breeding season, individuals tend to group near water bodies, making some nests of eggs above the riparian vegetation; while in the dry season it is most common to find individuals dispersed within the habitat (Mendoza-Roldan, 2017). Male *P. venusta* have an advertisement call with extremely low intensity, composed of a single or a sequence of pulsed notes (Bezerra et al., 2021).

Phyllomedusa venusta has been reported in various departments of Colombia, including Antioquia (Restrepo et al., 2017), Bolívar (Mendoza-Roldan, 2017), Boyacá (Ovalle-Pacheco et al., 2019), Cesar (Mendoza-Polo & Ochoa-Ramos, 2007; Rueda et al., 2008), Caldas (Acosta-Galvis, 2006), Córdoba (Romero & Lynch, 2012), Chocó (Lynch & Suárez, 2004), Cundinamarca (Duellman, 1974), Magdalena (Ruthven, 1992; Angarita et al., 2015), Santander (Bezerra et al., 2021), Sucre (Acosta, 2012) and Tolima (Acosta, 2000). Mendoza-Polo & Ochoa-Ramos (2007) mention the presence of the species in the foothills of the Sierra Nevada de Santa Marta (SNSM) based on an auditory record, which requires confirmation. Ruthven (1922) also mentions the presence of *P. tarsius* in the municipalities of Fundación and Aracataca in the department of Magdalena, but we cannot corroborate that it is *P. venusta* because there is no museum voucher specimen. The current study provides confirmation of the presence of this species in the SNSM (Fig. 1) and contributes new records to the known distribution of *P. venusta* in Colombia.

OBSERVATIONS AND DISCUSSION

During expeditions between June and August 2022, we observed *P. venusta* in three localities on the north-western flank of the SNSM. The localities include Río Piedra, Fundación, Department of Magdalena, Colombia (10° 31’16.3’’ N, 74° 04’14.0’’ W, 176 m a.s.l.) and the Arhuaca indigenous communities of Kankawarwa, vereda La Cristalina, municipality of Fundación, Department of Magdalena (10° 27’0.2’’ N, 73° 58’35.4’’ W, 295 m a.s.l.) and Umake, vereda el 50, municipality of Fundación, Department of Magdalena (10° 30’2.67” N, 73° 49’42.99” W, 1050 m a.s.l.) (Fig. 1).

Individual *P. venusta* were found on the vegetation by small ephemeral streams, perching at heights ranging from

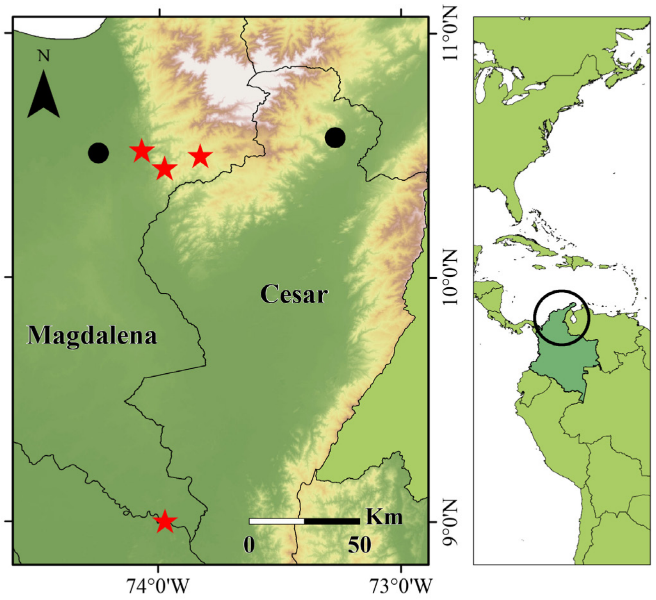

Figure 1. *Phyllomedusa venusta* records for the foothills of the SNSM; red stars = confirmed records with museum voucher to Departamento del Magdalena; black dots = unconfirmed records

Figure 2. Habitat of *Phyllomedusa venusta* in the locality of Río Piedra at 176 m a.s.l. in the Sierra Nevada de Santa Marta, Colombia

50 cm to 150 cm above the ground (Fig. 2). We collected and sacrificed four individuals in a 2 % lidocaine solution, obtained muscle tissue samples which were stored in 96 % ethanol. The specimens were fixed in 10 % formaldehyde solution, stored in 70 % ethanol and deposited in the Biological Collections Centre of the University of Magdalena with accession numbers CBUMAG-ANF: 01278 Male SVL = 6.961 cm; 01282 Male SVL = 6.852 cm; 01312 Male SVL = 5.73 cm; 01402 Female SVL = 8.3 cm.

The voucher CBUMAG: ANF: 01402 corresponds to a female of *P. venusta* which is larger and more robust than the males (Fig. 3A). Furthermore, nuptial pads were present on all collected males (Fig. 3B) while absent from the female (Fig. 3C). Our findings confirm the presence of *P. venusta* in the SNSM (Fig. 4), extending its known distribution range by approximately 169 km from the nearest confirmed locality reported for Departamento del Magdalena in the literature, with a collection voucher UIS-MHN-A-0769 (Dueñez-Gómez et al., 2004).

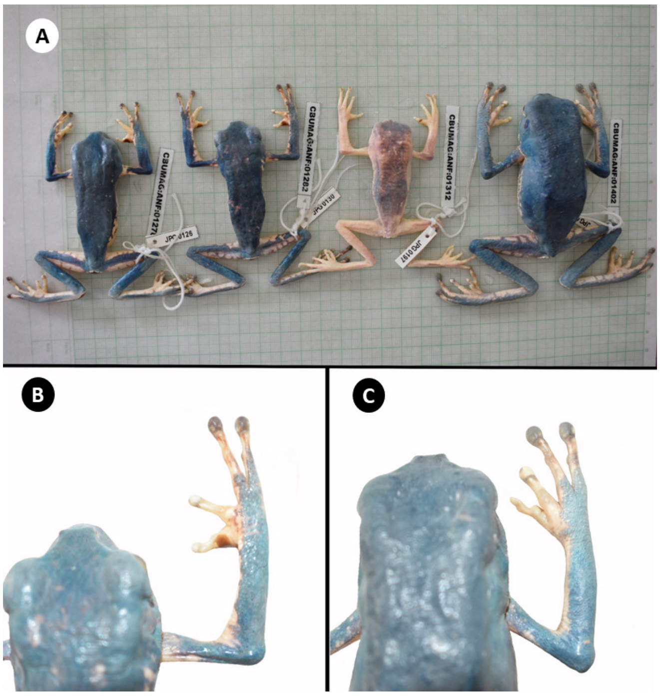

Figure 3. *Phyllomedusa venusta* CBUMAG voucher specimens – **A.** Three males and a larger female, **B.** A nuptial pad present on the first finger of a male, **C.** No nuptial pad present on the first finger of the female

During the fieldwork close to Umake, we recorded a release call of a female *P. venusta* using a Sennheiser ME66/K6 directional microphone connected to a Zoom H4N digital recorder. The female emitted this call when we attempted to manipulate her (Fig. 4A). The recording settings were at a sampling rate of 96 kHz and a resolution of 16-bit. All recordings were analysed using Raven Pro 1.5 software for Windows, following the method described by Köhler et al. (2017), with a FFT window size of 512 points. The female body temperature was recorded using an Extech thermohygrometer RH 101 and IR thermometer.

The release call was emitted at low intensity and consisted of two series of notes. The first series had three notes, and the second had five notes, with a time interval of 8.12 seconds between the two series. On average, each note had a duration of 0.12 seconds ± 0.05, with a dominant frequency of 1.02 kHz ± 0.17. The low frequency was 0.73 kHz ± 0.13 and the high frequency of 1.23 kHz ± 0.19. The duration between notes was 0.36 s ± 0.31 (Fig. 4B, Table 1). The recording of the female's release call was deposited in the sound collection of the Instituto de Investigaciones Alexander von Humboldt, Villa de Leyva, Colombia (AvH-CSA-36894). With the description of the female's release call, we are adding to the extensive vocal repertoire of this species (Bezerra et al., 2021), which will contribute to future studies on anuran communication.

Our records contribute to the richness of anuran fauna in the SNSM, which is considered one of the most important biodiversity hotspots in the world, and now having the

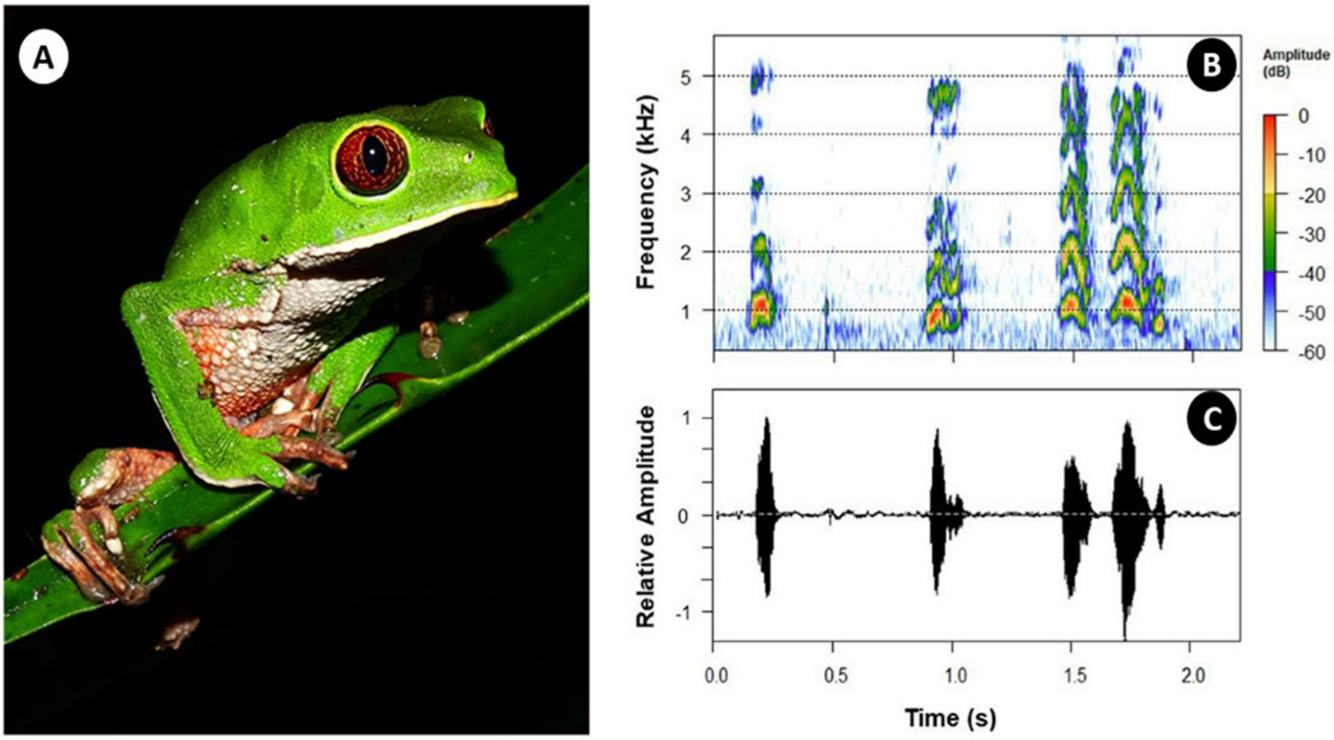

Figure 4. **A.** Adult female of *Phyllomedusa venusta* (CBUMAG: ANF: 01402, Female SVL = 8.3 cm), from the Umake locality at 1050 m a.s.l. in the Sierra Nevada de Santa Marta, and a recording of its release call – **B.** Spectrogram and **C.** Oscillogram

Table 1. Summary of the release call of a female *Phyllomedusa venusta* (shown in Fig. 3) from Umake locality in the Sierra Nevada de Santa Marta, Colombia – body temperature 28 °C

Call Series	Note no.	Note duration(s)	Dominant frequency (kHz)	Low frequency (kHz)	High frequency (kHz)	Inter-note interval (s)
1	1	0.16	1.21	0.89	1.47	0.78
	2	0.08	1.11	0.86	1.30	0.23
	3	0.10	1.14	0.8	1.35	-
2	1	0.10	1.05	0.72	1.21	0.62
	2	0.14	0.82	0.57	1.10	0.41
	3	0.12	1.04	0.68	1.30	0.07
	4	0.17	1.06	0.79	1.26	0.01
	5	0.03	0.70	0.53	0.83	-
Mean		0.12	1.02	0.73	1.23	0.36
SD		0.05	0.17	0.13	0.19	0.31
Min		0.03	0.70	0.53	0.83	0.01
Max		0.17	1.21	0.89	1.47	0.78

northernmost confirmed record of *P. venusta* in Colombia. *Phyllomedusa venusta* is classified as Least Concern (LC) on the IUCN Red List (IUCN, 2023). However, deforestation for crop and livestock expansion is increasing in the SNSM, negatively affecting the herpetofauna of the Colombian Caribbean coast, particularly *P. venusta*, which is a purely arboreal frog (Frost, 2023). We encourage conservation initiatives in the degraded habitat areas where *P. venusta* is found.

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SHORT COMMUNICATION

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A potential mimic of the venomous dipsadid snake *Philodryas olfersii*

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Mimicry is a defensive strategy that usually involves a harmless species (mimic) that emits the signals of a dangerous species (model) and thus deceives potential predators (Bates, 1862). This strategy is widespread across the animal kingdom, being common among snakes (Greene & McDiarmid, 2005). Some non-venomous snakes mimic venomous species because many predators actively avoid venom-injecting snakes (Pfennig et al., 2001). The best known case of mimicry in reptiles is that of the aposematic coral snakes in which the highly venomous brightly colored elapids (*Micrurus* spp.) act as mimetic models for other harmless snakes (Greene, 1981; Greene & McDiarmid, 2005). However, even cryptically coloured venomous vipers have been recognised as models for harmless species (Gans, 1961; Sazima, 1992; Wüster et al., 2004; Mebert, 2020). Viperid and elapid snakes have grooved or tubular fangs positioned anteriorly in the upper jaws that facilitate delivery of potent venoms during a bite, making them good candidates for mimetic models (Brodie & Brodie, 2004).

Here we suggest that an apparently cryptically coloured, rear-fanged dipsadid snake *Philodryas ofersii* is a mimetic model for a harmless snake. This is supported by the similarity of their dorsal colour pattern and geographic distribution. We observed and took photos of live specimens of *P. ofersii* and other sympatric green snakes. We also examined preserved snakes from the herpetological collections of the Instituto Butantan and the Universidade Federal do Rio Grande do Sul and noted their geographical origins. Additionally, we searched Google Images to determine whether or not the details of the colour pattern used here to support the mimicry hypothesis are also found in other green snakes elsewhere in the world.

Philodryas olfersii inhabits open and forested areas, occurring widely in South America from Colombia, French Guiana and Venezuela to Argentina and Uruguay (Nogueira et al., 2019). It is a slender, green and semi-arboreal snake, foraging both in vegetation and on the ground. This snake feeds on a variety of prey types, including birds (Hartmann & Marques, 2005; Sazima & Marques, 2007). Analysis of the gland secretion of *P. olfersii* has revealed the presence of the main toxin classes found in highly venomous viperids (Ching et al., 2006). The high toxicity of *P. olfersii* venom likely favours capture of birds, an important item of its diet (Sazima & Marques, 2007). This snake can easily bite humans resulting in severe pain and extensive oedema (Castro et al., 2021) and rarely even death (Salomão & Di-Bernardo, 1995). Thus, *P. olfersii* can inject its toxins into predators, making it a potentially dangerous species to prey upon.

Figure 1. Specimens of *Philodryas olfersii* (**A.** & **C.**) and *Erythrolamprus jaegeri* (**B.** & **D.**) – Northern forms (**A.** & **B.**) lack a vertebral stripe while the widely sympatric (see Fig. 2) southern forms (**C.** & **D.**) both have a brown vertebral stripe

Two distinct patterns of colouration can be readily recognized in *P. olfersii*. The first pattern has a dorsum that is completely green whereas the second pattern is also green but possesses a brown vertebral stripe (Arredondo, 2011; Marques et al., 2015; 2019) (Fig. 1). Individuals with brown stripes are distributed mainly south of 15º S while the uniformly green specimens occur mainly north of this latitude (Arredondo, 2011; pers. obs.) (Fig. 2).

Some South American green snakes such as *Erytholamprus jaegeri*, *E. typhlus* and *E. viridis* (Dipsadidae) occur in sympatry with *P. olfersii* (Marques et al., 2015; 2017; 2019; Nogueira et al., 2019). These terrestrial or semi-aquatic snakes are frog-eaters and do not have grooved teeth (Marques et al., 2015; 2017; 2019; Correa et al., 2016). Moreover, they are docile and do not bite when handled by humans (Borges-Martins et al., 2018; pers. obs.). Thus, these green snakes appear to be harmless to most potential predators. *Erythrolamprus typhlus*, *E. viridis* and the northern populations of *E. jaegeri* are uniformly or predominantly green (a few scales eventually have other distinct colours) (Souto, 2016; Nogueira et al., 2019). However, individuals in the southern populations of *E. jaegeri*

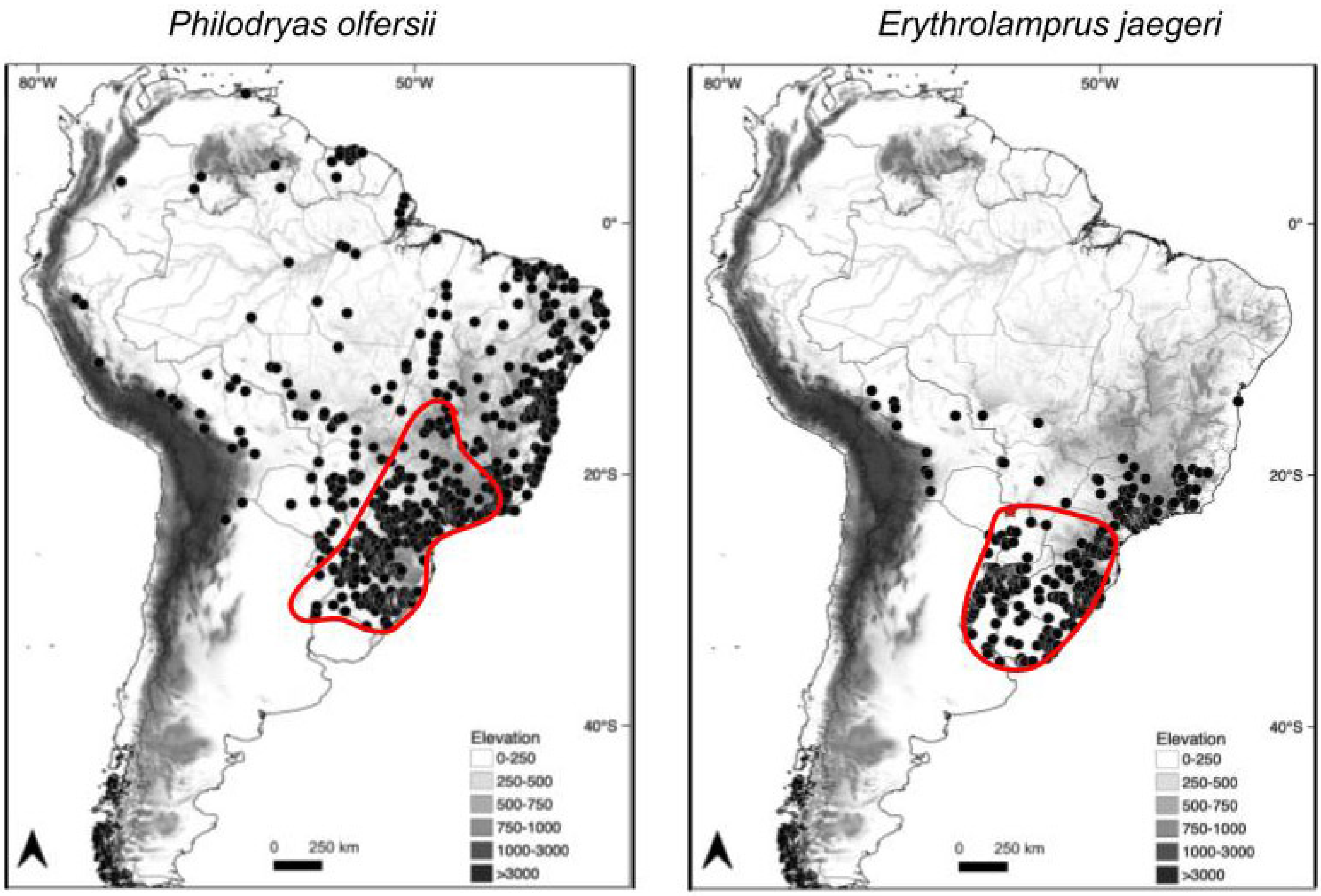

Figure 2. Distribution of *Philoryas olfersii* and *Erythrolamprus jaegeri*. The occurrence of specimens of both species with the brown vertebral stripe are delimited by the red line based on Arredondo (2012) and Souto (2016). Map adapted from Nogueira et al., 2019.

have a brown vertebral stripe similar to that of *P. olfersii* (Fig. 1) (Souto, 2016). Among specimens of *E. jaegeri* preserved in collections, part are almost uniform in colour with few dark-edged dorsal scales (in the northern range, < 25^{o} S) while others have uniform colour with or without a brown vertebral stripe (in the southern range, > 25^{o} S) (Fig. 2). This vertebral stripe was not detected in older preserved specimens. This may be due to the change in the colour of the snake in the preservative liquid, as the stripe was present in all specimens preserved in alcohol in the last three years as well as in all live specimens observed from the southern region. Souto (2016) suggests a taxonomic distinction between the southern and northern groups of *E. jaegeri*. Regardless of taxonomic problems, the wide area of sympatry of *P. olfersii* and southern populations of *E. jaegeri*, both uniformly green with a very similar brown vertebral stripe suggests the existence of a mimetic relationship between these two snakes. Although *P. olfersii* is a semi-arboreal species and *E. jaegeri* is semi-aquatic (Hartmann & Marques, 2005; Correa et al., 2016) potential predators of snakes roam these habitats and can be considered the selective agents where a mimicry complex can evolve. In this case, *P. olfersii* should be the mimetic model due to its potential to bite and inject its toxic venom into predators. Alternatively, a dark vertebral stripe may contribute to a countershading effect, which enhances camouflage by removing the cues associated with three-dimensional form (Donohue, 2020). If the brown vertebral stripe evolved to reduce the detectability by predators or prey, then the selective pressures may have operated independently in these two snakes, as a response to similar detectability processes. It is noteworthy, however, that our search revealed that a brown vertebral stripe is not a feature commonly seen in other green snakes, as in other species of *Philodryas* and *Erythrolamprus*, or in green species (which inhabit various habitats) in other genera, such as *Opheodrys*, *Oxybelis*, *Ahaetulla*, *Ptyas*, *Leptophis*, *Liochlorophis*, *Philothamnus*, *Dispholidus*, *Dendroaspis*, *Bothrops*, *Trimeresurus*, *Corallus* or *Morelia*. This rarity indicates that a brown vertebral stripe is not a common pattern in green snakes, which weakens the hypothesis of a disruptive/camouflage function. Conversely, the evolution of a similar and rare pattern in two different lineages of sympatric species – one of them with a highly toxic venom – seems to support the hypothesis of a mimetic complex.

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SHORT COMMUNICATION

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First record of the peninsula supple skink *Lygosoma peninsulare* in Thailand

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Figure 1. Dorsal view of *Lygosoma peninsulare* from Betong, Yala, Thailand

The peninsula supple skink *Lygosoma peninsulare* was described in 2018 from two specimens collected from Peninsula Malaysia (Grismer et al., 2018). The holotype was collected in 2016 in Kelantan state while a historical specimen was uncovered, originating from Bukit Larut (Boulenger, 1900; Grismer et al., 2018). The species is distinguished from other skinks on the Thai-Malay peninsula by having a robust yellowish-brown body, red tail, and distinctive dark eye patches. It is larger than other supple skinks on the peninsula with a maximum recorded snout-vent length of 119 mm. Despite its large size, the lack of records indicates its extreme rarity and very little is known of its natural history.

Herein we present the first record of *L. peninsulare* from Thailand (Fig. 1). On 6 April 2022 at 22:42 h, in Betong District, Yala, Thailand (5.85567˚ N, 101.25267˚ E, 606 m a.s.l.) during a herpetological survey in primary malayan mixed-dipterocarp rainforest, the authors were alerted to the presence of the lizard when a flash of red was seen leaping at the buttress of a large tree. The skink was jumping up towards a cicada nymph in the process of climbing the buttress, which it ferociously caught and swallowed. The skink was extremely fast-moving and capable of 'swimming' through leaf litter, rapidly, burying itself within ground debris.

A second specimen from Thailand was uncovered having previously been identified as *Subdoluseps bowringi*. A young individual (Fig. 2) was found on 22 November 2011 in a pitfall trap in the disturbed lowland forest of Khor Hong Hill, Hat Yai District, Songkhla, Thailand (7.02555˚ N, 100.51555˚ E, 240 m a.s.l.) by Jareeporn Pholmool. Although this forest is much drier than most in southern Thailand, we believe this species is supported through the dry season by the humid rivers and deep leaf litter found in its valleys.

Figure 1. Dorsal view of *Lygosoma peninsulare* from Hat Yai, Songkhla, Thailand

Knowledge of the ecology of *L. peninsulare* is very limited, we know that it is fossorial, spending most of its time under leaf litter, and will erupt out of the substrate to chase down invertebrate prey. It is nocturnal, corresponding with our observations of other *Lygosoma* sp. in Thailand. These observations show a surprising diversity of macrohabitat utilisation, between primary rainforest and disturbed seasonal forest.

The presence of *L. peninsulare* in Thailand is unsurprising, considering the holotype was found < 10 km from the border (Fig. 3), within the same continuous forest: the Belum-Temengor Forest Complex. This discovery takes the number of *Lygosoma* species present in Thailand to seven, although taxonomy remains under regular revision (Freitas et al., 2019). This adds to the growing body of herpetofaunal species being discovered for the first time in Thailand along the Malaysian border (Pawangkhanant et al., 2021) and demonstrates the need for further surveys in the region. We suggest the Thai common name: จิ้งเหลนเรียวมลายู (Jing Lhen Riow Malayu).

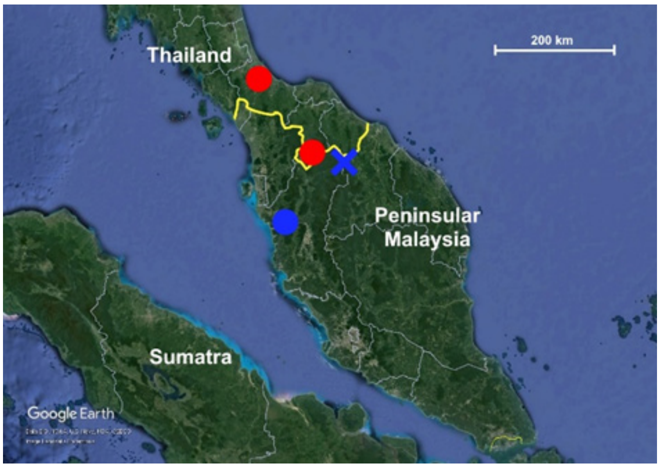

Figure 3. Records of *Lygosoma peninsulare* from the Thai-Malay peninsula. Red dots – records presented in this study from Betong, Yala and Hat Yai, Songkhla, Thailand; Blue symbols – records from Grismer et al., (2018) including the blue cross that designates the holotype locality.

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SHORT COMMUNICATION

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Hitchhiking freshwater clams: First report of *Sphaerium nucleus* attached to *Lissotriton helveticus* in France

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Phoresy is a specific form of commensalism where a mobile organism affords transport to a less mobile one (White et al., 2017). Many phoretic associations have been described (Clausen, 1976; Binns, 1982; Bartlow & Agosta, 2021) of which several examples involve freshwater molluscs; presumably because they have limited ability for long distance dispersal by themselves. Charles Darwin (1882) made pioneering observation of such dispersal in the freshwater mussel *Unio complanatus* attached to the toe of a duck, as well as supposedly *Sphaerium corneum* (then named *Cyclas cornea*) caught on the toes of newts (species not defined). Since then, several studies have reported phoretic associations between freshwater bivalves and lizards (Lopez et al., 2005), frogs and toads (Kolenda et al., 2017) and newts (Audibert et al., 2013).

Among amphibians, newts appear to be often used as 'hosts' by freshwater mussels for two reasons. First, mussels can attach easily to the toes of newts whereas attaching to the skin folds of anurans is more difficult (Kolenda et al., 2017). Second, newt species may move between ponds during the breeding season (Denoël et al., 2018) which would facilitate local mussel dispersal (Lopez et al., 2005). Consequently, there have been several reports of different newt species carrying small freshwater mussels of the genus *Sphaerium*. For instance, *S. corneum* has been found attached to the toes of *Lissotriton helveticus* (Laza-Martínez et al., 2012; Audibert et al., 2013), *Triturus marmoratus* (Boissinot & Migault, 2016), *Ichthyosaura alpestris* (Audibert et al., 2013) and *S. nucleus* was found to be attached to *L. helveticus* and *Triturus cristatus* (Wood et al., 2008). The frequency of this phenomenon may differ widely between sites. For example, in a study in Luxembourg very high mussel densities were found at one site of around 3000 per m², where 23 % of the 161 captured newts had *S. nucleus* or *Pisidium obtusale* attached to their toes while occurrence was less frequent at two other sites where mussel attachment to newt toes was recorded on 3.6 % and 7 % of newts (Wood et al., 2008).

In this study, we report observations of *S. nucleus* attached to the toes of *L. helveticus*. We sampled newts on 15 April 2022 around 23:00 h in two small forest ponds in France – Pond 1 of about 26 m² (0° 25'27.85'' W, 46° 8.0'49.56'' N, 70 m a.s.l) and Pond 2 of about and 75 m² (0° 25'29.28'' W, 46° 8.0'45.24'' N). In addition to *L. helveticus*, in Pond 1 we observed (but did not capture) *Rana dalmatina* and *Alytes obstetricans* and in Pond 2 *T. marmoratus*, *Triturus x blasii*, *Bufo spinosus*, *R. dalmatina*, *A. obstetricans* and *Pelophylax esculentus*. Both ponds were host to several aquatic plant species. In Pond 1, we captured 37 *L. helveticus* (21 females and 16 males), out of which 8 (21.6 % of individuals, 5 females and 3 males) had clams attached to their toes (Fig. 1) and 13 had wounded toes (35.1 % individuals, 8 females and 5 males). Clams were attached to the toes of forelegs (4 females and 2 males) and hind legs (1 female and 1 male). All individuals had only one clam attached. In Pond 2 we caught 69 *L. helveticus* individuals (57 females and 12 males). Surprisingly, while this pond was situated a hundred metres from the first pond and free living mussels were seen at the bottom, we did not find clams attached to *L. helveticus* toes.

Figure 1. *Sphaerium nucleus* found on a female *Lissotriton helveticus* in a pond in the Deux-Sèvres department, France

Freshwater clams clamped onto newt toes were collected and subsequently identified as belonging to the *S. corneum* group, within which it is sometimes difficult to assign a species name based on morphological characters alone. The specimens sampled exhibited a general shell shape intermediate between *S. corneum* (Linneaus, 1758) and *S. nucleus* (Studer, 1820). However, the high porosity of the shells and the size and shape of the hinge teeth allowed these specimens to be assigned to *S. nucleus* (Kořínková et al., 2008).

To our knowledge, this is the first report of *S. nucleus* attached to *L. helveticus* in France although this clam species has been observed attached to *L. helveticus* in Luxembourg

(Wood et al., 2008). In the same geographic area of our observation (6 km from our study sites), the closely related *S. corneum* was found on larger newt species (e.g. Boissinot & Migault, 2016), while in other parts of France and Spain, *S. corneum* has been found on *L. helveticus* (Laza-Martínez et al., 2012; Audibert et al., 2013). Such observations highlight the fact that both *Sphaerium* species can attach to different newt species, regardless of their size. However, we emphasise the complex identification of species from the *S. corneum* group which also include a third species, *S. ovale* (Férussac, 1807), whose taxonomic validity is still debated (Prié et al., 2021). The morphologic similarities of these taxa may obscure the identification of, but also the true diversity of, the freshwater clam species involved in these phoretic interactions.

Our report suggests that phoresy may be site specific. Indeed, this phenomenon can either be rare (Audibert et al., 2013) or frequent (Wood et al., 2008) within a population and with variations between sites (Wood et al., 2008). The observed differences between the two ponds in the frequency of mussel attachment to newts may be explained by the density of the mussel populations (a parameter we did not assess) and of the presence of other amphibian species.

'Host' selection by phoretic bivalves, if any, still needs to be explored. Indeed, Boissinot & Migault (2016) have shown that in a pond where *L. helveticus*, *T. cristatus* and *T. marmoratus* co-occurred, only *T. marmoratus* was affected, despite the fact that *L. helveticus* and *T. cristatus* were more abundant. This suggests that 'host' selection may occur, a process for which chemical orientation of the phoront has already been assessed to play a role in frog and lizard 'hosts' (Lopez et al., 2005). Wood et al. (2008) suggest alternatively that newt species behaviours and habitat use, or simply toe size and structure, must also impact the likelihood of attachment of sphaerid mussels. As mussel attachment can cause damage to individuals toes (Wood et al., 2008), this makes the relationship appear to be parasitic, because the mussels benefit by being facilitated in dispersal while the newts are harmed. Indeed, such damage may interfere with oviposition (Wood et al., 2008) and eventually reduce newts displacements; however these potential disadvantages are likely to be temporary as newts are capable of limb regeneration (Scadding, 1981).

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SHORT COMMUNICATION

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Observation of scale organs on the head of the slow worm *Anguis fragilis*

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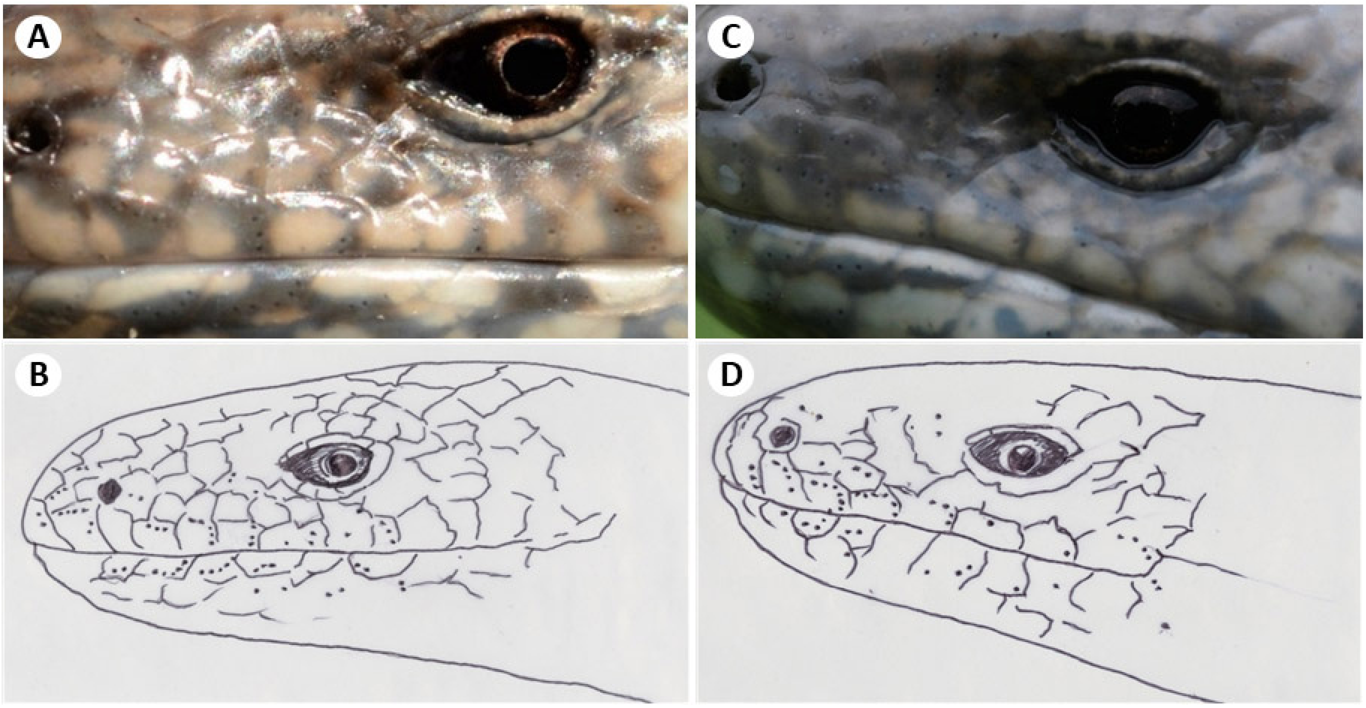

Figure 1. Lateral views of the heads of two male slow worms in pre-slough condition showing scale organs (black dots) – **A.** Close-up of part of the lateral view of the head of a male from Cheltenham, Gloucestershire and **B.** Tracing of the entire lateral view of A. to show the distribution of scale organs, **C.** Close-up of part of the lateral view of the head of a male from Hounslow, London, and **D.** Tracing of C. to show the distribution of scale organs

The existence of sensory structures, known as scale organs, in the skin of various kinds of reptile has been known since at least the second half of the nineteenth century (for example, Leydig, 1868). Of relevance to this note, their presence has been studied in at least three lizard families, namely Agamidae, Iguanidae and Gekkonidae, where the highest density of scale organs was found on the head, specifically on the frontal and lateral scales (Matveyeva & Ananjeva, 1995). In squamates more generally, the same tendency for a high density of sense organs to occur on the head is seen in the snakes *Leptotyphlops dilcis* and *L. munoal*, (Oreyas-Miranda et al., 1977). Receptor density on the head has been found to decrease from anterior to posterior and it has been suggested that this arrangement, in terms of functional ability, may be explained by the importance of the sensory fields surrounding the rostral part of the head, nostrils, eyes and ears (Matveyeva & Ananjeva, 1995).

The slow worm *Anguis fragilis* is a species of legless lizard belonging to the family Anguidae. Despite being widespread and locally common across much of Europe including Britain, many aspects of its biology remain poorly understood due to its cryptic nature, being largely fossorial and semi-fossorial and hence being most often encountered under refuges. Its occupancy of soil and leaf litter, and consequently its intimate contact with these media, would suggest that it might be well-provisioned with scale organs.

Evidence for possible existence of such scale organs in the slow worm was first noted by the author when reviewing a photograph of a large adult male in a pre-slough state briefly captured for photographic purposes near to Cheltenham, Gloucestershire, England on 11 April 2017. Detailed scrutiny of the image appeared to show many dark dots distributed mainly around the jaws of the slow worm, specifically mostly on the upper and trailing edges of the labial scales, although with others being scattered elsewhere on the head (Fig. 1A & B). The approximate diameter of each scale organ is estimated at between 50 and 100 micrometres. On 28 April 2023 an unusually large male slow worm was captured in Hounslow, London for biometric purposes and photographed; it was 454 mm long including an intact tail. Subsequent examination of this individual, also in a state of pre-slough, revealed a similar (but not identical) pattern of dots to the Cheltenham male (Fig. 1C & D). The similar distribution between the two individuals suggests that the structures were unlikely to be a result of pathology or other anomaly but could indeed be scale organs.

A subsequent casual internet search of sufficiently high-resolution images of slow worm heads has also revealed the presence of these putative sense organs, especially on large males in pre-slough condition. It is perhaps likely that the larger size of the male slow worm head makes it easier to resolve these structures (i.e. that this is a case of observer bias) and that closer examination may show them to be present also in females and the immature stages. Additionally, the pre-slough condition may make the scale organs easier to see, and hence explain why they appear to have been otherwise overlooked. As to their specific function, it is perhaps most likely in a fossorial or semi-fossorial species that the scale organs' primary purpose would be mechanoreception (this function has been shown in other lizard families – see for example Hiller, 1978) although other functions or even a multisensory function cannot be discounted. Further analysis of a wider sample of detailed photographs as well as photomicrography of preserved histological samples would be the next obvious steps in elucidating the precise nature of these structures. The author would be grateful for any further information relating to the presence of scale organs in slow worms and other anguid lizards at the correspondence address above.

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SHORT COMMUNICATION

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Attempted cannibalism by adult smooth snakes *Coronella austriaca* in France and Switzerland

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The smooth snake *Coronella austriaca* (Laurenti, 1768) is a small colubrid snake rarely reaching 90 cm in length (Speybroeck et al., 2016). It has a wide distribution in Europe, as far north as southern Scandinavia and in the south it reaches the Balkan, Iberian, and Italian peninsulas and the Mediterranean islands of Elba and Scilly (Speybroeck et al., 2016).

The diet of *C. austriaca* consists mainly of small mammals, insects, eggs and reptiles, including sometimes other snakes (Goddard, 1984; Rugiero et al., 1995; Reading & Jofré, 2013; Kolanek et al., 2020; Di Nicola et al., 2020; Escalante et al., 2023). The first evidence of cannibalism was collected from the study of faeces and stomach contents (Drobenkov, 1995; 2000; 2014; Luiselli et al., 1996). It was only recently that the first direct observations of cannibalism were reported from Poland between two juveniles (Kolanek & Bury, 2020) and between adults in England and Germany (Jofré & Reading, 2020; Rowland et al., 2021).

On 5 September 2020 at 17:47 h an adult male smooth snake was filmed attempting to subdue and ingest an adult female (BHS Video, 2023a). The observation was made on the edge of a mountainside forest in St Amarin town (47° 52′ N 7° 01′ E), near the Vosges mountains, on land surrounded by hedges, with wild and managed spaces. It was a sunny day without wind, the sky was partly cloudy. The snakes were on the cover of a gully pot containing water. The fight lasted 15 minutes where the male (estimated at 45 cm) tried to capture the head of the female with numerous bite attempts (Fig. 1). Eventually, the female managed to break free and moved away and the male went in the opposite direction. The environment seems conducive to snakes as others had already been seen in this location: two observations of *C. austriaca* (May 2015 and May 2019), and a barred grass snake *Natrix helvetica* in May 2009.

On 4 July 2023 at 19:50 h on a pedestrian path on the edge of the forest in Medeglia, Switzerland (46° 06′57″ N 8° 58′09″ E), two snakes were observed. It was a stormy day, the sky was periodically very cloudy, without wind, and temperatures approx. 18° C. The largest snake (approx. 60 cm) was biting near the neck of the second smaller snake (approx. 40 cm), probably trying to swallow it (Fig. 2). The victim coiled and squirmed, moving its tail rapidly in response to the attack, trying to free and defend itself (BHS Video, 2023b). As the snakes were on a path that was frequented by people and dogs the observer took the decision the move them some 15 m away. As a result, they stopped fighting, separated, and took refuge in a dry-stone wall. For this reason, we do not know the outcome of their interaction.

Figure 1. Attempted predation of an adult female smooth snake (*Coronella austriaca*) by an adult male – **A.** The male initiates the attack by grabbing the female in the tail area, **B.** Then in the middle of the body, **C.** In defence the female bites the male, and **D.** The female finally escapes (see BHS video, 2023a)

To the best of our knowledge, these are the first direct observations of attempted cannibalism between adult smooth snakes filmed in France and Switzerland. Jofré & Reading (2020) suggest that cannibalism in *C. austriaca* is a rare behavioural trait that can occur when an individual's body condition is low, possibly due to periods of food scarcity. At least in the case of the observation in France, it would seem to be a male attempting to eat a female. In European snakes, predation by males on adult females are known in other species such as the Montpellier snake *Malpolon monspessulanus* and the Aesculapian snake *Zamenis longissimus* (Glaudas & Fuento, 2022; Major et al., 2023).

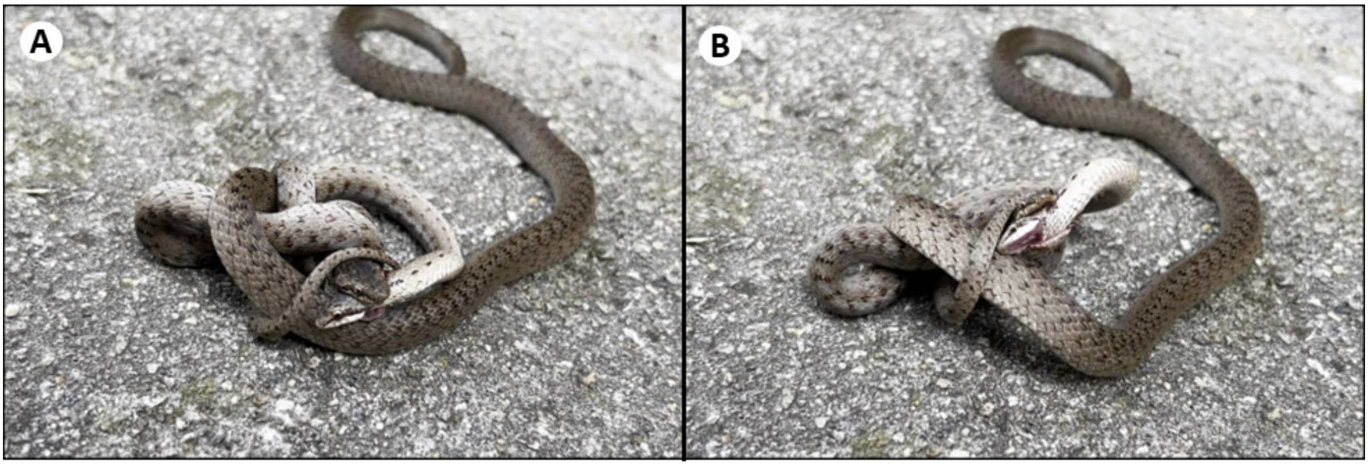

Figure 2. Attempted predation between two smooth snakes (*Coronella austriaca*) in Switzerland – **A.** The larger individual holds the smaller one around the neck, **B.** Then it attempts to ingest its victim (see BHS video, 2023b)

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Potential egg predators of the dwarf caiman *Paleosuchus palpebrosus* at the southern limit of the species' range in Brazil

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Egg predation poses a significant threat to small crocodilians that either lay few eggs or have few nesting sites (Amoah et al., 2021; Campos et al., 2015). This is the case for the dwarf caiman *Paleosuchus palpebrosus* that is known to lay only about 14 eggs per nest, in the mountains surrounding the Pantanal (Campos et al., 2022; 2015). This small clutch size is aggravated by a long incubation period, which in *P. palpebrosus* is 90 to 100 days (Medem, 1983; Magnusson, 1992); for most South American crocodilians it is around 60 to 70 days (Medem, 1983). Thus the nests of the dwarf caiman are exposed to a longer period in which they may be predated than nests of many other caiman species. Camera traps have been used previously to identify the potential predators of crocodilian eggs (Barboza et al., 2012; Campos & Mourão, 2014; Torralvo et al, 2017; Gonzáles-Desales et al., 2020) and to document the defense behaviour of females against egg predators (Campos et al., 2016).

Female *P. palpebrosus* remain close to their nests throughout the incubation period, often hiding in holes next to their nests (Campos et al., 2015). When in the proximity of potential predators females adopt a defensive posture, typically climbing on top of their nests (Campos & Sanaiotti, 2006). However, until this study, there was no photographic record of females taking care of their nests by streams around the Pantanal. This study reports the number of nests found in these areas during the nesting seasons of 2016 and 2017 and identifies the potential egg predators that visited these nests. Also, we recorded the behaviour of females attending their nests by the streams of the region Serra do Urucum. This area is located in the southern Pantanal, close to the southern limit of the species' distribution.

During the month of February in 2016 and in 2017 in the region Serra do Urucum, we searched on foot for *P. palpebrosus* nests in the riparian forests of the streams Pedras (19° 13'11" S, 57° 39'3" W), Banda Alta (19° 09'16" S, 57° 7'40" W) and Lajinha (19° 07'40" S, 57° W). The streams have narrow rocky bottoms and cold water, as described by Campos et al. (1995). In February 2016 and 2017, we installed digital camera traps, model Brushwell Trophy Cam, near four of the nests found each year. The cameras were programmed to take three photos at a time, triggered by motion sensors in continuous periods of 24 hours. The cameras were removed from the site after predation or egg hatching. In order to avoid any increase in predation rate that might result from our disturbance of the nests, we did not open nests and neither were eggs counted. The photos obtained were images of potential predators and/or defensive behaviour and nest opening by the female caimans.

Figure 1. Camera-trap photographs taken while monitoring the nests of *Paleosuchus palpebrosus* by streams of the southern Pantanal in Brazil – **A.** Nine-banded armadillo *Dasypus novemcinctus* on top of a nest, **B.** Female *P. palpebrosus* defends her nest against a feral pig *Sus scrofa*, **C.** Coati *Nasua nasua* on top of a nest, **D.** Female *P. palpebrosus* opening a nest

In 2016, we located two nests of *P. palpebrosus* in the streams of Pedras, one in the Banda Alta stream and another in the Lajinha stream. Nest 1 was attacked by a nine-banded armadillo *Dasypus novemcinctus* on 2 June 2016 at 00:05 h and again at 04:53 h, when it remained in the nest for 10 minutes (Fig. 1A; Table 1).

At nest 2, a nine-banded armadillo was photographed above the nest on 2 April 2016 at 03:24 h. A group of four capuchin monkeys *Sapajus cay* was recorded on 10 February 2016 at 14:21 h in the vicinity of the nest, one of which remained longer observing the nest, which until then had not been predated. The first appearance of the female caiman on top of the nest was on 11 February 2016 at 00:18 h, remaining there for 18 minutes. However, on 12 February 2016 at 23:09 h the camera recorded the presence of a nine-banded armadillo turning over the nest. In the photo, fragments of eggshells appear, indicating that possibly the nine-banded armadillo had access to the eggs. On 13 February 2016 at 03:11 h three or four pigs *Sus scrofa* were recorded close to the nest. The female *P. palpebrosus* was again recorded on

Table 1. Summary of the data obtained of potential predators visiting eight nests of *Paleosuchus palpebrosus* in streams of the southern Pantanal in Brazil, monitored using camera-traps during the nesting seasons of 2016 and 2017

Nest	Locality	Date	Time	Nest status	Recorded predators
1	Pedras	02-06-2016	00:05	predated	*Dasypus novemcinctus*
			04:53		
2	Pedras	10-02-2016	14:21	predated	*Sapajus cay*
		12-02-2016	23:09		*Dasypus novemcinctus*
		13-02-2016	03:11		*Sus scrofa*
		05-03-2016	09:42		*Sus scrofa*
		25-02-2016	02:15		*Nasua nasua*
3	Banda Alta	29-03-2016	06:46	predated	*Dasypus novemcinctus*
		30-03-2016	08:10		*Nasua nasua*
		30-03-2016	08:31–09:33		*Nasua nasua*
4	Lajinha	04-03-2016	00:04	predated	*Dasypus novemcinctus*
			21:04		
5	Pedras	27-04-2017	21:01	predated	*Procyon cancrivorus*
		03-05-2017	23:12		*Nasua nasua*
6	Pedras	30-05-2017	23:33	predated	*Dasypus novemcinctus*
7	Pedras	30-04-2017	04:38	hatched	
8	Pedras	25-04-2017	16:54	predated	*Dasyprocta azarae*
		27-04-2017	23:00		*Procyon cancrivorus*

5 March 2016 in the morning (09:42 h), defending her nest from the pigs (Fig. 1B). On 25 February 2016 at 02:15 h a coati *Nasua nasua* was recorded turning over the nest, which apparently had already been opened by previous predators (Fig. 1C).

Nest 3, located in the Banda Alta stream, began to be preyed upon by a nine-banded armadillo, recorded on the top of the nest on 29 March 2016 at 06:46 h. However, a group of four coatis were eating the eggs on 30 March 2016 around 08:10 h and again between 08:31 to 09:33 h; they completely destroyed the nest. Nest 4, in the Lajinha stream, was predated by nine-banded armadillos on 4 March 2016 at 00:04 h and returning at 21:04 h.

In 2017, five nests were found by the Pedras stream, and four of these nests were monitored by camera, resulting in photos of predators in three of these nests. Nest 5, supported by a dry trunk and the presence of termites, was preyed upon by a crab-eating raccoon *Procyon cancrivorus* on 27 April 2017 at 21:01 h, which remained for about 10 minutes on top of the nest. Coatis *N. nasua* also visited the top of this nest on 3 May 2017 at 23:12 h. Nest 6 was predated on 30 May 2017 at 23:33 h by a nine-banded armadillo *D. novemcintus* that remained on top of the nest for 10 minutes. Nest 7, after 105 days of incubation, the female was photographed opening the nest, moving her hind legs (Fig. 1D). Nest 8 was preyed on by an Azara's agouti *Dasyprocta azarae* and a crab-eating raccoon. The nest 9, which was not monitored using a camera trap, was not predated.

The predation rate of *P. palpebrosus* nests in the streams of region Serra do Urucum appears to be high, as seven of the nine nests monitored in this study were predated. The nests were visited by several mammal species including nine-banded armadillo, pigs, coati, Azara's agouti, lesser and giant anteaters, capuchin monkeys, raccoon and possibly others. The nine-banded armadillo was the most frequent in the nests, as it was photographed in six out the eight nests, followed by the coati, which visited three out the eight monitored nests. The photographic records of the nine-banded armadillo preying on the eggs were all at night, between 18:55 to 04:53 h, while the coati were diurnal between 09:31 to 14:14 h.

In the Amazon, nests of *P. trigonatus* are known to be predated by two species of Cingulata, the giant armadillo *Priodontes maximum* and the nine-banded armadillo (Campos et al., 2016). In the Pantanal, Campos & Mourão (2014) recorded both the nine-banded armadillo and six-banded armadillo *Euphractus sexcinctus* eating eggs of the Pantanal caiman *Caiman yacare*. Parental care is important in reducing egg predation and also in increasing hatching success among crocodilians (Lang, 1987). We recorded females protecting their nests against predators and *P. palpebrosus* is known to protect hatchlings for up to 21 months (Campos et al., 2012), one of the longest periods of parental care among crocodilians. Despite this it would seem that *P. palpebrosus* is unable to offer significant protection against egg predators.

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Defensive behaviour of the El Rincón stream frog *Pleurodema somuncurense* in Argentina

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Anurans exhibit numerous antipredator mechanisms to avoid predation. More than 30 behavioural categories (Kowalski et al., 2018) associated with various morphological and physiological modifications in body size, colour patterns and toxin production are known (Ferreira et al., 2019; Pedroso-Santos et al., 2020) although the most commonly reported are escape behaviours (active escape or fleeing) and death feigning (Toledo et al., 2011).

Reports of these behaviours often come from casual observations of individuals being manipulated in the field or laboratory. However, for more controlled observations there are techniques that can be used to trigger defensive responses in anurans, in particular simulations of predator attack such as hitting gently with sticks or with the forefinger on the head and/or the back (Green, 1988; Chiocchio et al., 2022), lightly pinching the head, arms and legs with blunt forceps (Blanchette & Saporito, 2016), by presenting a non-venomous snake to the frog (Villa, 1969; Toledo et al., 2011) or by using predator models (Bulbert et al., 2015).

Pleurodema Tschudi, 1838 is a Neotropical genus of leptodactylid frogs that currently includes 15 species. Three of them occur in southern South America (Faivovich et al., 2012; Ferraro, 2022): *P. bufoninum*, *P. somuncurense* and *P. thaul*, all within the *P. thaul* Clade. *Pleurodema bufoninum* and *P. thaul* are distributed in Argentina and Chile's terrestrial habitats and are categorised as of Least Concern (IUCN, 2016). *Pleurodema somuncurense* is an entirely aquatic microendemic species from the Argentinean Patagonia (Velasco et al., 2016) and is categorised as Critically Endangered (IUCN, 2016). *Pleurodema bufoninum* and *P. thaul* have well-developed lumbar macroglands, while *P. somuncurense* has a slightly protuberant patch of glandular tissue in this region (Ferraro et al., 2013). *Pleurodema bufoninum* and *P. thaul* exhibit deimatic anti-predator behaviour associated with their macroglands (Cei & Espina Aguilera, 1957; Kolenc et al., 2009; Ferraro et al., 2021). Deimatic behaviour includes four movements that the frog displays synchronically (Fig. 1A) – (i) 'crouching down' behaviour (Marchisin & Anderson, 1978; Toledo et al., 2011); (ii) rear elevation (Ferreira et al., 2019); (iii) body inflation (Noble, 1931) and (iv) eye-protection (Toledo et al., 2011). It was not known whether *P. somuncurense* also displayed deimatic behaviour, so we undertook in situ and ex situ experiments with adult and immature *P. somuncurense* to test for this.

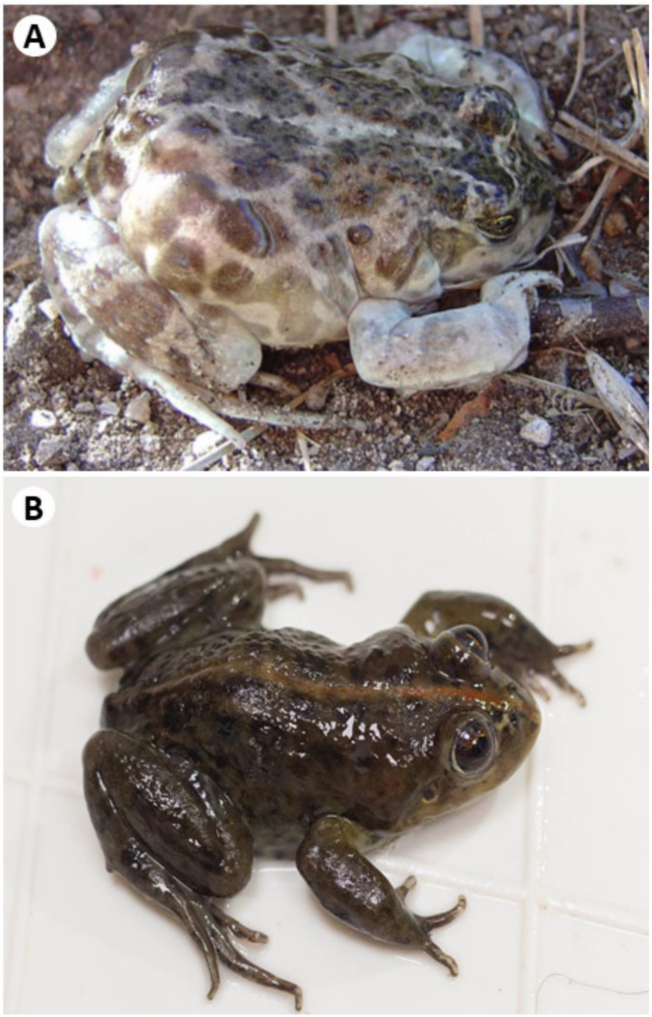

Figure 1. **A.** *Pleurodema bufoninum* showing deimatic behaviour, **B.** *Pleurodema somuncurense* not displaying deimatic behaviour following an ex situ test attempting to elicit a response

The in situ experiment was conducted in January 2015 in the Valcheta stream (Río Negro province, Argentina, 40.98° S, 66.62° W) at night, between 21:00–22:00 h. We captured individuals in their aquatic habitat and placed them on the ground, blocking their path to prevent their escape. Approximately two minutes later we delivered a mechanical stimulus by gently hitting the snout of the frog with a 20 cm stick three times and pressing the stick to the snout for five seconds. We examined 34 individuals of *P. somuncurense*: 13 males, 13 females and eight juveniles (less than 28 mm and without secondary sexual characters). The ex situ experiment was undertaken in 2020 with 30 adult individuals of *P. somuncurense* (without differentiating between males and females) obtained from a survival assurance colony at the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina. The study was the same as the in situ test but with the following differences: a) the individuals were removed from their aquariums and placed in a tray to prevent them from escaping and b) the testing was done during the day, between 12:00–13:00 h.

The in situ and ex situ experiments revealed similar results. None of the frogs showed any of the four defensive postures associated with deimatic behaviour, instead the *P. somuncurense* were quiescent or remained motionless (sensu Toledo et al., 2011) (Fig. 1B). However, during the fieldwork in the Valcheta stream (2013–2021), we observed that *P. somuncurense* displayed active escape or fleeing behaviour (Toledo et al., 2011; Ferreira et al., 2019) when approached or when a flashlight was shined on them. This behaviour involved submerging quickly into the water and hiding under the aquatic vegetation, occasionally also burying themselves in the mud at the bottom of the stream.

The absence of deimatic behaviour in *P. somuncurense* is probably related to its aquatic and nocturnal habits (Velasco et al., 2016). Deimatic behaviour is considered a plesiomorphic condition in *Pleurodema* (Faivovich et al., 2012; Ferraro et al., 2021). Consequently, the absence of deimatic behaviour in *P. somuncurense* is likely a secondary loss. This condition and the escape mechanism described herein differentiate *P. somuncurense* from the other two terrestrial species within the *P. thaul* Clade (i.e. *P. bufoninum* and *P. thaul*).

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SHORT COMMUNICATION

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First record of the Mizo rain snake *Smithophis atemporalis* from Bangladesh

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Bangladesh is known for its diverse ecosystems with 22 bio-ecological zones, including mangroves, wetlands, forests and rivers, which support a wide range of plant and animal species (IUCN, 2002). Among the bio-ecological zones of Bangladesh, the south-eastern hilly area is rich in wildlife resources, especially snakes (IUCN Bangladesh, 2015). Snakes play a significant role in ecological, environmental, economic and cultural sectors in Bangladesh (Jaman et al., 2020; 2021). However, the actual number of snake species is still unknown to us, and new species, including new distribution records, are being added (e.g. Hakim et al., 2020; Shome et al., 2020; Kajol et al., 2020; Mahamud et al., 2022; Barkat & Rabbe, 2022).

Smithophis is a natricine genus with four species that range from north-eastern India across north-eastern Myanmar to south-western China (Giri et al., 2019). Among the four species, *Smithophis atemporalis* Giri et al. 2019 has until now only been reported from Mizoram state (India) and has been assessed as a data deficient species (Fig. 1, Table 1)(Giri & Das, 2021). The only species previously reported from Bangladesh of this genus is *Smithophis bicolor* (previously known as *Rabdops bicolor*) from Alikadam, Bandarban, Bangladesh (Khan, 2015; 2018).

We present here a new country record of *S. atemporalis* from south-eastern Bangladesh close to the border with both India and Myanmar. On 20 October 2020 a single individual of this species (Fig. 2) was observed in a residence at one kilometre from Darjeeling Para towards Boga Lake (21° 57′51.4″ N, 92° 30′13.5″ E), Keokradong, Ruma Upazila, Bandarban, Bangladesh (Fig. 1). The snake crossed the road while we observed it closely and took photographs.

Smithophis atemporalis is bicolored with black trapezoids extending ventrally and white markings extending dorsally (Fig. 2). The scales are known to be iridescent and unlike other known *Smithophis* spp., the head lacks temporal shields. During the monsoon this snake can be seen around waterfalls and waterbodies. The diet includes earthworms and juvenile skinks.

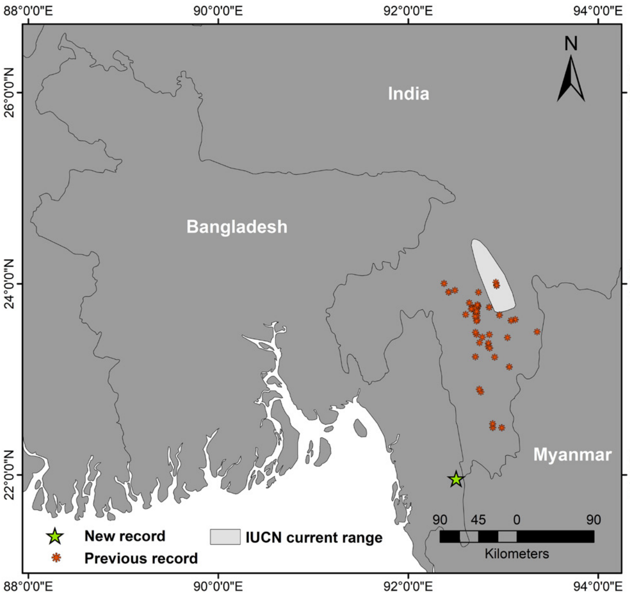

Figure 1. Past and present records of *Smithophis atemporalis* from Mizoram, India and Bangladesh

Since our record is based on relatively distant photographic evidence that precluded any detailed observation of the head scale pattern, identification was based on colour and patterning. We compared the current observation with the colour patterning of other *Smithophis* species in Asia (Table 1), and conclude that it is the first confirmed record of *S. atemporalis* for Bangladesh.

Previously, *S. atemporalis* was recorded from 60 different localities ranging from 71–1,235 m a.s.l. in four districts of Mizoram (Lalronunga et al. 2021; Ruatpuii et al., 2022) that are 76–226 km from the present location. The nearest location from the new record is Saiha, Mizoram, India (Fig. 1). *Smithophis atemporalis* is an interesting addition to the faunal list of Bangladesh. Little is known of this species in neighbouring India and further studies to investigate its occurrence, distribution and ecology in Bangladesh are needed.

Table 1. Comparisons of colouration, body pattern and geographical distribution of the four species of *Smithophis*. Adapted from Giri et al. (2019); Das et al. (2020); Vogel et al. (2020); Ruatpuii et al. (2022). Asterisk (*) indicates new record.

Species	**Colouration and body pattern**	**Distribution**
S. atemporalis	Bicoloured, blackish above, whitish below. Dorso-laterally, markings asymmetric with longer dark and shorter whitish markings with boundary between body markings wavy in the first quarter of the body and zigzag in the rest of the body.	India (Mizoram) Bangladesh*
S. bicolor	Bicoloured, dark brown or black upper parts, yellowish white below. The two colours strongly contrasting in short gaps and forming a jigsaw pattern (when viewed laterally). Some specimens are uniform blackish, while others show regular light vertical lines.	India, Myanmar, China, Bangladesh
S. linearis	The upper dorsal surface is dark greyish brown with bronze tints, pale ochre below. Upper and lower edges of each upper dorsal scale dark brown, forming ten narrow dark lines extending from back of head.	China, Myanmar
S. arunachalensis	Bicoloured, dark black-brown above, bright yellow below. Dorso-laterally, markings asymmetric with longer dark and shorter yellow markings, usually V-shaped when viewed laterally and trapezoidal when viewed from above. In first quarter of body the boundary between body markings wavy, rapidly becoming increasingly sharply zigzag towards the posterior.	India (Arunachal Pradesh)

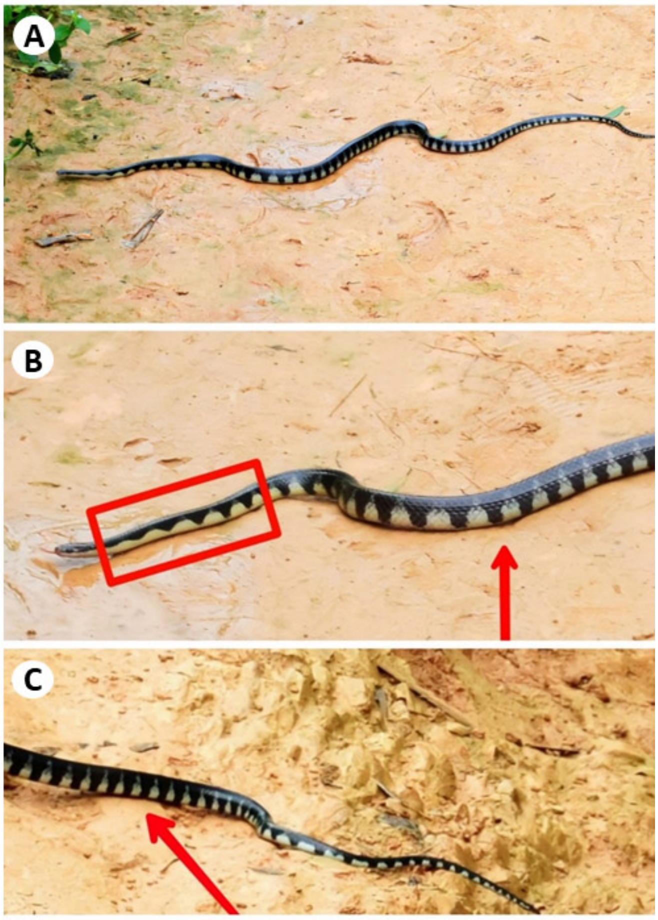

Figure 2. **A.** *Smithophis atemporalis* from Darjeeling Para towards Boga lake, Keokradong, Ruma Upazila, Bandarban, Bangladesh, **B.** The black markings do not extend to the lateral margins in the first tenth of the body so that there is a wavy effect between black and white (indicated in the box) while for the rest of the body the black markings reach the lateral margin creating a zigzag pattern, indicated by arrows in **B.** & **C.**

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SHORT COMMUNICATION

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Clutch size and hatchling morphology of Parrot snake *Leptophis liocercus* in the north-east Atlantic Forest of Brazil

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Parrot snakes (*Leptophis* spp) have been reported in both primary forests and disturbed environments in Brazil (Mattos et al., 2017). These arboreal colubrid snakes are active during the daylight and primarily feed on frogs, although some species may also consume lizards and small rodents (Albuquerque et al., 2007). The genus comprises 19 species distributed in Central and South America, including five species that occur in Brazil. *Leptophis liocercus* (Wied, 1824) was previously known as a subspecies of *Leptophis ahaetulla* but is now recognised as a distinct species (Albuquerque & Fernandes, 2022). It can be distinguished from its congeners by a series of characters, such as the head scales slightly edged with black and without black spots, and an unstriped dorsum, with dorsal colouration changing gradually from dark bluish green to light or dark brown, with scales narrowly edged with black (Albuquerque & Fernandes, 2022). When threatened *L. liocercus* is known to show aggressive behaviours such as biting, flattening their bodies and opening their mouths wide (Pontes & Rocha, 2008). Although some information on the reproductive biology is available for *L. ahaetulla*, *L. depressirostris* and *L. marginatus* (Rand, 1969; Dundee & Liner, 1974; Lewis, 2004; Cruz-Lizano et al., 2013; Linardi, 2016), nothing is known for *L. liocercus*. Herein, for *L. liocercus* we report the dimensions of a clutch of eggs and the emergent neonates found in an area of Atlantic Forest in north-east Brazil.

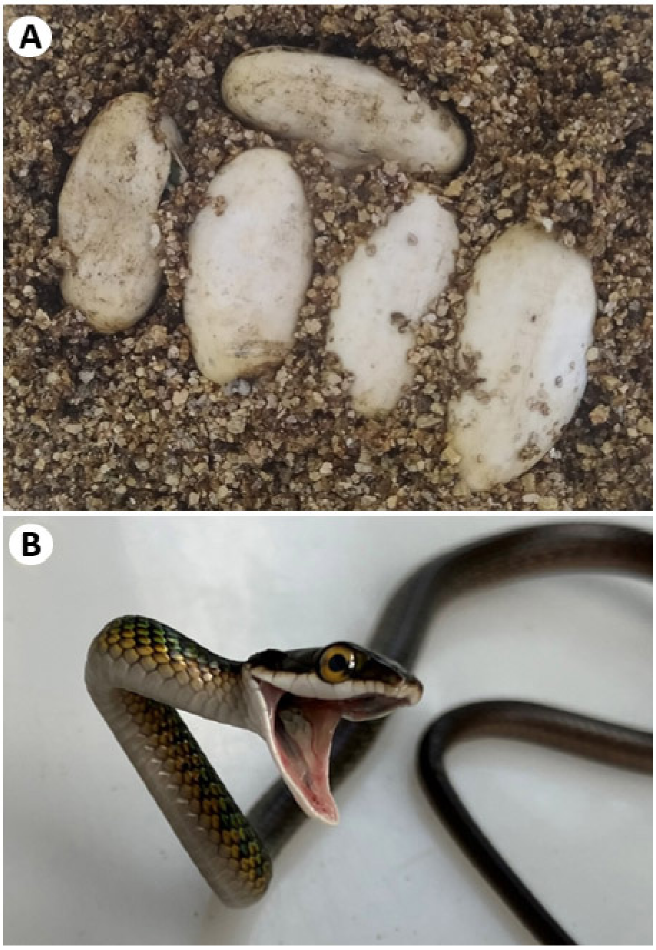

Figure 1. Eggs and hatchling of *Leptophis liocercus* – **A.** Five eggs, **B.** Hatchling exhibiting aggressive behaviour

On 29 September 2022, six eggs of *L. liocercus* were found in a rural area of the Mamanguape municipality, Paraiba state, north-east Brazil (6° 39.31’ S, 35° 07´47’ W; WGS 84; 44 m a.s.l.), about one kilometre from the Guaribas Biological Reserve. All eggs were found adhering to each other lodged inside a hollow brick, which was in a pile of bricks on a sandy floor outdoors. The eggs were collected and taken to the animal ecology laboratory of the Federal University (UFPB) under license SISBIO 74327-1. The eggs were incubated at a temperature of 20–22 °C, with approximately 60 % relative humidity, half-buried in vermiculite (Fig. 1A). Each egg was measured at the longest and widest point using a caliper (precision = 0.1 mm). Also, all hatchlings were measured using a digital caliper (precision = 0.01 mm), weighed using a spring scale (0.1 g) and sexed immediately after birth using a probe (Table 1S, see Supplementary Material). One individual was preserved in formalin 10 % and housed in the Herpetological Collection of the Universidade Federal da Paraíba. All other hatchlings were released in the Guaribas Biological Reserve.

The mean (± sd) dimensions of the eggs were 33.4 ± 1.8 mm long and 13.9 ± 0.9 mm wide. One egg was not fertilised and was found to be dehydrated after 16 days of incubation. On 1 December 2022 at 06:55 h, after 63 days (about 2 months) of incubation, two neonates started to hatch and had totally emerged after 5 hours and 20 minutes and 21 hours and 15 minutes respectively. On the same day, at 13:38 h and at 22:30 h other neonates started hatching, and the processes lasted for 4 hours and 40 minutes and 10 hours and 55 minutes, respectively. After a further 24 h the last snake had not started to cut though its egg shell, so we made a small incision in the egg with a scalpel. After 7 hours the snake had emerged completely. The mean (± sd) dimensions of the hatchlings were - total length 328.0 ± 18.5 mm and weight 3.8 ± 0.4 g. A summary of other body dimension can be seen in Table 1S. Of the five snakes, two were males and three females, the sexes were the same colour and appeared not to differ from the adult colouration, and on emergence all presented aggressive behaviour (Fig. 1B).

The clutch size of *L. liocerus* is similar to other *Leptophis* species: *L. ahaetulla* had one to eight eggs per clutch (Martins & Oliveira, 1999), *L. dibernardoi*, three to 12 eggs (Mesquita et al., 2013), *L. mexicanus*, two to six eggs (Censky & McCoy, 1988), *L. depressirostris*, five eggs (Lewis, 2004). Linardi (2016) reported a communal nest for *L. marginatus* with as many as 49 eggs, including at least 12 eggs that must have come from a single female since they were adhering to each other and in the same stage of development. Similar to our observation, these eggs were also inside a hollow cement brick 60 cm above the ground. Our observations on the mean length of the *L. liocercus* neonates, at 328 mm, were very similar to *L. marginatus* in south-east Brazil (318 mm - Linardi, 2016) but somewhat larger than *L. ahaetulla* in Panama (241 mm - Rand, 1969), and *L. depressirostris* in Costa Rica (278 mm - Lewis, 2004). Although clutch size, incubation period and hatchling dimensions appear to be similar among *Leptophis* species, the time of year when hatching occurs varied among species, which should reflect differences in the prevailing environmental conditions.

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SHORT COMMUNICATION

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Thigmothermic behaviour on paved paths after sunset by the weasel skink *Saproscincus mustelinus*

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Most lizards acquire body heat by basking in sunlight (Pianka & Vitt, 2003; Wilson, 2012) and use behavioural mechanisms to increase or reduce heat gain (Wilson, 2012). Another way that lizards may acquire heat is by direct contact with warm surfaces, a behaviour termed thigmothermy (Pianka & Vitt, 2003; Wilson, 2012). Thigmothermic lizards are generally nocturnal or live in shaded habitats (Wilson, 2012). Shade skinks of the genus *Saproscincus* (Eugongylini) dwell in forests and sheltered suburban gardens in eastern Australia (Wilson, 2012). The weasel skink *Saproscincus mustelinus* is surface active and dwells among leaf litter (Swan et al., 2017; Wilson, 2012). Its daily activity pattern appears to be variable as this skink has been said to be diurnal (Downes & Shine, 1999; Wilson, 2012) but active at dusk or shortly after dark on warm nights (Swan et al., 2017; Robertson & Coventry, 2019). Based on this information, recent revisions of several traits of the Scincidae classified the weasel skink as cathemeral, i.e. not strictly diurnal, nocturnal or crepuscular (Meiri, 2018; Slavenko et al., 2022).

Here I describe and illustrate weasel skinks lying on a warm paved path after sunset at a suburban site in south-eastern Australia, and suggest that this behaviour is a strong indication of nocturnal activity of this skink species.

I recorded skinks from 30 November 2022 to 15 April 2023, incidentally to my daily afternoon and evening walks on the Louise Sauvage pathway (33° 49′51″ S, 151° 04′15″ E″, 7 m a.s.l. to 33° 50′30″ S, 151° 03′56″ E, 6 m a.s.l.), Newington, New South Wales, Australia. This track is bordered by woodlots and paved with bitumen and small stone chips, with traffic of walking or running people,

Figure 1. Weasel skink *Saproscincus mustelinus* activity and death on a previously warmed paved pathway in south-eastern Australia – **A.** One adult individual lies after sunset with body and partly regenerated tail in close contact with the path surface, **B.** A roadkill adult individual scavenged by unidentified black ants at night, **C.** An adult individual forages on leaf litter at the pathway edge at night, and **D.** Another adult individual forages on a mulch layer in the morning

bicycles and electric scooters. I walked a round trip of about 500 m twice daily at a steady pace and looked for skinks on the path, which was warmed earlier by the sun. To hand touch, the path was warmer than the surrounding ground, with leaf litter and vegetation. I recorded skinks on the path from sunset to nightfall (16:59–20:02 h, the first figure in mid-autumn and the second one in summer) and photographed them with a digital camera equipped with 55–350 mm telephoto lens from a distance of 1–2 m. Observation sessions ranged 3–10 min, totalling about 15 h. After 15 April no skinks were recorded lying on the pathway. Additional selected images of live and road kill skinks are on file in the Coleção de Imagens (ZUEC-PIC 0825-0833) at the Museu de Diversidade Biológica, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

I sighted *S. mustelinus* motionless on the path after sunset 133 times. The mean (± sd) daily number of skinks sighted was 4.65 ± 2.73 (range 1–12 lizards) The postures displayed by the skinks while lying on the warm pathway varied little, their bellies and tails were in close contact with the path's surface and the head slightly elevated (Fig. 1A). As the path is bordered by woodlots, sometimes I mistook the lying skinks for sticks or dead curled leaves and the other way round. Occasionally an individual crossed the pathway, waving sinously the long tail. Most lying skinks seemed oblivious of passers-by, including those on bicycles and scooters and did not retreat even if they were in the middle of the path. Possibly due to the skinks' unwillingness to retreat I found 11 road kills (8.27 % of the total number of lying skinks sighted), the dead skinks being scavenged by ants on the same night or the next morning (Fig. 1B).

Besides those individuals applying themselves to the warm path, I observed two skinks foraging at night on the leaf litter at the pathway edge in the summer at 19:34 h and at 19:50 h (Fig. 1C), respectively. I also recorded four individuals active during the daytime. One adult foraged in the winter onset among leaf litter below a hunting perch of the Grey butcherbird *Cracticus torquatus* and was preyed upon by the bird (see Fig. 2D in Sazima, 2023) at 10:49 h. Another adult individual was moving on a shaded log in the autumn at 08:40 h, also in the autumn at 14:08 h a very small individual (apparently hatchling) laid on a shaded spot of the path, and in midsummer at 10:33 h an adult foraged in a mulch layer within a eucalypt stand (Fig. 1D).

As lying on a previously warmed pathway after sunset seems an unlikely behaviour for a diurnal lizard, acquisition of heat for nocturnal activity would be the simplest explanation for this behaviour in *S. mustelinus*. Another possibility would be that the skinks were asleep. I think that sleeping can be ruled out as all of them kept their eyes fully open and were alert. The skinks have semi-translucent eyelids and if they were asleep it would be expected that they do so with eyes closed. The observations reported herein document night and day activity of this skink species and qualify it as cathemeral (Meiri, 2018; Slavenko et al., 2022). From eight out of the nine subfamilies of scincid lizards that contain cathemeral species, Eugongylini has the smallest number (Slavenko et al., 2022), cathemerality being found in only 5 % of all lizard species (Meiri, 2018).

The variations in previous descriptions of the daily activity pattern of *S. mustelinus* (Meiri, 2018) most likely reflect the observational data of different authors under diverse circumstances and in different seasons of the year (Downes & Shine, 1999; Wilson, 2012; Swan et al., 2017; Robertson & Coventry, 2019). From my observations of individuals applying themselves to the warm path, plus those (admittedly scarce) recorded active at night and during the day, I suggest that this skink species is nocturnally active mostly in summer (warmer months), as indicated by Swan et al. (2017) and Robertson & Coventry (2019). It seems that it is diurnally active at least at the winter onset, but this remains to be verified. I was unable to confirm that this skink basks in sunlight (Downes & Shine, 1999), but I observed that it acquires body warmth by conduction (thigmothermy). However mosaic basking, using small patches of sunlight among otherwise shaded vegetation, has not been ruled out (Wilson, 2012).

The road kills I recorded support the statement of Wilson (2012) about shade skinks being unaware of passers-by and displaying little inclination to leave contact with warm surfaces. The number of skinks killed on the path is probably greater than I recorded, as early scavengers may spot and eat the carcasses in situ or carry them away. This view is exemplified by the scavenging behaviour displayed by the Australian raven *Corvus coronoides* (Sazima, 2020). Lastly, the small number of *S. mustelinus* individuals I recorded foraging at night or during daytime is probably due to their habit of dwelling among leaf litter (Swan et al., 2017; Wilson, 2012), which hampers direct visual observations. Information on this activity could be obtained in the future by using camera traps (Welbourne et al., 2020).

SUPPLEMENTARY NOTE

On 28 September 2023 I sighted a weasel skink on the paved path again, and since then I have seen skinks daily from 28 September to 19 October 2023. I saw skinks lying on the path after sunset 21 times, plus one road kill, and one individual lying on mulch at 09:27 h on a cloudy day (Fig. 2). Based on the previous observations and the recent ones, it seems that *S. mustelinus* is active at the study site mostly from Spring to Autumn.

Figure 2. Weasel skink *Saproscincus mustelinus* lying on mulch at a eucalypt stand on a cloudy morning

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SHORT COMMUNICATION

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Stiff-legged behaviour in the green and black poison frog *Dendrobates auratus* in response to simulated predation

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Predation pressure has led to the evolution of a diversity of antipredator strategies in nature. Stiff-legged behaviour is a defensive strategy that has been described in several neotropical, leaf-litter frog species of the families Brachycephalidae, Bufonidae, Craugastoridae, Cycloramphidae, Leptodactylidae, Microhylidae and Odontophrynidae (Sazima, 1978; Rocha et al, 1998; García, 1999; Toledo & Zina, 2004; Bertoluci et al., 2007; Menin & Rodrigues, 2007; Toledo et al., 2011; Mira-Mendes et al, 2016). The behaviour is usually preceded by short leaps and is characterised by frogs flattening their body and fully extending their fore and back limbs into a stiff (or rigid) position and remaining motionless. Given that this behaviour has only been described in cryptically-coloured frogs, remaining motionless is thought to reduce or prevent predator detection (Toledo et al., 2011); however, it is also possible that (1) remaining motionless would inhibit predators that use movement to detect prey or require live prey, and/or (2) the extended and rigid position would make it more difficult for the predator to handle and consume the prey. Although both possibilities are similar to the presumed functions of thanatosis (death-feigning), Toledo et al. (2011) distinguishes stiff-legged and thanatosis from each other mainly on the basis of limb extension and position. Anurans exhibiting thanatosis do not fully extend their limbs into a rigid position as in stiff-legged behaviour, but instead keep them in a loose (or relaxed) position (Toledo et al., 2010; 2011). In following Toledo et al. (2011), herein, we report the presence of stiff-legged behaviour in naïve, captive-raised, conspicuously-coloured *Dendrobates auratus* (Dendrobatidae) from a Panamanian lineage.

The natural range of the green and black poison frog *D. auratus* is from southern Nicaragua to Colombia (Savage, 2002). The species is characterised by its variable colours and patterns that, to predators with colour-vision, signal the presence of alkaloid defenses (Daly et al., 1992; Saporito et al., 2012; Stuckert et al., 2019). Body raising behaviour has been reported in captive and wild-caught adults of *D. auratus* from Costa Rica following simulated predation trials (Blanchette & Saporito, 2016; 2017).

We undertook laboratory tests of the defensive behaviour of juvenile *D. auratus* (5 months post-metamorphosis) in response to low and high rates of simulated predation; no frogs were harmed during these tests. To simulate predation, we disturbed eight frogs by picking them up and then releasing them using 7.5 cm pressure-sensitive forceps for three consecutive days, followed by one day of no such disturbance. The tests lasted for 23 days (25 September to 17 October of 2022). Once a frog displayed the stiff-legged behaviour, it was removed from the experiment. The three days in which there was simulated predation were referred to as 'predation periods'. Four frogs were subjected to low disturbance rates, in which they were picked up and released 12 times daily in each predation period (36 times) and four frogs were subjected to high disturbance rates, in which they were picked up and released 24 times daily in each predation period (72 times). Of the individuals exposed to the high disturbance rate one exhibited stiff-legged behaviour five days after the start of the experiment, two individuals displayed this behaviour after six days and the fourth individual after eleven days. Frogs exposed to the low disturbance rate also exhibited stiff-legged behaviour, but its appearance took a longer exposure to the simulated predation treatment. One frog began stiff-legged behaviour six days after the start of the experiment, while the other

Figure 1. *Dendrobates auratus* exhibiting stiff-legged behaviour in response to simulated predation (the brown particles present on the body of the frog are small pieces of moss from the experimental enclosures)

three individuals exhibited the behaviour after 22 days. Stiff-legged behaviour was identical in all *D. auratus* and consisted of frogs keeping their eyes open and assuming a stiff-and cross-legged position (Fig. 1). All individuals held the position even when further manipulated with forceps. On average, the behaviour lasted for five minutes, after which the frog returned to a normal, relaxed posture and behaviour.

Our results demonstrate that stiff-legged behaviour is not restricted to cryptically-coloured frogs and may be a more widespread defensive adaptation than previously thought. Its presence in conspicuously-coloured *D. auratus* also suggests that its defensive function extends beyond detectability (or camouflage), especially with respect to colour-visioned predators. Among conspicuously-coloured frogs, stiff-legged behaviour could function more similarly to thanatosis and limit predation to predators that require live prey and/or make it more difficult for certain predators to handle and consume the frogs. Stiff-legged behaviour in *D. auratus* may act as a supplementary defense, and in particular against colour-visioned birds, which are thought to be common predators of poison frogs (Saporito et al., 2007; Noonan & Comeault, 2009; Maan & Cummings, 2012; Rojas, 2017). Further, stiff-legged behaviour was induced in response to simulated predation, suggesting that its presence in wild populations of *D. auratus* (and other species) may depend upon the extent of predation pressure. All of the individuals in the present study were juveniles, and although it is possible that they express different defensive adaptations when compared to adults, Blanchette et al. (2016) reported body raising in both juveniles and adults of *D. auratus*. Surprisingly, none of the individuals in the present study exhibited body raising behaviour, which has been described in Costa Rican lineages of *D. auratus* exposed to similar predation treatments in both the lab and field (Blanchette & Saporito, 2016; 2017; Saporito unpublished data). The unexpected absence of body raising and presence of stiff-legged behaviour in this Panamanian lineage of *D. auratus* needs further study. Finally, field studies are required to determine if stiff-legged behaviour also occurs in wild individuals, and if so, its potential defensive function.

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NATURAL HISTORY NOTE

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First record of leucism in the Italian newt *Lissotriton italicus*

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We present here a record of leucism in the Italian newt *Lissotriton italicus*. This pigmentary anomaly involves partial or total absence of integumentary pigmentation, giving an individual a whitish to yellowish appearance, but with the eyes retaining normal colouring of the iris (Duellman & Trueb, 1994; Henle et al., 2017).

Lissotriton italicus is a tiny salamander (maximum total length: females 94.7 mm, males 80.4 mm) endemic to central and southern Italy (Scillitani & Tripepi, 2007; Di Nicola et al., 2019). With a total length of up to 95 mm for the larger females this species is the smallest of the European newts (Raffaëlli, 2007).

On 6 May 2009, during an entomological survey of the Sila Mountains (Calabria, southern Italy) (Mazzei et al., 2006) three leucistic *L. italicus* (1 male, 2 females) were observed in a pond close to the Machinella stream, in the municipality of Pallagorio (Province of Crotone, Calabria, southern Italy; 39° 19'30.67" N, 16° 53'39.97" E). Integumentary pigment of these individuals was almost completely lacking on body, head, limbs and tail (Fig. 1A). In the same pond and on the same date 14 normally pigmented adult specimens (8 males, 6 females) were observed (Fig. 1B).

To date there have been reports of albinism and leucism in at least 15 species of European salamanders (see Corsini et al., 2002; Modesti et al., 2011; Lunghi et al., 2017; Capula & Aloise, 2023 for the studied species). However, to our knowledge there is currently no record for *L. italicus*. Thus the case we report here is the first documented record of leucism for the species.

Figure 1. *Lissotriton italicus* from a pond in the municipality of Pallagorio (Province of Crotone, Calabria, southern Italy) – **A.** Leucistic adult female, **B.** Normally pigmented adult male

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NATURAL HISTORY NOTE

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Mindanao cross frog *Oreophryne anulata* found in a dormant pitcher of *Nepenthes copelandii* in the Philippines

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In the tropics, ecological interactions between animals and plants are complex. Many of these relationships are restricted to specific microhabitats, one of which is the water-filled cavities (phytotelmata) in plants. The fauna that uses phytotelmata has adapted their behaviours (i.e. foraging, refuging and breeding) to maximise their benefit from these microhabitats. In south-east Asia, phytotelmata include the pitchers of the plant family Nepenthaceae, especially those of the genus *Nepenthes*, which have been reported to have associations with insects, mammals and anurans. In the case of anurans, these have been observed particularly in Peninsular Malaysia and the island of Borneo (Fig. 1).

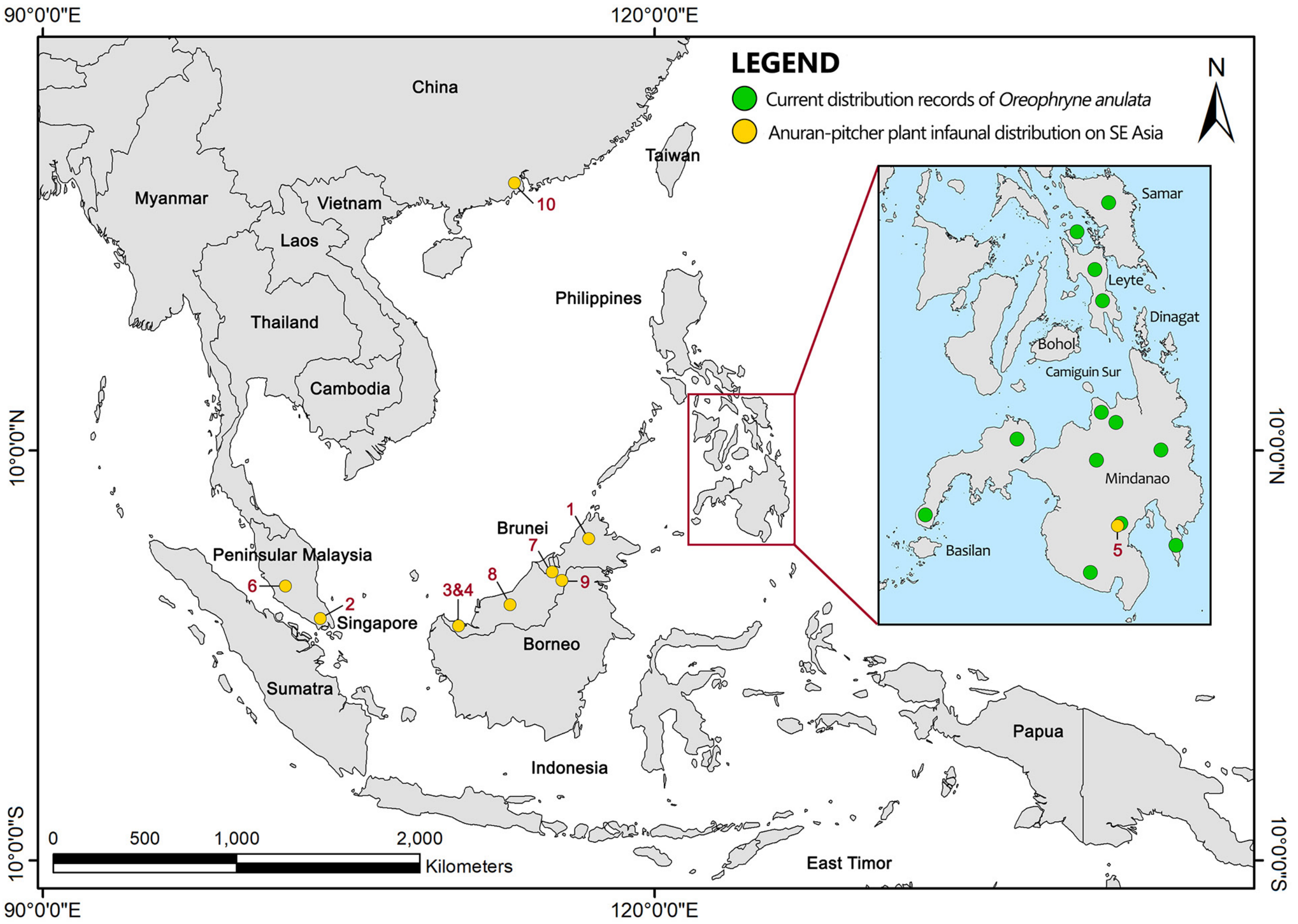

Figure 1. Distribution map of records of anuran associations with pitcher plants (*Nepenthes* spp.) in south-east Asia and the current distribution records of *O. annulata* in the Philippines. Anuran species and bibliographical references – **1.** *Kalophrynus heterochirus* Phillips et al., 2008; Das & Hass, 2010; **2.** *Kalophrynus pleurostigma* Lim & Ng, 1991; **3.** *Microhyla borneensis* Das & Haas, 2010; **4.** *Microhyla nepenthicola* Das & Haas, 2010; **5.** *Oreophryne anulata* This study; **6.** *Philautus aurifasciatus* Yong et al., 1988; **7.** *Philautus kerangae* Malkus &Dehling, 2008; **8.** *Philautus mjobergi* Phillipps & Lamb, 1988; **9.** *Philautus nepenthophilus* Etter et al., 2021; **10**. *Polypedates leucomystax* Hua & Lee, 2004

Figure 2. Observation of the frog *Oreophryne anulata* in a dormant pitcher of *Nepenthes copelandii* – **A.** Typical secondary montane forest habitat on Mt. Apo Natural Park, **B.** An active upper pitcher of *N. copelandii*, **C.** The dormant upper pitcher of *N. copelandii* in which *O. anulata* was found, **D.** *O. anulata* within the dormant pitcher, **E.** Close-up photo of *O. anulata* within the pitcher

In the Philippines, 115 species of anurans are currently recognised (Diesmos et al., 2015; 2020; Herr et al., 2021). Microhylid frogs are among these, and the genera *Chaperina* (Supsup et al., 2022) and *Kaloula* (Sanguila et al., 2016) are frequently linked to phytotelmata, but there are no previous reports of anurans using *Nepenthes* phytotelmata in the Philippines, apart from a dead toad *Pelophryne albotaeniata* that had probably drowned in a pitcher *Nepenthes leonardoi* on the island of Palawan (Sy & Tan, 2015).

From 19–23 March 2022, we conducted a rapid biodiversity assessment on Mt. Apo Natural Park. We used random visual surveys, microhabitat searches and opportunistic catching on pre-identified routes to assess the biodiversity in Sta. Cruz, Digos City, Davao del Sur of Mindanao Island (6° 59.0990′ N, 125° 14.6780′ E). Unexpectedly, we found a morphologically cryptic microhylid frog *Oreophryne anulata* (Stejneger, 1908) occupying the upper pitcher of *Nepenthes copelandii* in a montane secondary forest habitat (2,237 m a.s.l.; Fig. 2A). *Oreophryne anulata* is found on the islands of Biliran, Leyte, Samar, and Mindanao (IUCN SSC Amphibian Specialist Group, 2020); its known distribution in the greater Mindanao faunal region is shown in Figure 1. The frog was identified by its small size, well-developed disk on fingers and toes and faintly visible subarticular tubercles (Inger, 1954). Snout-vent length (21 mm) and weight (0.9 grams) were measured using a digital caliper and electronic scales (± 0.1 g). The specimen of *O. anulata* was collected and humanely euthanised with aqueous chloretone, then fixed with 10 % buffered formalin and subsequently preserved using 70 % ethanol (Simmons, 2002). It was deposited at the Central Mindanao University Zoological Museum (CMU MZ 3007.0). The pitcher plant species was recognised by its broad and robust leaves with canaliculate petioles and cylindrical and wholly infundibular upper pitchers (Gronemeyer et al., 2011; Pelser et al., 2011 onwards). The measurements of the pitcher were length 134 mm and width of lips 45 mm.

We observed *O. anulata* positioned upright, unharmed and partly submerged in the fluid within the pitcher (Fig. 2B–E). Notably, we did not detect any viscid fluid in the digestive zone, suggesting that this pitcher was dormant/sedentary. We can only speculate on the relationship between *O. anulata* and *N. copelandii*. It is possible that the frog may have fallen into the pitcher plant by accident and was unable to escape. Alternatively, it may be that the frog was actually using the pitcher as a refuge during the day to reduce the risk of predation. If this were the case, then possibly the frog was unaffected by digestive fluid, as the pitcher, being dormant, may have lacked digestive fluid, any digestive fluid present may have become diluted with rainwater/dew, and/or the frog’s own skin secretions may have conferred resistance to it. We recommend long-term and targeted field surveys to gain further insights into the relationship between *O. anulata* on *N. copelandii*.

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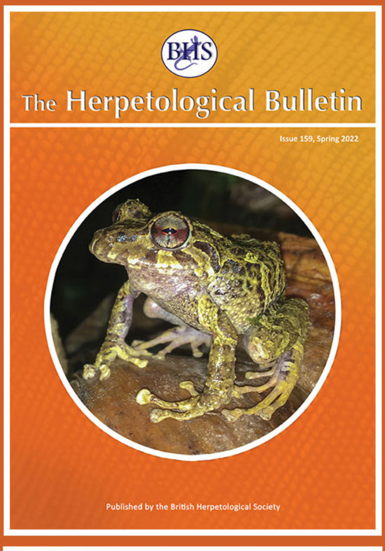
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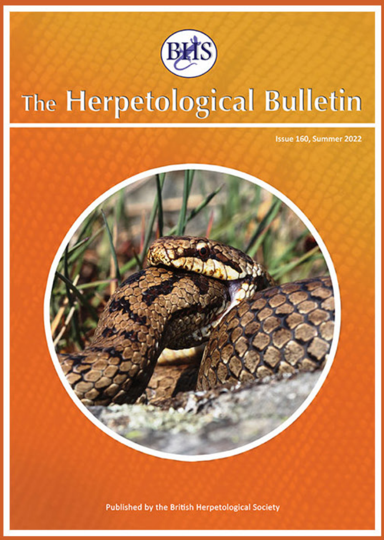
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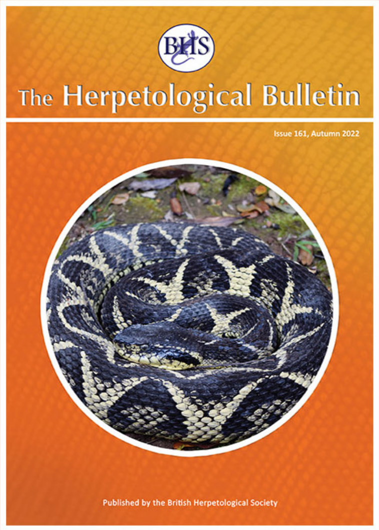
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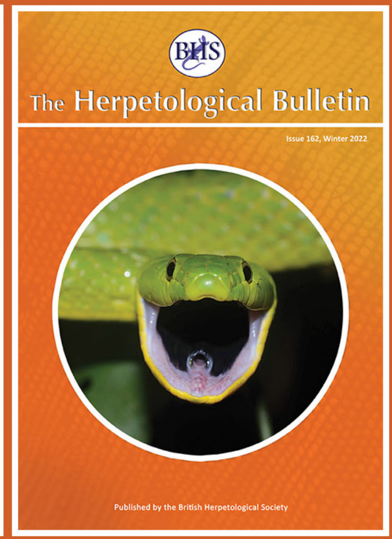
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