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Front Cover: A nose-horned viper *Vipera ammodytes* photographed by Angel Dyugmedzhiev in Bulgaria. There is an article about this species on p 1.

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RESEARCH ARTICLE

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Arboreal behaviours of the nose-horned viper *Vipera ammodytes*, with links to video evidence

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ABSTRACT – *Vipera ammodytes* is considered to be a predominantly terrestrial species although we report eight individual episodes of arboreal behaviour that include 1) copulation, 2) active hunting and feeding, 3) thermoregulation and/or waiting in ambush for prey, and 4) escape after capture and handling. To our knowledge, this is the first report of arboreal copulation in any *Vipera* spp., and the first report of active arboreal hunting by *V. ammodytes*. Arboreal thermoregulation and waiting in ambush for prey are difficult to distinguish from one another and vipers may combine these two types of behaviours. Arboreal escape after capture and handling is most likely the result of stress and it seems that it is rarely exhibited. It is clear that *V. ammodytes* can use arboreal habitats to its advantage and it is suggested that further study may demonstrate that arboreal activity is a more important component of the behavioural ecology of *V. ammodytes* than previously reported.

INTRODUCTION

European *Vipera* spp. have similar morphologies as well as many similarities in their behavioural ecology, e.g. upper thermal set points, activity patterns, food habits etc. (Saint Girons, 1978; 1980; Luiselli, 2006). Their diet consists mainly of small mammals and reptiles, although birds, arthropods and even amphibians are also occasionally preyed upon (Speybroeck et al., 2016). Even though *Vipera* spp. are considered as mainly terrestrial (Phelps, 2010), some arboreal behaviours and activities have been reported: 1) active hunting and feeding (Groen et al., 2020); 2) thermoregulation (Saint Girons, 1975; Marx, 2022); 3) escaping from predators (Marx, 2022).

The nose-horned viper *Vipera ammodytes* (Linnaeus, 1758) is a medium-sized viper, distributed in the southern parts of Central and Eastern Europe and parts of Asia Minor to the Lesser Caucasus (Speybroeck et al., 2016). In Bulgaria, it is distributed throughout most of the country, except in the high mountains, intensively cultivated agricultural, and urbanised land (Stojanov et al., 2011). It is considered that *V. ammodytes* is mainly a sit-and-wait ambush predator whose diet depends on individuals' age and size. Juveniles prey mainly on small lizards and centipedes and as they grow in size their diet becomes more diverse, including also small mammals and more rarely frogs, snakes and birds (Beshkov, 1977; Luiselli, 1996; Anđelković et al., 2021). The species is considered predominantly terrestrial (Phelps, 2010), although some arboreal activity has been documented (Schweiger, 2013). In the current report, we document several aspects of arboreal activity and behaviour of *V. ammodytes* in Bulgaria.

MATERIALS & METHODS

Most of the data were collected during a long-term study (2013–2024) in various parts of Bulgaria. Vipers were located by active search along with inspection of potential hideouts during March–November when the species is active (Dyugmedzhiev et al., 2022). Co-ordinates for detected vipers were taken with a hand-held GPS device (Garmin eTrex 20; precision: 5 m). The temperature of the substrate on which the vipers were found (Ts) as well as the air temperature at 15 cm (T15) above the ground were measured with a quick-reading thermometer (TOPELEK TECP022AH; precision: 0.3 °C). The average between the values of Ts and T15 were used to estimate the temperature of the microhabitat (Dyugmedzhiev et al., 2021). The hour of the day in which each viper was found as well as the meteorological conditions (wind, cloudiness and rain) were recorded. When possible, vipers were captured, measured (precision: 0.5 mm), weighed (precision: 0.01 g), photo documented for individual identification (Dyugmedzhiev et al., 2018) and then released at the site of capture. The process of handling the snakes usually lasted less than 30 mins. Age group of the vipers was estimated based on individual total length (SVL + TL) as follows: neonates/juveniles – up to 28 cm; subadults – 28–46 cm for females and up to 49 cm for males; adults – over 46/49 cm for females and males, respectively (Dyugmedzhiev et al., 2020). The sex of vipers was determined by the body colour pattern, the length and width of the tail and SVL/TL ratio (Tomović et al., 2002).

Data on the predation of nestling birds (see below) were collected in 2015 during the course of filming the documentary 'Vrachanski Karst Reserve', which is dedicated to the biodiversity of this protected area.

OBSERVATIONS & DISCUSSION

Eight individual observations on arboreal activity and behaviour of *V. ammodytes* were made during the course of the study, categorised as 1) copulation; 2) active hunting and feeding; 3) thermoregulation and/or waiting in ambush for prey; and 4) escape after capture and handling. The specifics of each observation are presented below.

Copulation

On 12 May 2024, AD and MS found two adult *V. ammodytes* copulating on the branch of a Jerusalem thorn *Paliurus spina-christi* at 18:28 h, near Slivnitsa Village (41° 41'10" N, 23° 9'53" E; 183 m a.s.l.), south-western Bulgaria. The shrub was situated next to a path and above a stone wall, marking the yard boundary of a property with beehives. We recorded a quick video from approximately 1.5 m distance from the vipers, after which we retreated about 5 m away, hiding behind a bush, from where we continued to observe and film the vipers. The weather was strongly windy, cloud cover was 70%, and the microhabitat temperature was 19.1 °C. The vipers were situated proximately 100 cm above the ground. On the initial moment of observation, the male (SVL = 56.9 cm, TL = 8.2 cm, W = 140.22 g), was laying vertically on an upper branch, with its head positioned up and left, and the hind part of its body slightly coiled around that of the female, and its tail entangled around the females' tail with one of the hemipenes inserted in the female's cloaca (Fig. 1; BHS video 2025a, Part 1). The female was lying horizontally, with its head and first half of the body positioned below and to the left on the lower branch, and the hind part of its body was positioned on same branch as the male (Fig. 1; BHS video 2025a, Part 1). Throughout the period of copulation, the female did not stay still and altered its position many times. These alterations included 1) remaining in the same overall position as in the initial period of observation, but with its head positioned to the right (BHS video 2025a, Part 2), 2) climbing down and up many times, between its initial position, the lower branches and the upper branch on which the male was positioned (BHS video 2025a, Part 3), and 3) returning to its initial position. In contrast, the male stayed relatively still during the entire copulation, only slightly adjusting the back of its body and its tail, keeping its position around the female's body, and adjusting its head to balance for stability (BHS video 2025a, Parts 2&3). The copulation continued for 21 minutes from first observation after which the two snakes started to disentangle themselves with the male making spiral movements on the same branch (BHS video 2025a, Part 4) but with its head and front body positioned to the right, and the female climbing down from the bush and disappearing in the grass and stones on the ground beneath it. Once completely disentangled from the female, the male also started climbing down towards the ground and was then captured for measurement. After it was released, it disappeared in the grass and stones, as did the female.

Figure 1. A pair of *Vipera ammodytes* copulating in a Jerusalem thorn tree *Paliurus spina-christi* about 100 cm above the ground. Red dashed arrow indicates the female snake (F); blue continuous arrow indicates the male snake (M). Note that the lower blue arrow appears to be pointing at the female's body but actually this is part of the male's body lying in front of the female, this is more easily seen in the accompanying video (BHS video, 2025a).

Even though *V. ammodytes* is a species that is relatively easily found and observed in-situ, observations on its copulation are not very common and in the few reported cases, it occurred on the ground (Beshkov, 1977; Čubrić & Crnobrnja-Isailović, 2023). To the authors' knowledge, this is the first reported case on arboreal copulation in the species, as well as in the *Vipera* spp.generally. There was no obvious reason for why this copulation took place on the branches of the shrub. Indeed, there were possible disadvantages in this position since the vipers appeared much more exposed to avian predators on the branches and due to strong wind the branches were shaking making the shrub an unstable surface for copulation.

Active hunting and feeding

On 5 May 2015, BN and IHN used a pre-set action camera (GoPro 3+) to film an adult female *V. ammodytes* that was predating long-tailed tit *Aegithalos caudatus* nestlings between 17:59 h and 18:17 h in Vrachanski Balkan Nature Park (43° 11'24.66" N, 23° 31'16.59" E; 514 m a.s.l.), close to Zgorigrad village, north-western Bulgaria. The weather was mostly sunny, warm (air temperature about 25 °C) and calm, without wind. The nest was situated at 140 cm above the ground, comparatively well concealed in the interior of a hawthorn bush *Crataegus monogyna*. The viper climbed to the nest while the parents were not present, placed its

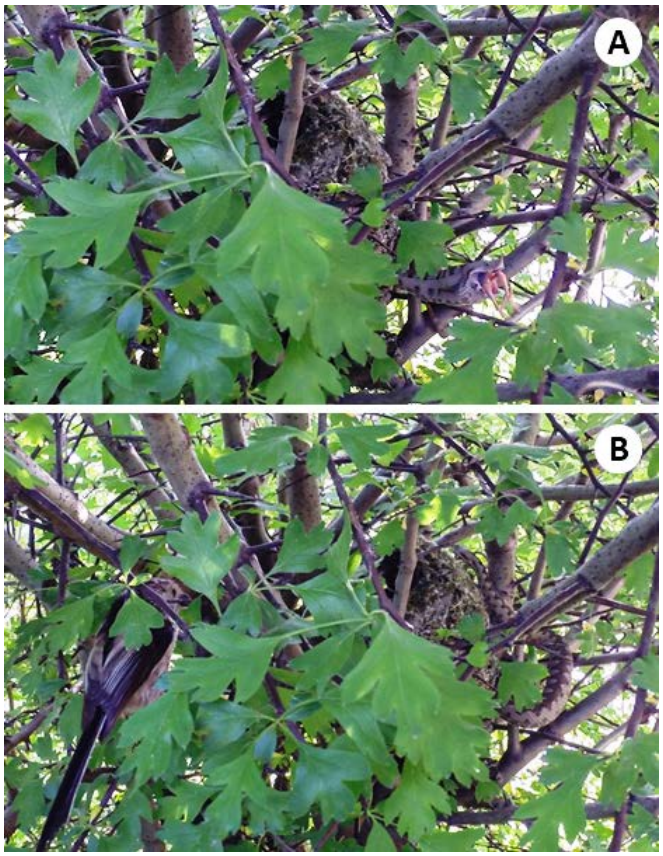

Figure 2. Predation of nestlings long tailed tits by an adult female *Vipera ammodytes* – **A.** The viper ingesting the first nestling, after dragging it out of the nest, **B.** The viper is crawling on top of the nest in front of one of the parents, before entering it a second time to capture the second nestling. More detail of this behaviour is shown in BHS video (2025b).

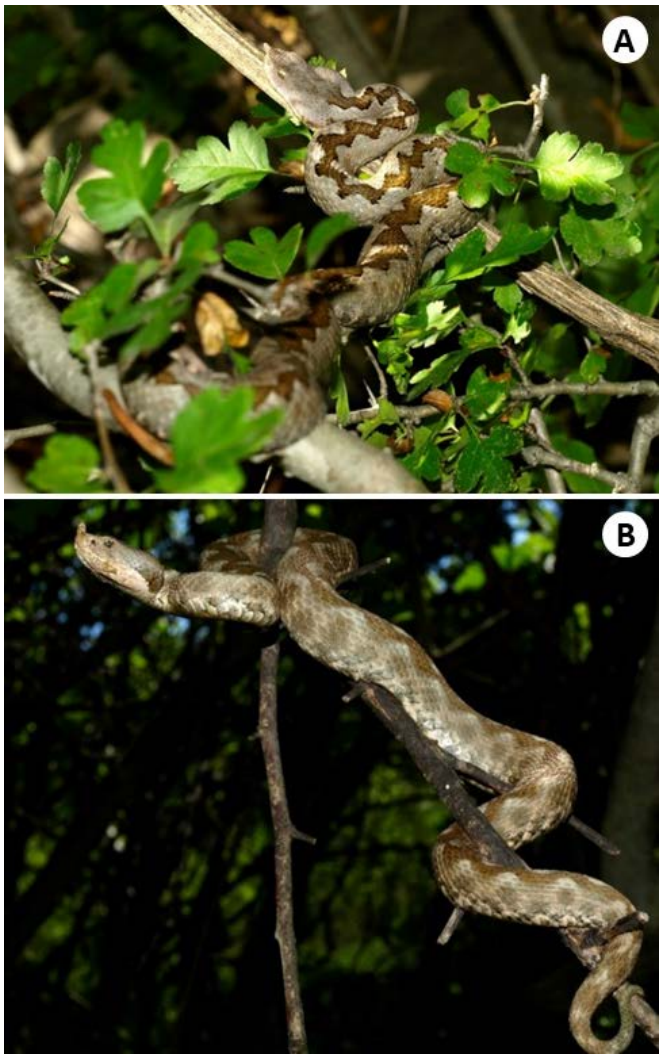

Figure 3. Two adult female *Vipera ammodytes* demonstrating – **A.** Probably waiting in ambush for prey, **B.** Escaping after capture and handling which resulted in it climbing up a tree

head inside it, bit and dragged out one of the nestlings and then started to swallow it (Fig. 2; BHS video, 2025b). While swallowing, one of the parents returned with food in its beak. The parent panicked, jumping from branch to branch around the nest, while the viper continued to swallow its nestling (BHS video, 2025b); this process took about 11 minutes. After devouring it, the viper captured another one, while the parent was still moving around the nest, after which, the snake climbed down from the nest whilst still holding the second nestling in its mouth; the viper went out of sight 2.5 minutes after capturing it. The long-tailed tit nestlings appeared to be about 4 days old. After the viper left, the parents approached the nest and continued feeding the remaining nestlings, which were left unharmed (BHS video, 2025b). The total number of nestlings in the nest remained unclear, at least two were left after the snake retreated.

It is well documented that birds make up a small part of the diet of *V. ammodytes* (Beshkov, 1977; Luiselli, 1996; Cattaneo, 2010; Anđelković et al., 2021; Tomović et al., 2022), however, to the best of our knowledge direct observations on bird predation until now have been lacking. Other European *Vipera* spp.are considered as mainly terrestrial sit-and-wait ambush predators but there have been similar observations of them as arboreal predators. For example, the northern viper *Vipera berus* has been reported in Britain preying on goldfinch *Carduelis carduelis* (Springwatch, 2014) and reed bunting *Emberiza schoeniclus* (Springwatch, 2024) and in the Netherlands preying on the Eurasian blue tit *Cyanistes caeruleus* (Groen et al., 2020); this species also occasionally preys on the nestlings of ground-nesting birds (Pielowski, 1962; Luiselli & Anibaldi, 1991; Groen et al., 2020). *Vipera ursinii* is also known to occasionally prey on fledgling birds in Italy, especially on those species that nest in dwarf juniper bushes (Agrimi & Luiselli, 1992).

Thermoregulation and/or waiting in ambush for prey

We report three different cases of this type of behaviour. On 29 September 2019 at 11:56 h near Gara Lakatnik Village (43° 5'20" N, 23° 23'2" E; 450 m a.s.l.), north-western Bulgaria, an adult female viper (SVL = 50.6 cm, TL = 5.8 cm, W = 69.15 g) was found by AD, NS and Kostadin Andonov in a light, sparse deciduous forest with shrub undergrowth, growing on a rocky/stony area. The weather was calm with light wind, cloud cover about 20% and microhabitat temperature 26 °C. The viper was positioned approximately 60 cm above the ground, stretched out between a thin tree, a shrub and a vine, with its body

being in partial shade. The back of its body was coiled on the stem of young cherry plum tree *Prunus cerasifera*, the middle part of the body was positioned horizontally on a thin branch of a hawthorn shrub and the front part and head of the viper were positioned in an upward position on a thin Traveler's joy vine *Clematis vitalba* (Fig. 3A). The viper was photographed in-situ and then captured for measurement. After its release, it hid beneath the rocks and stones.

On 31 May 2013 at 14:50 h in the Kresna Gorge (41° 45'53" N, 23° 9'20" E; 210 m a.s.l.), south-western Bulgaria, a juvenile female (SVL = 21.4 cm, TL = 2.5 cm, W = 8.56 g) was found by AD on a branch of a Jerusalem thorn in a stony area, overgrown with shrubs, situated between a dirt road and a railway. The weather was calm with light wind, cloud cover 50% and microhabitat temperature 30.5 °C. The viper was coiled on itself and positioned on a small branch in shade, approximately 20 cm above the ground. Following the sighting, the viper was captured for measurement and released back on site where it hid beneath some stones.

On 30 September 2024 at 12:54 h near Cherni Vit Village (42° 50'22" N, 24° 11'40" E; 606 m a.s.l.), northern Bulgaria, an adult female (SVL = 53.1 cm, TL = 6.1 cm, W = 31.82 g) together with four neonate vipers (as well as the shed skins of the latter) were found by AD. They were around a small hole between the roots of a shrub-sized hornbeam *Carpinus* sp. on a rocky/stony hill with scattered shrubs and trees. The weather was calm with light wind, cloud cover was 20% and microhabitat temperature 27 °C. While all neonates were laying on the ground (three of them basking and one hiding under a small stone), the female was stretched on the branches of the shrub approximately 30 cm above the ground, with its body exposed to the sunlight. Immediately after the sighting, the female as well as three of the neonates were captured for measurement, while the fourth neonate escaped into the hole. The second half of the female's body was thinner than the first half and had a long furrow, indicating that it was post-parturition and that the four neonates were most likely her offspring. After their release, all individuals hid in the hole, where the first neonate had escaped.

It is very difficult to distinguish between arboreal thermoregulation and/or waiting in ambush, therefore, we have presented them together. Furthermore, vipers might often combine these two types of arboreal behaviours. For instance, during colder days the ground warms up more slowly than the air above it, resulting in the temperatures of the ground often being lower than those of the air (Dyugmedzhiev et al., 2021). Therefore, on cold days vipers may climb onto branches where they can bask in a more favourable thermal environment, as already reported for *Vipera aspis* (Saint Girons, 1975; Marx, 2022), and wait in ambush for birds. During hot days, on the other hand, vipers escape from the high temperatures either by retreating to shelters (i.e. under stones, in holes, etc.), or to microhabitats that are in the shade, where they could hunt for prey (Dyugmedzhiev et al., 2021; 2022). In these sites, vipers might climb on the branches of trees or shrubs to wait for birds, or lay on the ground to hunt for small mammals or lizards that are also trying to escape from the heat. We speculate that this is precisely the case with the adult female observed around Gara Lakatnik Village. The day of the observation was hot, with microhabitat temperatures in the sun exceeding 28–29 °C, however, temperatures in the shade, where the viper was found were lower and much more tolerable (about 26 °C). The height at which the viper was resting, together with its peculiar position and placement, indicates that most likely it was waiting in ambush for birds. Furthermore, ambient temperatures in sunny places above the ground might often be more tolerable than those on the ground during warm and hot days, because of the cooling effect of the wind. Thus, vipers that stay in sunlight may find more suitable thermal conditions on the branches of shrubs where they can wait for birds or lizards. Such may have been the case with the observation of the adult female around Cherni Vit Village.

In the case of the juvenile female viper, observed in the Kresna Gorge, it seems more plausible that the viper was thermoregulating, instead of waiting for prey. This observation was made in a particularly hot day (i.e. microhabitat temperature of 30 °C in the shade). As suggested above, the thermal conditions 20 cm above the ground may have been more tolerable than those on the ground. While it seems unlikely that such a small viper was waiting to predate a bird, it may well have been waiting for lizards that may also climb on those lower branches to escape from the heat of the ground.

Escaping after capture and handling

We report three different cases on this type of behaviour.

On 19 July 2014 at 13:55 h near Balsha Village (42° 51'20" N, 23° 15'8" E; 713 m a.s.l.), central-western Bulgaria, a gravid adult female viper (SVL = 48.4 cm, TL = 5.7 cm, W = 149.8 g) was found by AD in an abandoned quarry overgrown with a mixture of grasses, shrubs and scattered trees. The weather was calm without wind, cloud cover was about 80% and microhabitat temperature was 20.7 °C. The viper was hidden beneath a stone under a shrub-sized hornbeam. The snake was captured, measured, colour-marked and photo-documented, with the entire process lasting around 20 minutes, after which it was released at the same spot. Right after its release, however, the viper did not hide under the stones, but instead started climbing on the inner branches of the shrub. It climbed until it reached approximately 60 cm above the ground after which it stopped and remained there. The viper was then left alone. At 16:04 h AD checked the area again and found the viper still on the shrub, only slightly moved so that its body would be exposed to sunlight. The viper was not disturbed. At 18:00 h, AD checked the area one last time and observed (without disturbing it) that the viper had now descended to the ground and was basking in front of the same shrub.

On 11 May 2017 at 11:25 h in the Kresna Gorge (41° 45'58" N, 23° 9'8" E; 213 m a.s.l.), south-western Bulgaria, an adult female (SVL = 53.4 cm, TL = 6.7 cm, W = 95.35 g) was found by AD on a stone pile overgrown with a mixture of tree- and shrub-sized hornbeam trees. The weather was calm with light wind, cloud cover of 20%, and microhabitat temperature was 18.5 °C. The viper was on the stones with most of its body positioned in the shade under a shrub. Similarly to the previous case, on its release following the measurement procedures, the viper started climbing on the inner branches of the shrub. It climbed until it reached a vertical part of the

branch, approximately 75 cm above the ground, after which it stopped and remained there, partially coiled around it (Fig. 3B). The viper stayed in this position for about 1 minute after which it moved the front part of its body downwards, to the part of the branch where its tail was. During this period the viper was photographed quickly and was then left alone. No further observations were made.

On 12 April 2024 at 14:50 h (local time) near Banichan Village (41° 37'34" N, 23° 43'40" E; 563 m a.s.l.), south-western Bulgaria, a pre-shedding adult male (SVL = 59.2 cm, TL = 8.1 cm, W = 151.44 g) was found by AD in a shrubbery area overgrown with Jerusalem thorn. The weather was calm with light wind, cloud cover of about 50%, and microhabitat temperature was 25.3 °C. The viper was on the ground, in partial shade under one of the shrubs. On release post-measurement, the viper climbed on the inner branches of the shrub until it reached approximately 100 cm above the ground after which it stopped and remained there. The viper was left alone. On the way back, at 17:20 h, AD checked again the area, however the viper could not be found.

The arboreal behaviour shown after capture and handling is most likely a result of stress. Usually, vipers are well aware of the characteristics of their habitat, and the most common ways of getting away from their enemies is either by escaping through the vegetation and/or hiding in various protective shelters in close proximity, such as under stones, in rock crevices, holes, etc. It appears that when under stress, they might choose alternative escape routes, such as climbing on branches of shrubs. This behaviour, however appears to be very rare in *V. ammodytes*, since it was exhibited in only 3 cases from out of more than 1,000 captured and handled vipers during the course of the study. Arboreal escape has already been reported for *V. aspis*, where several individuals were reported to climb on trees at heights of 70–190 cm while escaping from the observer, without being previously captured (Marx, 2022). The author speculated that this escape tactic is a result of behavioural plasticity in the species allowing it to adapt to changing environmental conditions caused by the predation pressure of wild boar's abundant population.

Vipera ammodytes is generally considered to be a terrestrial species (Phelps, 2010) but arboreal activity of *V. ammodytes* and other *Vipera* spp.is difficult to observe. Consequently, further study may well demonstrate that arboreal activity is a more important component of the behavioural ecology of *V. ammodytes* than previously reported.

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RESEARCH ARTICLE

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An analysis of the illegal trade of non-marine chelonians in West Bengal, India: Study based on a seven-year confiscation history

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ABSTRACT – This study of the illegal trade in non-marine chelonians (NMC) in West Bengal is based on 133 confiscation reports from various online news sources and from Divisional Forest Department Offices obtained in the period 2017–2023. A total of 33,317 NMC seizures were identified, spanning nine different species, three Endangered, three Vulnerable, one each of Near Threatened and Least Concern, and one invasive alien. The vast majority of seizures were of two species, the Indian flapshell turtle *Lissemys punctata*, which is a soft shelled species and accounted for 94% of identified seizures, and the Indian star tortoises *Geochelone elegans* that accounted for 5.2% of NMC. Confiscations were from 15 districts of West Bengal of which North 24 Parganas district was found to be a particular hotspot accounting for 57% of cases. The supply chain of *L. punctata* extended beyond West Bengal to Uttar Pradesh (50%) and Odisha (25%), whereas *G. elegans* were smuggled exclusively from Tamil Nadu. Additionally, 349 kg of body parts were seized. All confiscated items were meant either for domestic consumption or for illegal supply to markets in Bangladesh. Significantly more confiscations were recorded during the winter season. NMC seizures remained more or less constant throughout the COVID-19 pandemic with an increase in the involvement of women in illegal trading. West Bengal is a significant hub for the illegal trade of NMC and stringent conservation measures are required, in collaboration with various stakeholders, to prevent regional extinction of the species involved and the detrimental impacts of the local release of seized NMC.

INTRODUCTION

Globally, non-marine chelonians (NMC) are the most commonly trafficked group of reptiles among illegally traded wildlife (Rosen & Smith, 2010; Auliya et al., 2016). There is a vast and developing market in East and Southeast Asia for the trading of NMC (Chen et al., 2009; Harrison et al., 2016; Sy & Lorenzo, 2023) and unrestricted trade presents the greatest threat to the majority of Asian chelonian species (Cheung & Dudgeon, 2006; Rhodin et al., 2018; Stanford et al., 2020). They are traded for a variety of purposes such as food, traditional medicine and as pets (Shepherd et al., 2020). More than 50% of Asian NMC are currently threatened with extinction despite their protection under national laws, and their inclusion in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendices (Cheung & Dudgeon, 2006). With the evidence of both illicit domestic and international trade of NMC, India holds significant responsibility to address the ongoing Asian chelonian crisis (Mendiratta et al., 2017).

India, a megadiversity country, has the sixth highest freshwater turtle and tortoise biodiversity in the world, with the lower Gangetic plain being recognised as a turtle diversity hotspot (Buhlmann et al., 2009; Mittermeier et al., 2015). Over 78% (22 species) of Indian NMC are in threatened categories of the IUCN Red List (Mendiratta et al., 2017). All these species are also protected under the Wild Life (Protection) Act, 1972 of India (WPAI) so that the harvesting and trading of these species, whether presented as live, dead or as body parts, are punishable offenses (Bhupathy et al., 2014; Mendiratta et al., 2017). More than 11,000 NMC were illegally traded in India every year from September 2009 to September 2019 (Badola et al., 2019), with one study reporting that 54% of the Indian NMC species have been traded illegally (Mendiratta et al., 2017).

Soft-shell turtles (Family Trionychidae) are primarily hunted and traded for their meat (Krishnakumar et al., 2009), whereas hard-shell turtles (Family Geoemydidae) and tortoises (Family Testudinidae) harvested in India are trafficked largely for commercial pet markets in Southeast Asia and China (D'Cruze et al., 2015; Leupen, 2018). Three major trade routes are known to exist in India and one of the most active and popular is from Kolkata to Bangladesh and other Asian countries (Bhupathy et al., 2000). Despite being a well-known turtle trade route and a part of the Gangetic plain in the Kolkata zone of West Bengal, there are no scientific reports regarding the rates of NMC trade or seizure. The aim of the present study was to identify the species of NMC that are seized, their frequency, volumes, trading hubs, routes, and trade networks in West Bengal.

MATERIALS & METHODS

A thorough Google search was undertaken of online bulletins for information on seizures of NMC from West Bengal in the period January 2017 to December 2023. A variety of sources, including published literature, online media reports, blog posts, YouTube videos and other social media platforms and

records from non-governmental organisations (NGOs) were explored to find reports of NMC confiscations. Additionally, during the specified period, the Wildlife Crime Control Bureau (WCCB) eastern region was visited along with several Divisional Forest Offices (DFO) and range offices, for the collection of data on seized NMC. The search terms used were 'confiscated turtle', 'turtle seizure', 'confiscated tortoise', 'tortoise seizure', 'wildlife trade', 'turtle poaching' and 'tortoise poaching' in West Bengal, and also included were searches in the Bengali language (such as 'kochhop bajeapto' = chelonian confiscation, 'kochhop kenabecha' = chelonian trade, 'bonnoprani bajeapto' = wildlife confiscation in Paschim Bongo = West Bengal). Reports written in the English language and reports translated from the Bengali language into English were retained for further scrutiny. All reported seizures were carefully checked to avoid duplication. Whenever contradictory data were encountered in reports on the same seizure from different sources, the most comprehensive data sources were accepted for further analysis and duplicates were disregarded. Essential details from each record were extracted, such as the date and location of seizure, species seized, type of commodity (live or dead animals, meat or body parts), product quantity (number or kilograms), the intended purpose of trade (meat, pet or traditional medicine) and the routes of trafficking, if available. Furthermore, to record the exact location of each seizure, we documented additional information such as the mode of transportation and final destination of the cargo. Animals seized from public transport, pet stores, warehouses or markets were classified as part of the commercial trade. In cases where species identity could not be verified, it was designated as 'unidentified'. From the seizure records, sources of origin and sites of poaching were compiled. The data from Divisional Offices can be found in the Supplementary Material A.

Data on the geographic locations of confiscations and their collection points were used to generate maps illustrating trading hubs and routes. Arc GIS 10.8.2 software was employed for analysing and plotting the collected data, and graphical representations of results were generated in MS Excel 2019. To test for statistically significant difference between the three seasons, namely summer (March–June), monsoon (July–October) and winter (November–February); and between the 15 districts of West Bengal, NMC confiscations were subject to the non-parametric Kruskal-Wallis test using SPSS-26, this showed heterogeneity between both seasons and districts which justified pairwise post-hoc testing using the Mann Whitney U test.

RESULTS

Between January 2017 to December 2023, 133 confiscation reports of NMC involving West Bengal were obtained and analysed which accounted for seizures of 33,317 individuals from a total of nine species. Dead turtles numbered 1,666, while 420 specimens could not be identified due to being incomplete body parts (Table 1). The greatest number of confiscations was recorded in 2017, and the lowest in 2018 (Fig. 1). From 2019 onwards the rate was relatively constant

Table 1. Non marine chelonians (NMC) confiscated in West Bengal, 2017–2023 and IUCN Red List status

Species	No. of individuals confiscated	No. confiscated as % of all identified NMC	IUCN Red List category (updated 2024)
Lissemys punctata	30,872	93.84%	Vulnerable
Geochelone elegans	1,712	5.20%	Endangered
Nilssonia gangetica	268	0.81%	Endangered
Nilssonia hurum	36	0.11%	Endangered
Pangshura tentoria	1	0.003%	Least Concern
Pangshura tecta	3	0.009%	Vulnerable
Chitra indica	2	0.006%	Near Threatened
Pangshura smithii	2	0.006%	Vulnerable
Trachemys scripta elegans	1	0.003%	Invasive alien species
Total identified	32,897		
Unidentified	420		
Grand total	33,317		

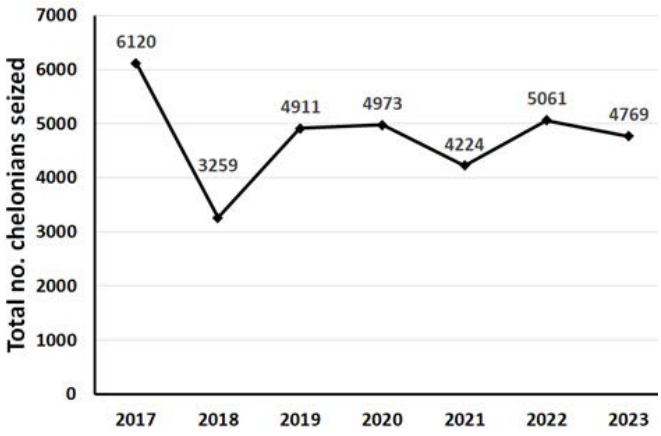

Figure 1. Number of non-marine chelonians confiscated in West Bengal, 2017–2023

suggesting that there was no impact of the COVID pandemic on the seizure rate (Fig. 1). The Indian flap-shelled turtle *Lissemys punctata* was the most frequently confiscated turtle, accounting for 93.87% of all identified NMC, followed by the Indian star tortoise *Geochelone elegans* which accounted for 5.20% (Table 1). The remaining seven species made up less than 1% of the identified NMC and included a single specimen of the invasive alien species *Trachemys scripta elegans*, which was confiscated in Siliguri. Of the eight native confiscated species, three *Nilssonia gangetica*, *Nilssonia hurum* and *Chitra indica* are categorised as Endangered, three *L. punctata, Pangshura tecta* and *G.*

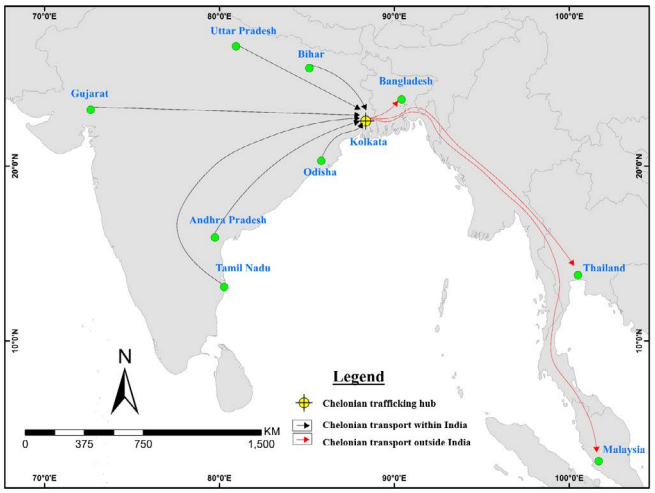

Figure 2. Transport routes of the illegal non-marine chelonian trade within and outside India

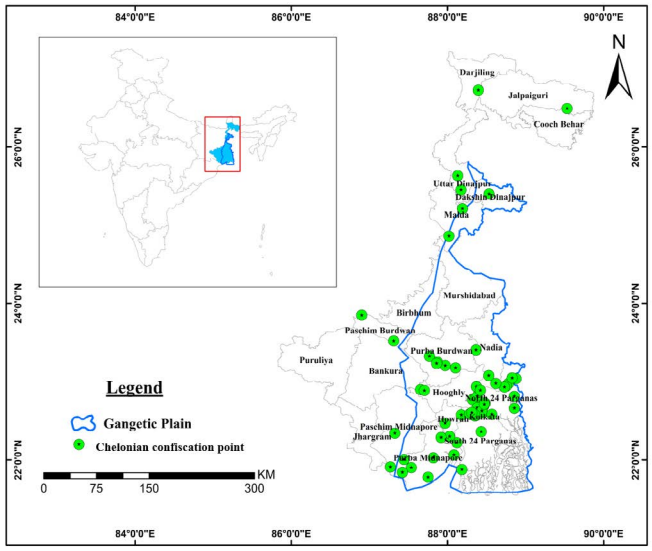

Figure 3. Non-marine chelonian confiscation points in West Bengal identified during the current study

Table 2. Number and percentage of non-marine chelonians (NMC) illegally entering in West Bengal from other Indian states, 2017–2023

NMC source state	No. of confiscated NMC originating outside West Bengal	% of confiscated NMC from identified source states
Uttar Pradesh	22	55%
Tamil Nadu	4	10%
Andhra Pradesh	1	2.5%
Gujarat	1	2.5%
Orissa	10	25%
Bihar	2	5%
Total NMC from identified source states	40	
Total NMC from unidentified source states	93	
Grand total confiscated NMC	133	

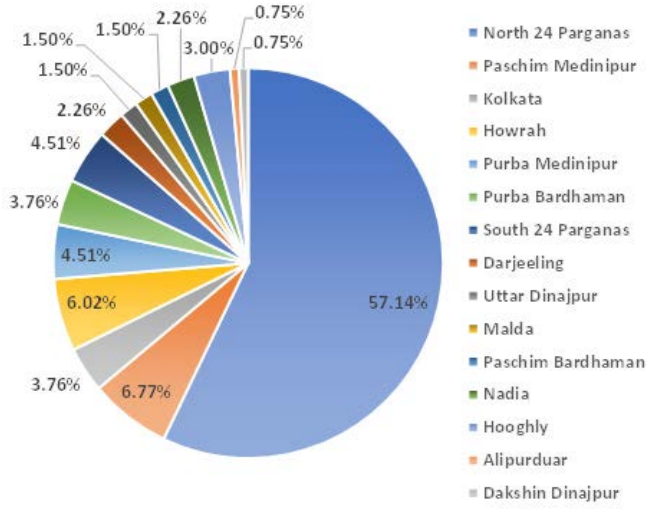

Figure 4. Percentage of non-marine chelonian seizures among the various districts of West Bengal, 2017–2023

elegans as Vulnerable, and *Pangshura smithii* as Near Threatened and *Pangshura tentoria* as Least Concern as per IUCN Red List (Table 1). A full listing of the conservation status of these species is give in Supplementary Material B Table 1S.

All the NMC were confiscated from markets, fish transport vehicles, rail stations, bus terminals and trucks, where they were stored secretly. In one instance, two individuals of *L. punctata* were seized from a marquee of the Goddess Durga where they had been displayed but were not for sale and so were categorised as non-commercial. The majority of the confiscated wild turtles were imported to West Bengal from different states of India (Fig. 2). Uttar Pradesh accounted for most of the identified imports (55%) followed by Odisha (25%) and Tamil Nadu (10%). The states of Bihar, Andhra Pradesh and Gujarat together accounted for the remaining 10% of the identified sources (Table 2). All the confiscated tortoises in West Bengal originated from Tamil Nadu, the southernmost state of India. Among all NMC seizures in West Bengal, 86.47% were from the lower Gangetic plain, and more than 67% of those were from districts sharing an international border with Bangladesh (Fig. 3).

Seizures were reported across 15 districts in West Bengal. North 24 Parganas district in southern West Bengal accounted for over half (57.14%) of all incidents (Fig. 4). There was a statistically significant difference in the number of confiscated NMC among the 15 districts (Kruskal-Wallis, $H = 40.651$, $p < 0.001$), and pairwise comparisons revealed that North 24 Parganas district accounted for significantly more confiscations when compared to other districts (Fig. 5) and the heat map further reinforces this finding (Fig. 6). More than half of the seizures (59.26%) were of NMC consignments

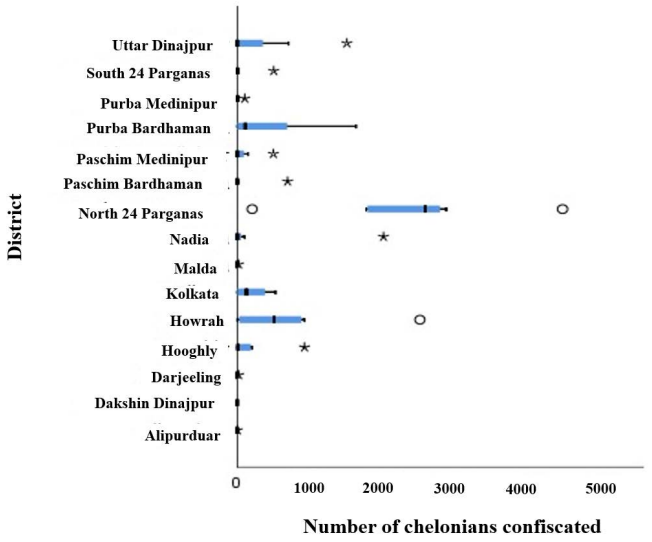

Figure 5. Number of non-marine chelonians confiscated in various districts of West Bengal 2017–2023, ° = outlier, * = extreme outlier

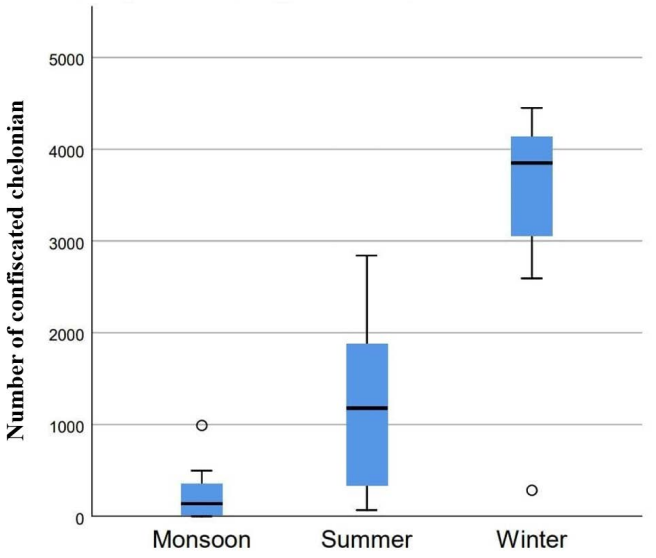

Figure 7. Number of non-marine chelonians confiscated during different season in West Bengal, 2017–2023, ° = outlier

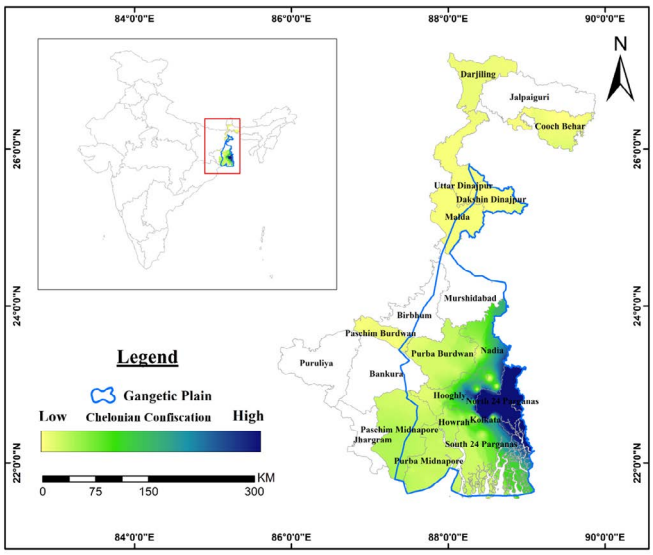

Figure 6. A heat map of non-marine chelonian confiscations from the districts of West Bengal, 2017–2023

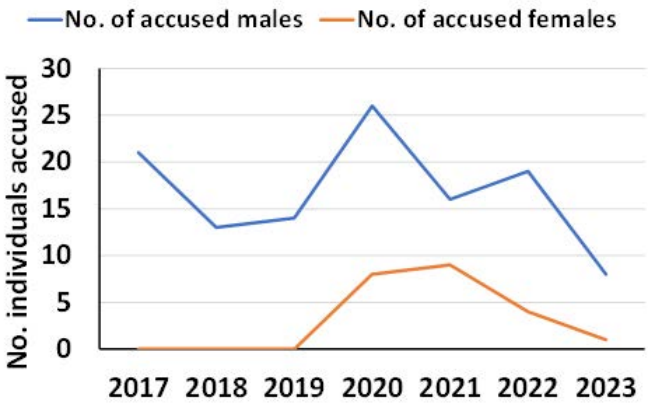

Figure 8. Gender split of people accused of involvement in non-marine chelonian trafficking

in transit by road (38.89%) and rail (20.37%). The remaining 40.74% were confiscated from markets, residential houses, hotels or warehouses. Of the 17 consignments confiscated in transit, 14 were destined for Bangladesh, two for Thailand, and one for Malaysia (Fig. 2). Three confiscations, one each in Kaliachak (Malda district), Bangaon (North 24 Parganas district) and Gangarampur (Dakshin Dinajpur district) resulted in the confiscation of a total of 349 kg of body parts, intercepted while being smuggled to Bangladesh. There was a statistically significant difference in the number of confiscated NMC between seasons (Kruskal-Wallis test, $H = 11.896$, $p < 0.003$), and pairwise comparisons indicated that the number of confiscations was significantly higher during the winter season (Fig. 7).

Both men and women were involved in the trafficking of turtles. In 2020, more people, particularly women, were involved in turtle trafficking in comparison to other years (Fig. 8) with a rise in women's participation from 2020 to 2022 which more or less coincides with the period of the COVID pandemic.

DISCUSSION

The lower Gangetic plain has been recognised as a priority area of NMC conservation (Mittermeier et al., 2015) and the results of the present study has highlighted it as a significant hub for national and international NMC trafficking and markets (Figs. 2, 3 & 6). Demand for them as food, traditional medication and as pets, has accelerated the decline and extinction of over half of the known species of NMC (Stanford et al., 2020), yet this has received little attention from conservation biologists and remains understudied (Stengel

et al., 2011; Stanford et al., 2020). In India, during a five year period (2011–2015) there were 223 confiscation reports with seizures totalling 58,442 live NMC (Mendiratta et al., 2017) while in a seven year period (2013–2019) there were 268 reports with seizures totalling 74,000 live or dead individuals (Sengottuvel et al., 2024). In the present study covering the seven year period 2017–2023, West Bengal alone accounted for 133 confiscation reports detailing the seizure of 33,317 individuals that included six Vulnerable/Endangered species (Table 1).

In the present study *L. punctata* accounted for about 94% of identified seizures; it is likely that these were being trafficked for food. This species is currently listed as Vulnerable on the IUCN Red List (Rahaman et al., 2021), protected under Schedule I of the WPAI, and is widely harvested and trafficked for its meat and supposed medicinal value in India and in other Asian countries (Das, 1991; Choudhury et al., 2000; Krishnakumar et al., 2009; Bhupathy et al., 2014; Mendiratta et al., 2017; Mandal et al., 2024). It is now the most traded species, potentially due to its non-aggressive behaviour and small size. It might experience a fate similar to *Batagur baska* that was once an abundant species in the mangroves of West Bengal and Odisha (Mallick et al., 2021), which being very palatable was over-exploited in these regions (Moll et al., 2009); *B. baska* is listed as Critically Endangered on the IUCN Red List (Praschag & Singh, 2019) being limited to Sundarban area and likely extirpated from Odisha (Mallick et al., 2021). In this context, conservation of *L. punctata* should be prioritised.

Geochelone elegans, which accounted for about 5% of all identified seizures, is largely trafficked for the international pet trade (D'Cruze et al., 2015). Smuggled *G. elegans* in West Bengal originated exclusively from Tamil Nadu. There are indications that the smuggling route of this species is currently shifting (Stoner & Shepherd, 2020). Once commonly trafficked through Chennai (Sengottuvel et al., 2024), *G. elegans* is now rerouted to Kolkata, and subsequently to Malayasia (where it is an exotic species) either directly or via Bangladesh, most likely due to increased detection risk in Chennai (Stoner & Shepherd, 2020).

In West Bengal, North 24 Parganas is the largest hub of illegal trading, accounting for 57.14% of identified seizures and has also emerged as a soft-shell turtle trafficking hub because this district shares 280 km of international border with Bangladesh (Sengottuvel et al., 2024). Other important trafficking centres are Paschim and Purba Medinipur, Howrah and Kolkata districts. West Bengal is evidently a major hub for meat trading rather than for the pet trade. Approximately 150 kg of turtle meat is traded daily in the local fish markets of Purba and Paschim Medinipur districts (Bindra, 2023). Moreover, *L. puncata* are illegally harvested and sold in Paschim Medinipur district for their meat (Mandal et al., 2024). It was also found that the bulk of the supply of soft-shell turtles in West Bengal originated outside the state with 50% from Uttar Pradesh and 25% from Odisha. This confirms recent suggestions that Uttar Pradesh is the major supplier of soft-shelled turtles to consumers across India (Sengottuvel et al., 2024). Historically, the primary transportation methods of soft-shell turtles reported for West Bengal was by road (Choudhury & Bhupathy, 1993). The present study has demonstrated that this is still the case. However, rail transport has been also suggested as an easier route of entry due to the lack of trained personnel at entry and exit points (Shepherd et al., 2007) coupled with inadequate scanning equipment at railway stations (Emogor et al., 2021). Significantly more turtles were confiscated during the winter season, likely because an increase in demand during the festive season in West Bengal when indigenous people consume turtle meat. Moreover, the capture of ectotherms is easier in the winter season due to their lowered metabolic rate and activity level (Litzgus & Hopkins, 2003). Likely because of the lack of alternative sources of income during the COVID pandemic, turtle trafficking saw an increased participation of women during this period. In contrast, there was apparently a remarkable decrease in poaching cases of NMC in India during the lockdown period, even though the total number of wildlife poaching cases more than doubled between pre-lockdown (10 February to 22 March, 2020) and lockdown periods (23 March to 3 May, 2020) (Badola, 2020).

From 2018 to 2020, the red-eared slider *T. s. elegens* was traded in large quantities in India at very low prices (USD 0.36 to USD 0.48 per individual) due to ready availability and their better survival rate and small size which make them suitable for clandestine transportation (Pragatheesh et al., 2021). This North American species is recognised as one of the top 100 most invasive species worldwide (Dupuis-Desormeaux et al., 2022). In India, it has also been reported from Gujarat, Karnataka, West Bengal, Punjab, Hyderabad, Rajasthan and Goa (Chaudhuri et al., 2018; Jadhav et al., 2018; Vyas, 2019), and has been reported as a predator of native turtle species (Vyas, 2020). Although only one case of trade in this turtle was recorded in this study, the presence of *T. s. elegans* might have a profoundly destructive impact on the local ecosystem. Similar observation has also been made in Rajarhat wetland, Kolkata (Chaudhuri et al., 2018).

The present study unequivocally indicates that West Bengal serves as a significant hub for the illegal trade of NMC, with North 24 Parganas district as its epicentre. As such, enhanced anti-poaching measures are imperative in West Bengal, encompassing advanced poacher identification techniques such as patrol dogs, conducting efficient searches in trains, rail stations, trucks and airports using modern devices and quick response enforcement strategies. Trafficking of NMC shells, both carapace and plastron, to meet the increasing demand for traditional medicines in Southeast Asia and China is well known (Compton, 2000; Sundar, 2004; Chen et al., 2009; Das & Singh, 2009). The confiscation of body parts, however, is far more difficult compared to live specimens as it demands good species identification skills, which are a clear training need.

It is crucial to note that following seizure many live turtles are released into local water bodies, which could potentially have detrimental impacts on the ecosystem. It is essential to establish adequate infrastructure for housing and rehabilitating confiscated turtles. Authorities must conduct thorough assessments of the health and source of origin of the confiscated individuals before release to mitigate the risk of disease transmission, disruption of population genetics,

and adverse effects on local biodiversity. Harvesting for illegal trafficking, along with other threats such as climate change, threaten the extinction of these chelonians in the wild. As a contribution to the long-term survival of these species, trafficking must be controlled and coupled with thorough monitoring and implementation of national conservation legislation.

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The roar of the tiger stream treefrog *Hyloscirtus tigrinus*: advertisement call, distribution update, novel colouration patterns and comments on natural history

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ABSTRACT – *Hyloscirtus tigrinus* is a rare, Endangered and colourful stream treefrog inhabiting the paramos and high Andean forest of south-western Colombia and northern Ecuador. During 2021–2023, we undertook fieldwork in Colombia at the Puracé National Park and surrounding areas, which yielded observations on the distribution, behaviour, advertisement call, colour pattern variations and phylogeny of this species. We report two new localities for *H. tigrinus*, which extends the north-eastern margin of its range by 18 km and gives a total of 10 known localities; these are restricted to between 2,638–3,470 m a.s.l.. Recordings of the male advertisement call indicate a single low-pitched pulsed note without frequency modulation, composed of two to six pulses. The call is emitted at a rate of 37.8–48.3 calls/min, with a dominant frequency between 1,636.52–1,894.92 Hz, and resembles that of *Hyloscirtus larinopygion* and *Hyloscirtus antioquia*, but *H. tigrinus* shows a lower number of pulses per call. Like other *Hyloscirtus* spp., the passive defensive behaviour of the species may include immobility, crouching and contraction. The populations from the new localities exhibit a light brown to reddish-brown dorsal colouration with black reticulate pattern or scattered blotches, which represents the most divergent colouration observed in the species. Phylogenetic and morphological analyses provide evidence that the most northerly populations, which exhibit divergent colouration, belong to *H. tigrinus*. Genetic distances for gene 16S (0.293–0.298%), indicate they are closely related to populations from Ecuador but remain distinguishable from them.

INTRODUCTION

The *Hyloscirtus larinopygion* (Amphibia, Hylidae) species group is a clade that comprises 19 Neotropical anurans species (Rivera-Correa et al., 2024), distributed in Andean ecosystems of Colombia, Ecuador and Peru, which are confined strictly to Andean creeks and streams in restricted elevational ranges (Coloma et al., 2012; Ron et al., 2018; Frost, 2024). The species within the group are relatively large (SVL >60 mm) and are colourful, ranging from dark to grey/brown tones contrasting with bright marks. They are nocturnal, with apparently seasonal activity and are remarkably elusive (Duellman & Hills, 1990; Coloma et al., 2012). Knowledge of the diversity and systematics of this group has increased in the last two decades (e.g. Coloma et al., 2012; Almendariz et al., 2014; Ron et al., 2018; Reyes-Puig et al., 2022; Rivera-Correa et al., 2024) but certain species still lack comprehensive information regarding their natural history.

Hyloscirtus tigrinus Mueses-Cisneros & Anganoy-Criollo 2008, is an Endangered, rare and colourful frog, part of the northern clade of *H. larinopygion* group (Coloma et al., 2012; IUCN SSC Amphibian Specialist Group, 2018; Ron et al., 2018). This medium-sized (SVL ~ 57 mm) frog is distinguished from its congeners by having a distinctive colour pattern: the dorsal region is yellow-green or yellowish-brown with transverse black stripes, sometimes reticulated, with or without mid-dorsal stripe; the flanks are yellow-green with black reticulation, and the iris may be coloured light grey or yellow with black reticulation (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). This species is known from eight localities in high Andean ecosystems of central and centro-oriental cordilleras in the departments of Cauca, Huila, Putumayo and Nariño in south-western Colombia, and the province of Sucumbíos in the cordillera oriental of northern Andes of Ecuador, at altitudes of 2,640–3,470 m a.s.l. (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011;

Coloma et al., 2012). In Colombia, *H. tigrinus* has been associated with the riparian vegetation of streams and lakes in high Andean and subparamo ecosystems (Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011). The potential threats to *H. tigrinus* are logging, burning, unregulated use of land for cattle raising and agricultural activities, pesticide use, invasive species (i.e. *Oncorhynchus mykiss*), unregulated tourism, and chytrid fungus (Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012).

The available information about the species includes adults and tadpole morphology, conservation status, geographical and ecological distribution and systematics (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012; Rivera-Correa & Faivovich, 2013; Rivera-Correa et al., 2016; Ron et al., 2018; Reyes-Puig et al., 2022; Sánchez-Nivicela et al., 2023). Herein, we contribute the first information on the advertisement call and the defensive behaviour of the species and provide an updated phylogeny for the *H. larinopygion* species group. We also present new records of *H. tigrinus*, updating its geographical distribution and describing the external morphology and additional colour patterns of the specimens collected.

MATERIALS & METHODS

New records and defensive behaviour

Our field work was undertaken during three visits to the Guanacas-Puracé-Coconucos and the Sotará paramo complexes in the Andean region of south-western Colombia. In the Guanacas paramo, we visited two localities - in February 2021 La Sabana (2° 25'53.06'' N, 76° 14'00.75'' W; 3,090 m a.s.l.) and in February 2023 Quebrada La Marquesa (2° 28'00.73'' N, 76° 12'56.14'' W; 3,180 m a.s.l.). Both are located in the village of Río Sucio, municipality of Inzá in the department of Cauca. In December 2021, in the Sotará complex we visited the Laguna La Magdalena, located in the Puracé National Park, municipality of San Agustín, department of Huila (1° 56'10.55'' N, 76° 36'22.64'' W; 3,460 m a.s.l.). At each site, we conducted visual and auditory encounter surveys (Crump & Scott, 1994) along the streams, creeks and lakes of Andean forest and subparamo ecosystems (Cuatrecasas, 1958). Geographical co-ordinates and altitude at survey sites were obtained with a Garmin GPSMAP 64s (map datum WGS 84). We described the defensive behaviour of the species based on observations during visual encounters and handling of specimens.

We photographed the captured individuals with a Nikon D610 with AF-S VR Micro-Nikkor 105mm f/2.8G IF-ED and external flash. Collected specimens were euthanised using benzocaine (Chen & Combs, 1999), fixed in 10% formalin, preserved in 70% ethanol (Cortez et al., 2006), and stored at the herpetological collection of the Museo de Historia Natural de la Universidad del Cauca (MHNUC-HE-AN) and the Amphibians Collection of the Museo de Historia Natural de la Universidad de Caldas (MHN-UCa-Am). These voucher specimens are listed in the Supplementary Material.

External morphology

We examined the external traits and took morphometric measurements for six collected specimens according to the definitions described by Duellman (1970) and Heyer et al. (1990). All measurements were taken with a digital caliper and rounded to the nearest 0.1 mm. The description of nuptial pads follows the terminology of Luna et al. (2012). Sex was determined by direct examination of gonads through a dorsolateral incision, while colour patterns were described from photographs of live specimens. We compare these morphological traits with those described for *H. tigrinus* by Mueses-Cisneros & Anganoy-Criollo (2008) as well as variations reported subsequently by other authors.

Phylogenetic analyses and genetic distances

Some individuals from the studied populations showed markedly different colour patterns relative to the known variation in *H. tigrinus*. To confirm their identification and update the phylogeny of the *H. larinopygion* group, we sequenced the mitochondrial genes 12S rRNA, tRNA-Val and 16S rRNA of two individuals we collected from Río Sucio, Colombia (MHN-UCa-Am 1869–1870). We also included one specimen from Santa Bárbara, Ecuador (QCAZ 38421). Tissues obtained from the specimens collected were deposited in the collection of the Museum of Zoology QCAZ at Pontificia Universidad Católica del Ecuador. Our phylogenetic analyses are based on the same matrix used by Ron et al. (2018), including the recently described *Hyloscirtus sethmacfarlanei* and *Hyloscirtus arcanus* (Reyes-Puig et al., 2022; Rivera-Correa et al., 2024; sequences from GenBank, https://www.ncbi.nlm.nih.gov/genbank). The sequences were aligned with MAFFT, E-INS-i algorithm (Katoh & Standley, 2013). Phylogenetic relationships were inferred under maximum likelihood (ML) with software IQ-TREE 2.2.0.7 (Nguyen et al., 2015; Minh et al., 2020) under default settings. We partitioned the matrix by gene and each partition was analysed under model GTR + R + I. To estimate branch support, we made 1,000 ultrafast non-parametric bootstrap searches (-bb 1000 command; Hoang et al., 2018) and 1,000 replicates for the SH-like approximate likelihood ratio test with the -alrt 1000 command (Guindon et al., 2010). We considered that branches with bootstrap values > 94 and SH-aLRT values > 79 had strong support. Uncorrected genetic distances for the gene 16S were calculated with Mesquite 3.7 (Maddison & Maddison, 2021).

Advertisement call description

We obtained call recordings of adult males from La Sabana and Laguna La Magdalena populations following the standard procedures described by Arcila-Pérez et al. (2024). All recordings were digitised in WAV format at a sampling frequency of 44.1 kHz and 16 bits amplitude resolution using a Tascam DR-40X handheld digital recorder connected to a Rode NTG1 unidirectional condenser microphone positioned about 1–2 m from the calling males. We recorded the air temperature (T) and humidity (RH) with an HTC-2 digital thermohygrometer (accuracy ± 0.1 °C and 1%), while the body size (SVL) of the captured individuals was measured with a SPI super polymid dial caliper (± 0.1 mm). Calls were

analysed using the software Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics, 2024); temporal parameters were measured from the waveform (in ms) and spectral parameters in spectrograms generated from the Fourier transformation with a Blackman algorithm, window length of 256 samples, overlap of 80% and a DFT of 1,024. We elaborated calls figures of waveforms, spectrograms and power spectra with Seewave v.2.1.8 package (Sueur et al., 2008) in the R environment using the RStudio interface (R Core Team, 2023; Posit Team, 2024).

We followed the note centred approach and terminology suggested by Köhler et al. (2017) and Emmrich et al. (2020) for the classification and description of the advertisement call. The following quantitative parameters were measured: call duration (= note duration), intercall silent interval, call repetition rate (calls per minute), number of pulses per call, pulse duration and pulse repetition rate (pulses per second), dominant frequency, low frequency (= 5% frequency), high frequency (= 95% frequency) and frequency bandwidth (= difference between high and low frequencies). We deposited the call recordings at the Colección de Sonidos Ambientales, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia (IAvH-CSA; listed in the Supplementary Material). We reviewed published information about the advertisement calls described for the stream treefrogs of the *H. larinopygion* group and compiled a comparative table showing the variation of commonly measured temporal and spectral traits.

Geographic distribution update

We compiled the historical localities of *H. tigrinus* from the literature (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012) and web portals such as Anfibios del Ecuador and the Global Biodiversity Information Facility (Ortiz & Páez-Rosales, 2022; GBIF.org, 2024). We generated an updated map and estimate of the extent of occurrence for the species using the geometric convex hull polygon, calculated from Minimum Bounding Geometry algorithm in QGIS 3.34.3-Prizren (IUCN SSC Red List Technical Working Group, 2024; QGIS.org, 2024).

RESULTS

New records and defensive behaviour

We documented 11 individuals of *H. tigrinus* at La Sabana from 10–18 February 2021. The vegetation in these areas is characterised by shrubs of *Baccharis* spp., *Blechnum loxense*, *Clethra* spp., *Diplostephium* spp., *Espeletia hartwegiana*, and *Pentacalia* sp., with emergent trees of *Clusia multiflora*, *Hedyosmum* spp. and *Weinmannia* spp. in a subparamo ecosystem. Two individuals were encountered on the ground in gallery forests along streams at least 5 m wide. The first individual was observed at 22:30 h near a small puddle beside the Río Negro (Fig. 1A). Upon capture, this individual exhibited defensive behaviour, bending its limbs close to the body, and remaining immobile for ten minutes until placed in a plastic bag (Fig. 1B). The second individual was vocalising at sunset (18:20 h) in the shrubs of a Río Negro tributary, approximately 1 km away from the first, but eluded capture.

Figure 1. *Hyloscirtus tigrinus* in situ - **A.** Male from La Sabana (left) and its Río Negro gallery forest habitat (right), **B.** Defensive behaviour of the same individual from La Sabana, **C.** Male from La Magdalena (left) and its subparamo ecosystem habitat (right).

The remaining nine individuals were detected near to streams and creeks in the Andean forest. One of these individuals was found on the ground about 2 m from the Quebrada San Antonio, another tributary of the Río Negro. Other individuals were detected near to creeks perched between 1.5–5.0 m high in the branches of shrubs or bamboo (*Chusquea* sp.). On 12 February 2023, in Quebrada La Marquesa, one individual was detected vocalising and captured on branches 1.5–2.0 m above the ground in the Andean forest, and a second was observed on nearby branches.

In the northern part of the Laguna La Magdalena wetland, several males were heard vocalising on 7 December 2021, near a border of subparamo vegetation, predominantly *Chusquea tessellata*, *Baccharis* spp., *Blechnum loxense*, *Clethra* spp. and *Diplostephium* spp. However, dense vegetation impeded observation. We located one male vocalising within mosses covering a shrub, about 1.40 m above the ground (Fig. 1C). The first detection occurred around 19:30 h, and the individual was recorded and

Figure 2. Colour pattern variation in *Hyloscirtus tigrinus* from Laguna La Magdalena- **A.** MHNUC-HE-AN 1410, and La Sabana, **B.** MHNUC-HE-AN 1351, **C.** MHNUC-HE-AN 1352, **D.** MHN-UCa-Am 1870. For a detailed description of these specimens see Supplementary Material.

captured at 23:48 h. On 8 December 2021, another individual was observed walking on the ground under shrub vegetation, using natural tunnels formed by the vegetation as a refuge.

External morphology

The six individuals collected, four from La Sabana (MHN-UCa-Am 1869 –1870, MHNUC-HE-AN 1351–1352), one from Quebrada La Marquesa (MHN-UCa-Am 1868) and one from Laguna La Magdalena (MHNUC-HE-AN 1410), were adult males with an SVL between 55.6–56.9 mm. They exhibited the diagnostic characters reported in the original description of the species (for details see Supplementary Material Tables 1S & 2S). Dorsally, their skin texture was shagreen while ventrally it was finely areolate but less so towards the throat; the surface of hind limbs was strongly areolate. The snout was rounded in dorsal view and truncate in lateral view. The tympanum and tympanic annulus were visible, with a prominent supratympanic fold extending from below eye across upper and posterior margins of tympanum towards posterior end of mouth and down to the arm insertion. Forearms were robust, axillar membrane absent, ulnar tubercles and folds absent, nuptial pads absent, fingers and toes with lateral fringes, disks on fingers and toes rounded, disks colouration black with cream or brownish spots, tarsal tubercles absent, webbing membrane on hands absent, calcar tubercle present, webbing membrane on feet present (I 2+-2- II1- -3 III 2+ 3IV 3--2V).

We observed four different colour patterns in life on the individuals collected (Fig. 2). Detailed descriptions of these colour patterns are presented in the Supplementary Material.

Phylogeny and genetic distances

Hyloscirtus tigrinus is sister to a clade comprising *Hyloscirtus psarolaimus, Hyloscirtus criptico, Hyloscirtus pacha* and *Hyloscirtus staufferorum*. Within *H. tigrinus*, the Colombian samples are sister to each other, and the resulting clade is sister to a clade of the Ecuadorian population (Fig. 3). Branch lengths are extremely short, suggesting low genetic differentiation between populations. Branch support for *H. tigrinus* is strong (ultrafast bootstrap = 100, SH-aLRT = 100). Genetic distances between the Colombian and Ecuadorian populations range from 0.293 to 0.298%. Intrapopulation distances range from 0 to 0.145%.

Advertisement call

We analysed 306 calls from four male *H. tigrinus*, two from La Sabana (102 calls) and two from Laguna La Magdalena (204 calls). The advertisement call consists of a single low-pitched pulsed note without frequency modulation (= non-frequency modulated pulsed call), with a duration of 142.88 ± 28.38 ms (61.9–216.6 ms) and composed of two to six pulses (188 of the 306 calls were composed of four pulses). The call is emitted at a rate of 37.8–48.3 calls/min with inter-call silent intervals of 1290.52 ± 293.18 ms (669.0–3024.2 ms). The pulses had a duration of 14.59 ± 4.32 ms (3.2–44.8 ms) and a repetition rate of 25.39 ± 1.91 pulses/s (17.34–31.77 pulses/s). The call had a dominant frequency of 1702.95 ± 68.34 Hz (1636.52–1894.92 Hz) and a bandwidth of 234.75 ± 45.45 Hz (172.26–344.53 Hz). Commonly, the highest energy is found in the second and third pulses. However, the first pulse may exhibit varying amplitudes, ranging from high to low. The final pulse consistently presents the lowest amplitude (Fig. 4). Similar to *Hyloscirtus antioquia* and *Hyloscirtus larinopygion*, the advertisement call of *H. tigrinus* is audible to the human ear as a faint melodious ‘brrrrr’ and resembles the sound of a stridulating cricket.

The males from Laguna La Magdalena were recorded at air temperature of 9.0 °C in the subparamo ecosystem and presented calls commonly composed for four to five pulses of longer duration and lower repetition rate (3.2–44.8 ms and 17.3–31.8 pulses/s) when compared with those from La Sabana (5.2–25.6 ms and 21.0–29.7 pulses/s; Fig. 4A & B). The recordings from La Sabana were obtained in the Andean forest ecosystem at air temperatures of 12.1–13.4 °C and showed calls without strong amplitude modulation between pulses, so the silent inter-pulse intervals were not always distinguishable. These calls had a shorter duration (76.2–154.2 ms) when compared with the calls from Laguna La Magdalena (61.9–216.6 ms) and were usually composed for three to four pulses; a male from this population showed the highest values of dominant frequency for the species, 1,808.8–1,894.9 Hz (Fig. 4C & D). Call variation in temporal and spectral traits of the four recorded males is summarised in the Supplementary Material Table 3S.

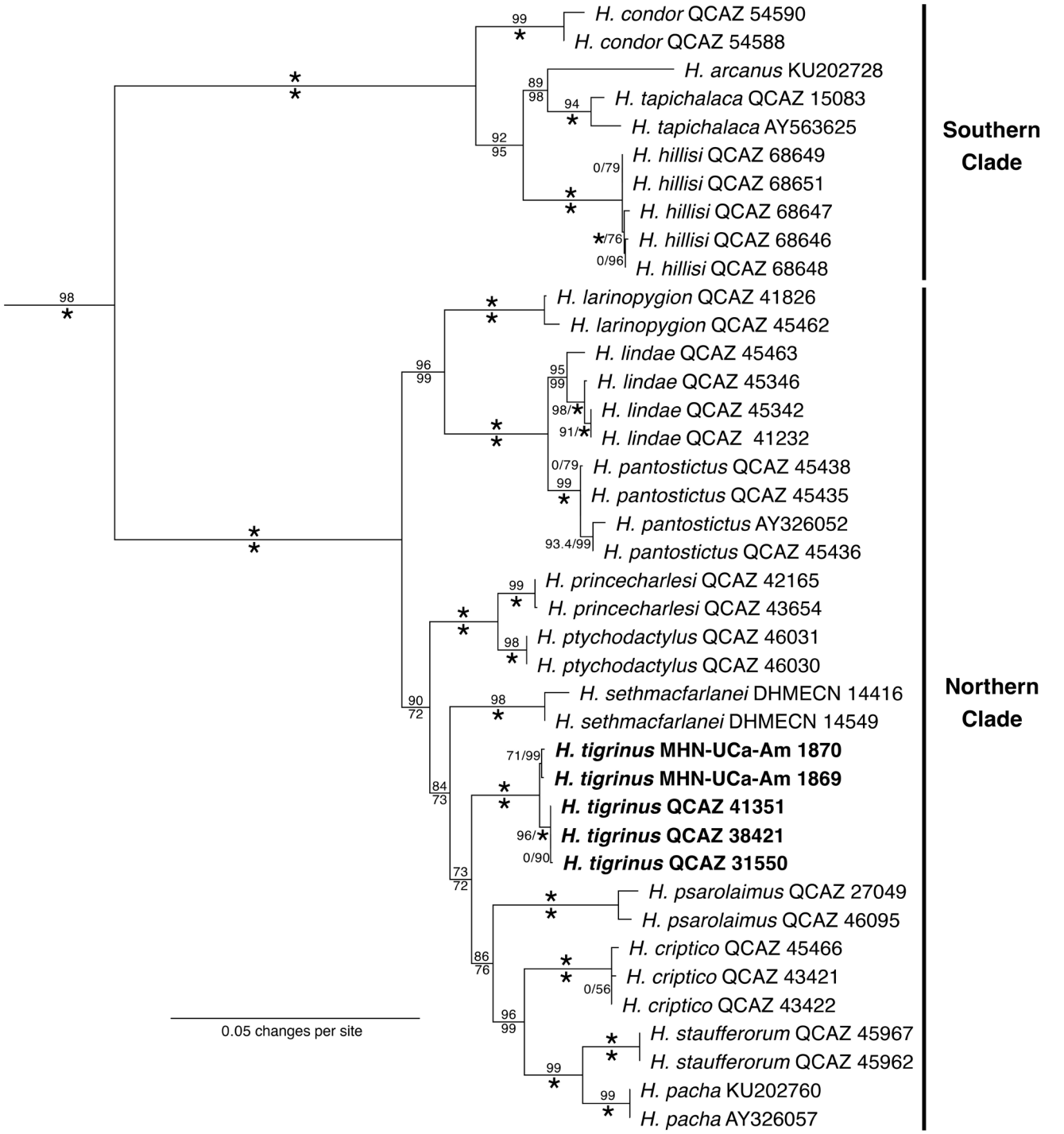

Figure 3. Phylogenetic relationships of species in the *Hyloscirtus larinopygion* group (updating Ron et al., 2018), inferred from the mitochondrial genes 12S rRNA, tRNA-Val, and 16S rRNA under maximum likelihood. Bootstrap values are shown above the branches and SH-aLRT values below. Values of 100% are represented by an asterisk.

Geographic distribution update

Based on our fieldwork and the geographical data associated with historical records, *H. tigrinus* is present in ten known localities in the Andes of south-western Colombia (departments of Cauca, Huila, Nariño and Putumayo) and northern Ecuador (province of Sucumbíos). The populations reported in the village of Río Sucio correspond to the northern most distribution of *H. tigrinus*, with Quebrada La Marquesa being the most northerly locality known for the species. The lowest altitudinal records of *H. tigrinus* have been from Santa Bárbara (Sucumbíos; 2,638 m), while Laguna La Magdalena corresponds to the highest altitudinal record (Huila; 3,467 m). The Minimum Bounding Geometry algorithm applied to these localities estimated the extent of occurrence of *H. tigrinus* to be 2,119.94 km^2 (Fig. 5).

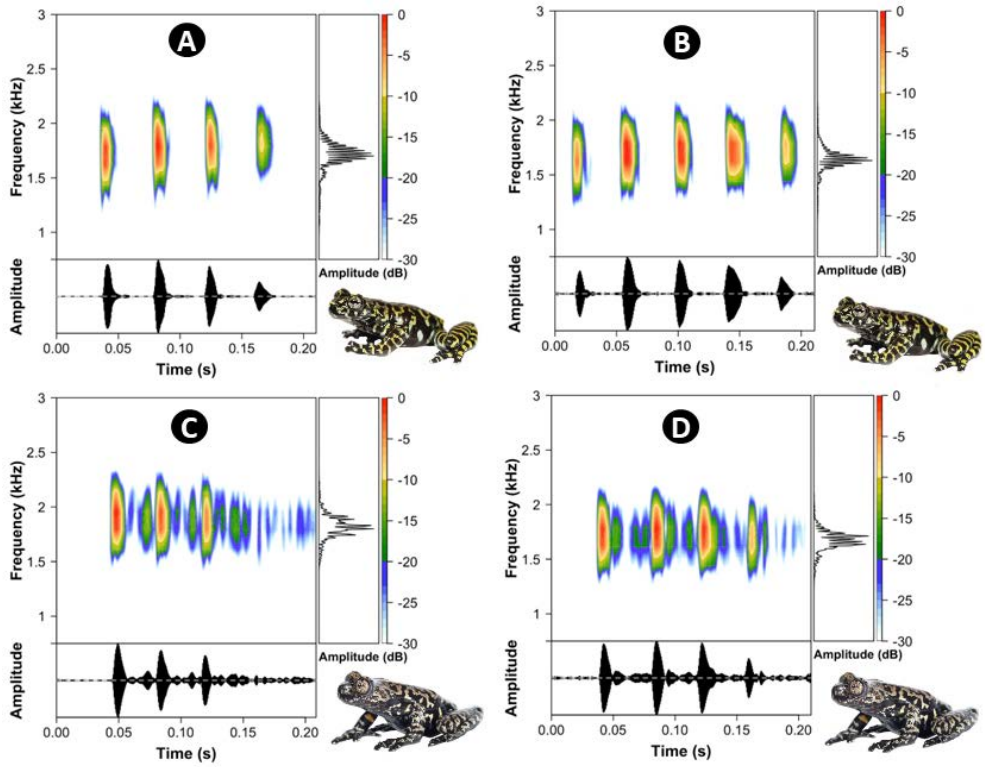

Figure 4. The advertisement calls for *Hyloscirtus tigrinus* from Laguna La Magdalena- **A.** IAvH-CSA-39385, **B.** IAvH-CSA-39386 and from La Sabana, **C.** IAvH-CSA-39383, **D.** IAvH-CSA-39384). For each male the spectrogram (top), oscillogram (bottom) and power spectrum (right) are shown.

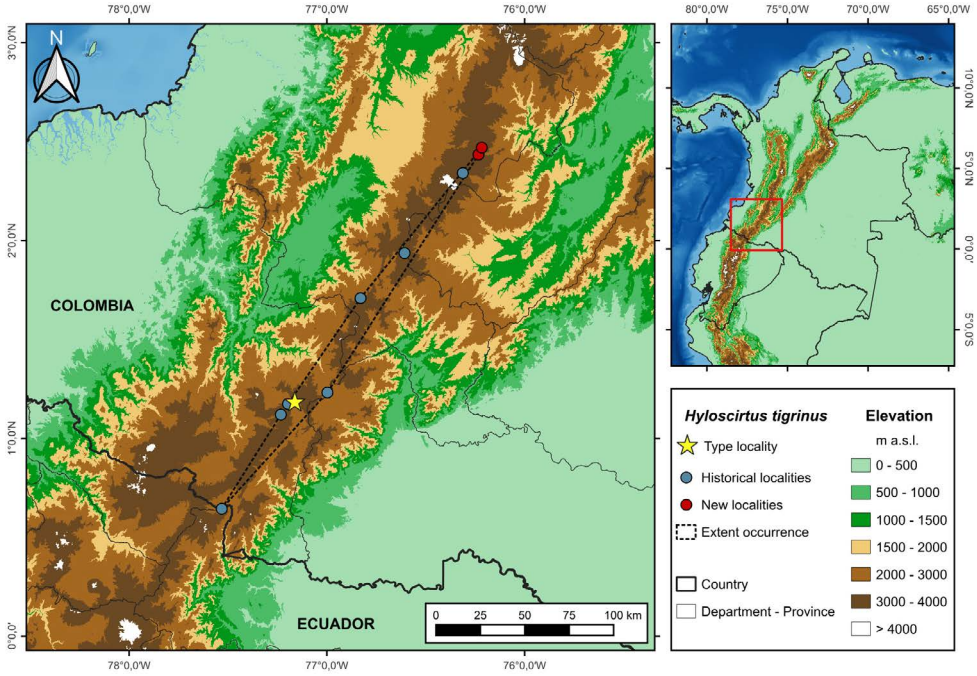

Figure 5. Locality records of *Hyloscirtus tigrinus* and the estimated extent of the species occurrence

DISCUSSION

As with other species of the *H. larinopygion* group, the individuals of *H. tigrinus* that we observed were associated with undisturbed montane streams and exhibited nocturnal activity (Coloma et al., 2012). They used dense vegetation as refuges and perches, where most individuals were observed in areas with dense branches at various heights, ranging from 0.5 m to 3–4 m above the ground. Based on our records from village of Río Sucio, we have extended the distribution range of *H. tigrinus* by 18 km in a straight line from the previous most northerly locality (Termales de San Juan, Puracé National Park, Cauca; Fig. 5). Likewise, this area is dominated by Andean forests and subparamo ecosystems, as has been

previously documented for the species (Mueses-Cisneros & Perdomo-Castillo, 2011).

The records from Laguna La Magdalena suggest that, among the species of the *H. larinopygion* group, *H. tigrinus* reaches the highest altitude (3,467 m), followed only by *H. larinopygion, H. antioquia* and *Hyloscirtus tolkieni* which are found up to 3,200 m (Rivera-Correa & Faivovich, 2013; Sánchez-Nivicela et al., 2023). Additionally, the Santa Bárbara locality in Sucumbíos represents the lowest recorded altitude for the species at 2,638 m, where *H. tigrinus* occurs in sympatry with *Hyloscirtus pantostictus, Hyloscirtus psarolaimus* and *Hyloscirtus lindae* (Coloma et al., 2012). Similarly, certain populations from Nariño and Putumayo, may coexist with *H. lindae* (Mueses-Cisneros & Perdomo-Castillo, 2011). In contrast, *H. tigrinus* has not been found in sympatry with other members of the *H. larinopygion* group at the known localities from its northern distribution (Cauca and Huila) (Mueses-Cisneros & Perdomo-Castillo, 2011; this study). The village of Río Sucio also corresponds to a historical locality for *Hyloscirtus caucanus* (km 66–67 road Popayán - Inzá; 2,660–2,700 m), where the paratypes and tadpoles referred in the species description were collected (Ardila-Robayo et al., 1993). However, during our fieldwork in La Sabana and La Marquesa, restricted to elevations above 3,000 m, we did not record *H. caucanus* or any other species of the *H. larinopygion* group coexisting with *H. tigrinus*. Further expeditions are needed to identify additional populations of this rare and endangered species, as well as to understand the distribution patterns of the species of *H. larinopygion* group that inhabit the high Andean ecosystems of south-western Colombia and northern Ecuador.

Defensive behaviour

The defensive behaviour observed in the new record of an individual from the village of Río Sucio corresponds to a passive strategy to avoid detection by predators (Toledo et al., 2010; 2011), previously documented for some species of the northern clade of *H. larinopygion* group (Rivera-Correa & Faivovich, 2013; Bejarano et al., 2015; Duarte-Marin et al., 2019; Reyes-Puig et al., 2022). Other species such as *H. psarolaimus* and *H. lindae* also display immobility, crouching and contracting (Bejarano et al., 2015), while *H. larinopygion*, *H. antioquia* and *H. sethmacfarlanei* may enhance these behaviours by emitting distress calls (as documented in *H. larinopygion*) and producing odoriferous and noxious secretions (Rivera-Correa & Faivovich, 2013; Duarte-Marin et al., 2019; Reyes-Puig et al., 2022). Some defensive behaviours such as body-rise, contracting and production of secretions, have also been reported in members of the southern clade like *Hyloscirtus diabolus* and *Hyloscirtus tapichalaca* (Kizirian et al., 2003; Rivera-Correa et al., 2016), and particularly *Hyloscirtus condor* exhibits additional defensive strategies such as biting and spine aggression (Almendariz et al., 2014).

External morphology

The morphological characters that we examined in the specimens from Río Sucio and La Magdalena mostly match those described for the type series and other additional specimens (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). However, our findings regarding nuptial pads and calcar tubercle align with those reported by Rivera-Correa & Faivovich (2013), suggesting that *H. tigrinus* lacks nuptial pads and may have a fleshy calcar tubercle that is poorly developed.

The variation in dorsal colouration of the type series of *H. tigrinus* and certain individuals from nearby localities in Nariño and Putumayo, include patterns ranging from yellow to green tones and presence of transverse black stripes or reticulate blotches (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). A subtle variation is reported in a female from Laguna La Magdalena (Montezuma & Mueses-Cisneros, 2009), which shows an intense yellow hue and more intricate reticulated black pattern (Mueses-Cisneros & Perdomo-Castillo, 2011). The male (MHNUC-HE-AN 1410) registered at the same locality during our fieldwork shares a similar colour pattern, but the flanks and some upper areas of the forelimbs and hind limbs show a smooth transition from dark yellow to a green-yellowish hue. Additional variation corresponds to the individual from Termales de San Juan (Puracé National Park, Cauca), which exhibits a darker brown dorsal tone with a yellow mid-dorsal stripe and irregular black spots, although it maintains yellow colourations on the forelimbs and hind limbs (Mueses-Cisneros & Perdomo-Castillo, 2011).

The specimens from the village of Río Sucio show ventral colouration and conspicuous orange markings similar to that of individuals reported in the type locality (Castelví Natural Reserve; Mueses-Cisneros & Perdomo-Castillo, 2011). With our observations, the *H. tigrinus* populations located in the northern part of the distribution limits represent the most divergent colouration observed in the species. The colouration patterns of these individuals resemble that described for *H. caucanus*, however, they can be differentiated from *H. caucanus* by external ulnar tubercles and yellow discs on the fingers and toes (Ardila-Robayo et al., 1993). A recent taxonomic key to identify species within the *H. larinopygion* group (Sánchez-Nivicela et al., 2023), uses the yellow, green or greyish cream colour with thick black reticulum or stripes to identify *H. tigrinus*, but our findings suggest the need to include brown to reddish-brown colourations to account for the extensive colour variability of this species.

Phylogeny and genetic distances

The phylogeny we describe is generally consistent with previous publications (e.g. Coloma et al., 2012; Ron et al., 2018; Reyes-Puig et al., 2022; Rivera-Correa et al., 2024). However, there are differences in some relationships within the northern clade. In our phylogeny, as well as those of Ron et al. (2018) and Coloma et al. (2012), *H. tigrinus* is sister to the clade *H. psarolaimus* + *H. criptico* + *H. pacha* + *H. staufferorum* while in Reyes-Puig et al. (2022) it is only sister to *H. criptico* + *H. pacha* + *H. staufferorum*. However, support in Reyes-Puig et al. (2022) is much weaker than in Ron et al. (2018) or Coloma et al. (2012), suggesting a less reliable topology. Despite the remarkable geographic variation described in the colouration patterns of *H. tigrinus* (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros

& Perdomo-Castillo, 2011; this study), populations from marginal localities exhibit very low genetic distances for gen 16S (0.293–0.298%). These values align with the divergence range (0.2–0.9%) observed for the same gene among conspecific populations of sequenced members within the *H. larinopygion* species group (Coloma et al., 2012). Although the population distances of the 16S gene in *H. tigrinus* are comparable to those observed in *H. criptico* (0.2%; Coloma et al., 2012), the geographic separation between *H. tigrinus* marginal populations is significantly greater (250 km). This geographic distance is even greater than that of *H. lindae* populations (122 km), which display the highest genetic divergence within the group (0.9%; Coloma et al., 2012). Likewise, the intrapopulation distances observed for the gen 16S in *H. trigrinus* (0–0.145%) are considerably lower than those reported for *H. sethmacfarlanei* (0.4%; Reyes-Puig et al., 2022). Therefore, the most northerly populations reported in this study, despite exhibiting divergent colouration, correspond to *H. tigrinus*, and the genetic results support and corroborate the morphological findings, confirming that these populations belong to the same species.

Advertisement call

The description of the advertisement call of *H. tigrinus* increases to 11 the number of species with documented acoustic data in the *H. larinopygion* species group (Table 4S in Supplementary Material). The integration of this evidence within recent phylogenetic hypotheses (Fig. 3; Rojas-Runjaic et al., 2018; Ron et al., 2018; Rivera-Correa et al., 2024), shows a pattern consistent with previous findings (i.e. Rivera-Correa et al., 2017), where non-pulsed calls are suggested as the ancestral character of *Hyloscirtus*, while pulsed calls have several independent origins in the northern clade of the *H. larinopygion* group: *H. tigrinus, H. psarolaimus* and the clade *H. lindae* + *H. pantostictus* + *H. larinopygion* + *H. antioquia*. However, for a comprehensive understanding of the evolutionary trajectories of these acoustic traits in the northern clade, acoustic data needs to be collected for *Hyloscirtus princecharlesi, Hyloscirtus pychodactylus* and *H. sethmacfarlanei*, in addition the phylogenetic positions and advertisement calls of *H. caucanus, Hyloscirtus sarampiona* and *H. tolkieni* should be addressed.

The detailed analysis of the advertisement call of *H. tigrinus* shows similarities in call duration with *H. psarolaimus* (Coloma et al., 2012), but the lowest number of pulses per call in *H. tigrinus* and the higher dominant frequency in *H. psarolaimus* allows the pulsed calls of these closely related species to be distinguished. The advertisement call of *H. tigrinus* resembles the call duration, calls per minute and dominant frequencies of *H. larinopygion* and *H. antioquia* (Rivera-Correa et al., 2017), which share similar altitudinal distribution and habitat conditions in the Andean cloud forest and subparamos (Rivera-Correa & Faivovich, 2013). However, *H. tigrinus* exhibit the lowest number of pulses per call reported in the northern clade of the *H. larinopygion* group, and despite its wide range of variation in pulse duration, the mean values suggest that *H. tigrinus* tends to produce shorter pulses than *H. antioquia* and *H. larinopyion* (Tables 3S & 4S in Supplementary Material).

The acoustic variation reported here for *H. tigrinus* and its 'basal' position relative to the clade *H. criptico* + *H. pacha* + *H. staufferorum*, might offer insights into the evolution of call structure among these related species. The individuals recorded in Andean forest near to streams (La Sabana), emitted calls of shorter duration with fewer pulses, weak amplitude modulation between pulses and slightly higher dominant frequency, in contrast to those recorded near to lakes in subparamo (La Magdalena) where dense vegetation and flowing water noise are less prevalent (Table 3S in Supplementary Material). The abiotic noise produced by streams constitute the main directional selective force of spectral components of the advertisement calls in *Hyloscirtus* spp. (Vargas-Salinas et al., 2024), whereas the temporal components, particularly in allopatric distribution patterns, seems to be shaped by genetic drift and weak selective pressures related to species recognition and reproductive isolation (Rivera-Correa et al., 2017; Vargas-Salinas et al., 2024). However, other selective forces, such as the attractiveness of calls to females, territorial signalling to other males, detectability by predators or level of abiotic noise, need to be considered as additional factors that may shape the evolution of advertisement calls in stream treefrogs (Vargas-Salinas & Amezquita, 2014; Röhr et al., 2016; Vargas-Salinas et al., 2024), and need to be addressed to better understand the evolutionary history of acoustic signals in the *H. larinopygion* group.

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RESEARCH ARTICLE
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A survey of turtles along the Da River in Vietnam, with insights into the Critically Endangered *Rafetus swinhoei*

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ABSTRACT – Vietnam harbours one of Asia's richest turtle biodiversities, yet knowledge of the distribution and abundance of Vietnamese turtles remains sparce. This study focuses on the turtle community along the Da River in northern Vietnam. Using a combination of field surveys, interviews and floating camera traps, we recorded 44 individuals across four species, showing that *Pelodiscus* sp. is locally by far the most abundant species, but that that the diversity of the turtle community is quite depleted. We found also the invasive *Trachemys scripta elegans* in the study area. Out of 90 interviewees, we obtained seven reliable reports of recent *Rafetus swinhoei* sightings, thus providing promising leads for conservation efforts.

INTRODUCTION

Vietnam is home to some of Asia's most diverse turtle communities (Buhlmann et al., 2009; Ennen et al., 2020), yet many species face significant threats due to habitat loss, hunting and insufficient conservation efforts (Rhodin et al., 2018; Stanford et al., 2020). Among these, the Yangtze giant softshell turtle *Rafetus swinhoei* stands out as one of the world's most endangered species (Le Duc et al., 2020a; Luiselli et al., 2024). Despite the threatened status of most Vietnamese chelonian species, scientific data on their ecology and distribution in Vietnam remains sparce (Ducotterd et al., 2022; Le et al., 2024).

The Da River, known locally as the Black River, offers a unique ecosystem that may harbour crucial populations of turtles, including *R. swinhoei* (Le Duc et al., 2020b; Van Pham et al., 2022). However, comprehensive studies on turtle diversity and abundance in this area are lacking. The current study, based on a short term survey along the Da River, helps to fill this knowledge gap by documenting turtle species and identifying areas of potential occurrence for *R. swinhoei*. By integrating field surveys and interviews with local communities, we contribute data that will guide future conservation efforts in the area.

MATERIALS & METHODS

Study area

The study lasted 24 days (16 September to 9 October 2024), in Son La Province, Vietnam, with a total field effort of 576 man hours. Fieldwork consisted of a combination of direct observations, camera trapping and interviews with local residents and fishermen. The survey focused on a 65 km stretch of the Da River upstream from the Son La Dam and the Nam Mu River (Fig. 1A). The Da River originates in Yunnan Province, China, and flows through Vietnam before joining the Red River in Hanoi. This region features diverse aquatic habitats, including deep pools, sandy riverbanks and rocky waterfalls (Dao et al., 2010).

The study area is characterised by dramatic changes in the river bed width and depth by season, due to the effects of the dam control (Fig. 1B).

Field protocol

Camera trapping. Sixteen floating camera traps were deployed at two sites along the Da River. We assembled the camera traps which consisted of a camera trap (COOLIFE H881 – 21 MP 1080 HD [Coolife® Corporation] – 125° - 49 LEDs with a 32GB memory micro-SD card) placed on a floating structure and baited with a mixture of fish, squid, dry shrimp and bovine intestine.

Direct observation. To make direct turtle sightings and to check the camera traps, the research team covered approximately 380 km by boat along the 65 km of the Da and the Nam Mu Rivers. We inspected fishermens' catches and documented any individuals seen in the wild. Each turtle sighting was georeferenced, and the animal was identified to the lowest taxonomic level as possible. For *Pelodiscus*, we decided to avoid species identification as two closely related species may occur in Vietnam *Pelodiscus sinensis* and

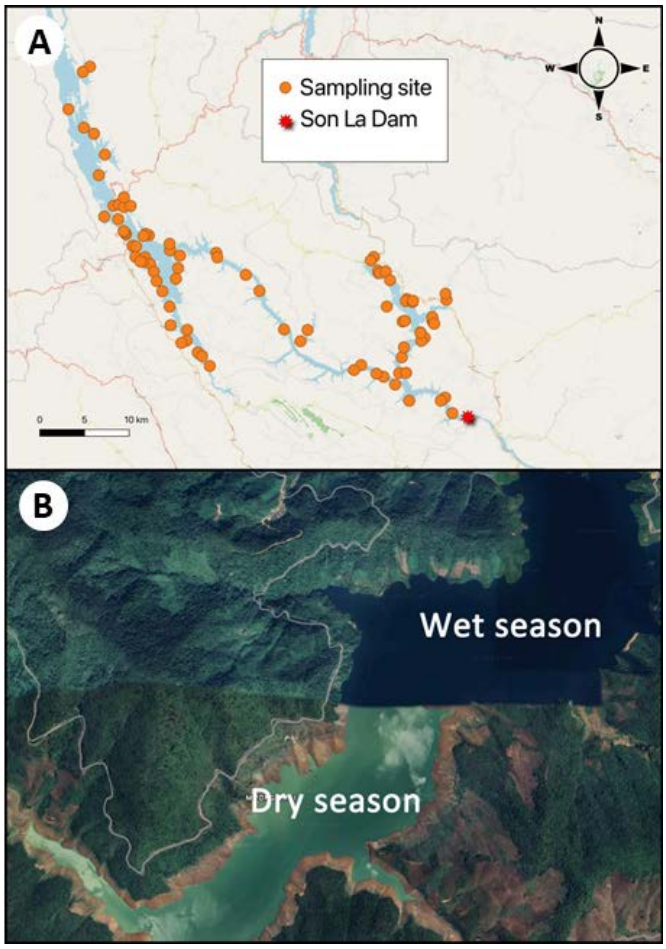

Figure 1. Map of the Da River system - **A.** The sampling sites used in the study, **B.** Detail of the dramatic changes in the banks and width of the river bed between dry and wet season (from Google Earth)

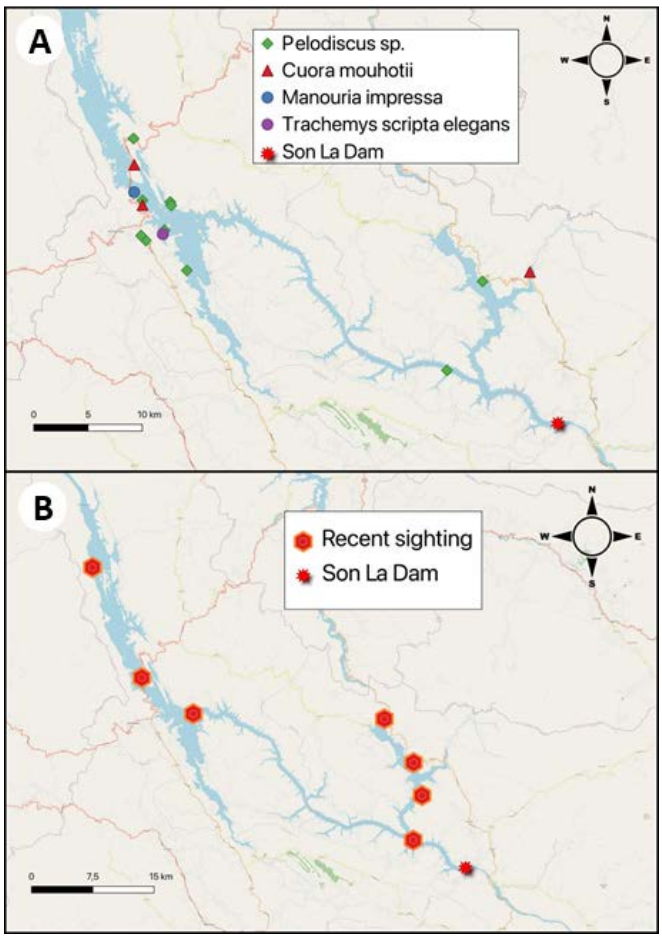

Figure 2. Records of turtles species along the Da River in Vietnam - **A.** Turtle species record by direct observation during the study, **B.** Records of *Rafetus swinhoei* according to reliable interviewees

Pelodiscus variegatus (Farkas et al., 2019), but the species boundaries are so poorly known in these taxa that we prefered to avoid specific identification until there is more compelling evidence of the true species.

Face-to-face interviews. Ninety local residents and fishersmen were interviewed using a standardised questionnaire (that can be found in the Supplementary Material of Le Duc et al., 2020b). We also interviewed a retired professional hunter of *R. swinhoei*, Mr. Đức, 75 years of age, to gather anecdotal evidence and historical insights into turtle populations along the Da River system.

RESULTS

During our survey, a total of 44 turtle individuals, representing four species, were recorded directly - *Pelodiscus* sp. (38 specimens), *Cuora mouhotii* (4 specimens), *Manouria impressa* (1 specimen) and the alien invasive *Trachemys scripta elegans* (1 specimen). Of our total sightings, 86.4% were *Pelodiscus* sp. The precise locations at which these species were recorded are presented in Figure 2.

Observations of *Pelodiscus* sp. included individuals with diverse colour patterns, consistent with the description of both *P. sinensis* and *P. variegatus* (Fig. 3), suggesting a wide phenotypic variability within the population and no support for a clear morphological difference between the two supposed species.

Information obtained through the 90 face-to-face interviews provided some interesting details concerning the turtle communities in the study area. Our interviewees confirmed that *Pelodiscus* sp. is the most commonly encountered turtle in the area, accounting for about 90% of reported catches. This aligns well with our field observations and suggests that this species is not currently under significant threat in northern Vietnam. Additionally, reliable reports indicated the occasional presence of *Palea steindachneri* which was however not seen in our surveys. Indeed, most of the fishermen described clearly the two abundant species of the area as Ba ba gai *P. steindachneri* and Ba ba tron *Pelodiscus* sp. Of particular interest were seven independent reports of recent *R. swinhoei* sightings (Table 1 & Fig. 2B). Key findings for these latter interviews include:

1. Four sightings occurred during the dry season, and for the three others the interviewees were unable to remember the season.
2. A notable sighting in 2017–2018 involved a 50 kg individual in the Nậm Nhạt River.
3. Nesting behaviour was described, with eggs laid in a sandy-grassed areas between March and May (dry season).

Figure 3. Phenotypic variability within *Pelodiscus* sp. in the study area, the turtles in the upper row are not the same individuals as in the lower row

Table 1. Synthesis of the face-to-face interviews reporting the recent sighting of *Rafetus swinhoei* at the study area

Date of last sighting	Season	Observation	Record co-ordinates	Interview number	Dimensions/ weight	Comment
March 2024	Dry season	Sighting	N 21.705445, E 103.613708	1	1.2 x 0.6 m	We had placed camera traps in this area
March-April 2021–2023	Dry season	Sighting	N 21.826282, E 103.555547	1, 15 & 61	60 cm	We had placed camera traps in this area
2020–2021	?	Sighting	N 21.660253, E 103.900175	34 & 58	70–80 kg	Seen by 3 people
9 June 2024	Dry season	Sighting	N 21.612277, E 103.934666	56	100 kg	Frequent sighting by the interviewee
2020	?	Sighting	N 21.576318, E 103.944730	55	30 kg	
2019	?	Sighting	N 21.527299, E 103.934280	48	50–60 kg	
2014	Dry season	Sighting	N 21.665853, E 103.674602	68	100 kg	

Historical accounts from a retired hunter

A retired hunter provided historical insights into *R. swinhoei* along the Da River system as follows:

1. He and his father (a professional *Rafetus* hunter himself) frequently caught *Rafetus* from 1970 to 1982, with the largest individual weighing about 80 kg.
2. Nesting sites were characterised by sandy-grassed riverbanks, with nests containing 100–130 eggs buried about 30 cm deep.
3. *Rafetus* exhibited specific nesting behaviours, such as creating a U-shaped path to conceal its nest. According to his description, *Rafetus* females tried to hide the nest from potential predators by moving in different directions in the long grass, so the predator (whether an animal or a human) was often confused by following turtle's path.

He recounted detailed feeding habits and behavioural aspects for the species. The species shows a clear tendency to feed upon carrion. For instance, he had seen a *R. swinhoei* eating a dead buffalo in the river. The turtle also eats fish, shrimp and water plants but the interviewee did not remember clearly about food composition in the *Rafetus* stomach. According

to him, it is a calm animal, but moves very fast to water when disturbed by humans. Its favourite living area is the slow, deep running water after a rocky waterfall. The living area always has a sandy bank.

Many times he and his father killed these turtles on the sandy bank, where they bury themselves under the sand. In these cases, hunters often find *Rafetus* individuals by looking for their tracks along the sandy riverbank. They discriminate their tracks very easily from those of other sympatric turtles due to their much larger size. Once the signs are spotted, the hunters will search the spot where the turtle is hidden below the sand by slowly walking barefoot and also probing a thin stick into the sand to find the animal underground. If the stick bounced back, the signal of a softshell, hunters would quickly dig the animal out and turn it on its back to stop it from moving.

His last sighting of *R. swinhoei* was in 2017–2018 in the area of Nậm Nhạt river, with an individual that was about 50 kg weight. He also suggested that the running river in Mường Tè district and Nậm Muc river have more *Rafetus* left than elsewhere in Vietnam.

DISCUSSION

Species diversity and abundance

Our study has shown that the Da River is inhabited by an extremely simplified community of turtle species (three or four species if we also consider *P. steindachneri* whose presence seems certain) compared to the potential high diversity that characterises the water basins of northern Vietnam (Ducotterd et al., 2022). Furthermore, only *Pelodiscus* sp. can be said to be widespread, abundant and dominant, further demonstrating the high ecological impairment with regard to the turtle communities in the study area. The presence of *C. mouhotii* and *M. impressa*, though less common, suggests that the terrestrial forested banks of the riverine system may guarantee appropriate habitats for these two Endangered species (IUCN, 2024).

The seven reported sightings of *R. swinhoei* suggest that this Critically Endangered species may still inhabit the Da River and its tributaries. Historical and anecdotal evidence from the former hunter provides a quite detailed description of the nesting, feeding and behavioural ecology of this species, offering crucial information for targeted conservation efforts. The dry season appears to be a critical period for *Rafetus* activity, potentially linked to nesting or feeding. This is also the season when field surveys should be concentrated to increase the probability of observing this species in the wild, since the dramatic lowering of the water level makes it relatively easy for the fishermen (and hence other observers) to spot these animals while basking, or while just resting motionless on the sandy bottom of the waterbodies.

Conservation implications

The Nậm Nhạt, Mường Tè district and Nậm Muc Rivers emerge as priority areas for conservation action. These regions feature the slow, deep water and sandy banks preferred by *R. swinhoei*.

This study provides the first assessment of turtle diversity along the Da River, with a particular focus on the elusive *R. swinhoei*. While *Pelodiscus* sp. dominates the local turtle community, anecdotal reports and historical accounts offer some hope that *R. swinhoei* still persists in the region. Future research should prioritise systematic surveys of identified hotspots, combined with eDNA analyses to confirm species presence (Seimon et al., 2024).

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SHORT NOTE

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Recent observations on green turtle *Chelonia mydas* movements and nesting in the Egyptian Red Sea

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ABSTRACT – Conservation of sea turtles requires identifying vital habitats such as nesting beaches and feeding grounds. Through a citizen science programme that records flipper tags and takes ID photos, we identified a new green turtle migration path between Zabargad Island and Abu Dabbab bay, Egypt. This bay also experienced nesting in 2022, but not in 2021 and 2023. The identification of this migration route supports an earlier assessment that Zabargad is one of the most valuable green turtle rookeries in the Red Sea and that Abu Dabbab bay is an important feeding bay for sea turtles. We also recorded an event in which a tagged green turtle was harvested for consumption, which suggests that females may be vulnerable to this threat during the nesting season. Our data justifies protecting any historic nesting beach in the Red Sea based upon its potential as future nesting activity could be the result of turtles returning from a hatching event 20–30 years ago and nesting beaches may not be used each year.

INTRODUCTION

Conservation of sea turtles requires identifying vital habitats such as nesting beaches and feeding grounds (Godley et al., 2008; Shimada, et al., 2021a; 2021b; Tanabe et al., 2023). Green turtles *Chelonia mydas* are a globally endangered species, in which very little is known about their populations in Egypt's Red Sea (Hanafy, 2012; Attum, 2014; Mancini et al., 2015; Fouad et al., 2022). Green turtles are particularly vulnerable to anthropogenic disturbances at their nesting beaches, inter-nesting habitat and feeding grounds due to their dense aggregations (Godley et al., 2008). Our knowledge of green turtle movement patterns between nesting and feeding grounds in the Red Sea is understudied compared to other regions (Godley et al., 2008), with our knowledge of these patterns being less studied in Egypt (Attum et al., 2014) than on the eastern side of the Red Sea in Saudi Arabia (Al-Mansi et al., 2021; Shimada et al., 2021b; Tanabe et al., 2023).

Green turtle nesting on Egypt's mainland beaches is characterised by low density, widely dispersed nests (Hanafy, 2012; Shimada et al., 2021b). In contrast, Zabargad Island in the Red Sea has a much higher nesting density and thus is believed to be the most important green turtle rookery in Egypt (El-Sadek et al., 2016; Hanafy, 2012; Shimada et al., 2021b). Red Sea islands were historically safe nesting sites for sea turtles, when compared to the mainland, given the lack of mammalian predators and remoteness from anthropogenic disturbance (Attum & Nagy, 2024). We report on a new migration path from Zabargad Island to

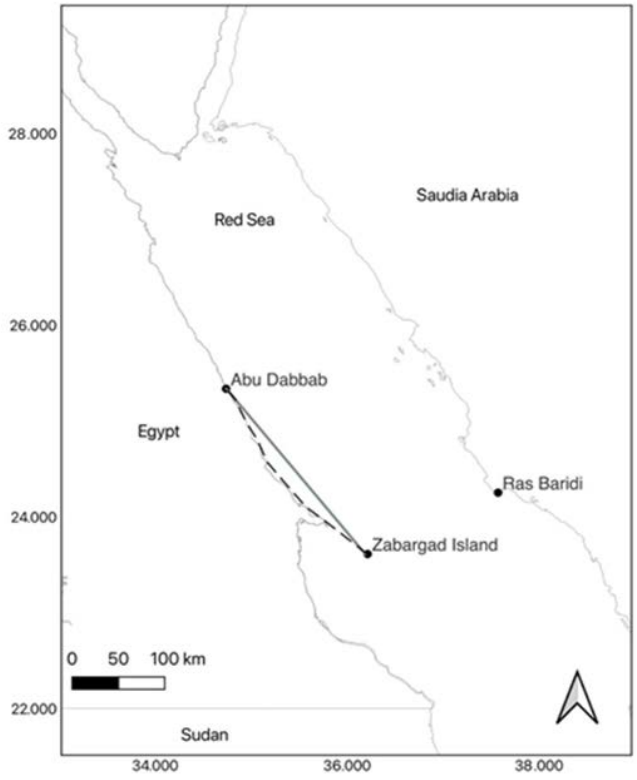

Figure 1. Map of Red Sea showing direct (solid line) and proposed (hashed line) migration route between Zabargad Island and Abu Dabbab bay, Egypt

the Abu Dabbab bay feeding ground, discovered by using a combination of a flipper tag and photo ID. We also report on recent nesting activity on a historic nesting beach in Egypt.

MATERIALS & METHODS

For identification purposes, turtles were photographed at popular snorkel and dive sites by an ongoing citizen science programme, The Red Sea Project (Fouad et al., 2022). Important identification features are facial and carapace profiles or unique scars and other morphological features (Jean et al., 2010; Reisser et al., 2008). Turtles identification was then undertaken manually or by using the Internet of Turtles online platform (https://iot.wildbook.org), which employs the Hot Spotter algorithm (Dunbar et al., 2021) and a multispecies deep learning approach (Otarashvili, 2024).

Sea Turtle Monitoring Sites

Zabargad Island

Zabargad Island (4.72 km^2) is located in the southern Egyptian Rea Sea, approximately 52 km from the mainland (Fig. 1). Green turtles nest on the sandy beaches located at the southern (2.5 km) and eastern side (1.5 km) of the island (El-Sadek et al., 2016). Human access is limited mostly to liveaboard dive and fishing boats, both legal and illegal. On Zabargad Island in 2008, 2009, 2010, 2012, 2013 and 2014, walking surveys of nests were undertaken usually between July–August for a minimum of three nights (El-Sadek et al., 2016). Metal (Monel) tags were attached at the trailing edge of the front flipper as turtles returned to the water, as part of the programme of the Red Sea Protectorates (Egyptian Environmental Affairs Agency).

Abu Dabbab bay

Green turtle nesting activity was monitored in Abu Dabbab bay (Fig. 1) between 2021 and 2023. Abu Dabbab is a small seagrass bay (0.17 km^2; width 317–430 m) that is part of a complex of feeding grounds used by sea turtles (Fouad et al., 2022; Mancini et al., 2015; Montagna et al., 2023). The bay is also a historic nesting beach for green turtles (Hanafy, 2012). Nesting was observed opportunistically during walks or brought to our attention by hotel and dive centre management as part of an outreach programme.

OBSERVATIONS

Zabargad Island

A female green turtle EG 0197 (curved carapace length 109 cm) was tagged at Zabargad Island on 26 August 2013. She has been recorded in Abu Dabbab for almost twenty years (2005, 2006, 2011, 2012, 2022, 2023 and 2024). The straight-line distance between Zabargad Island and Abu Dabbab bay is 241 km. If we assume that EG 0197 moved in a similar path to previously satellite-tagged turtles, she crossed the Red Sea, moved north-west towards the Berenice peninsula, which is the shortest distance between Zabargad Island and the mainland. She likely then followed the coast north to Abu Dabbab (Al-Mansi et al., 2021; Attum et al., 2014). This proposed distance between the feeding ground and nesting site would be approximately 248 km. Although, the exact migration path of this turtle is unknown, the final destination is the point of maximum displacement for 99 % of sea turtles (Hays & Scott, 2013).

Green turtle EG 093 was tagged between July–August 2008 and found dead a few days later on the same nesting

Figure 2. Green turtle (EG 0197) showing position of flipper tag (red arrow) and detail of the tag in the inset

beach on Zabargad Island. She was apparently harvested for consumption by local fishermen. Although turtles or their eggs are not regularly consumed in the Red Sea (Mancini, 2015), fishermen are known to occasionally consume eggs (El-Sadek et al., 2016). Our record indicates that in more remote locations, some fishermen do consume green turtles. This observation also suggests that females may be vulnerable to fishing during the aggregations of the nesting season (Attum et al., in press).

Abu Dabbab bay

In 2022, four live nesting events were observed (11/08, 12/09, 19/09 and 02/11), in addition we also observed the tracks and covered pits of three additional nesting events (16/8, 24/09 and 28/09). Nine additional emergences, false crawls, were recorded for a nesting success rate of 44 % (7 nests/16 total nesting emergences). No nests were observed in 2021 or 2023.

DISCUSSION

The use of flipper tags and photo-ID has increased our understanding of migration routes of green turtles nesting at Zabargad Island. The identification of this migration route between Zabargad Island and Abu Dabbab bay supports an earlier assessment that that Zabargad Island is one of the most valuable green turtle rookeries in the Red Sea (Shimada et al., 2021b). Five turtles tracked from their nesting beach in Zabargad used disparate feeding grounds spanning the length of the northern and southern Red Sea (Attum et al., 2014). The identification of this migration path also further highlights the importance of Abu Dabbab as a vital feeding ground for sea turtles in the Red Sea that nest in Egypt and internationally (Montagna et al., 2023).

Current nesting observations also suggest that Abu Dabbab is an intermittent and low-density nesting site. The nesting succuss of green turtles in Abu Dabbab is on the lower range of values for green turtles (46–65% Hanafy, 2012; 40–52% Bourjea et al., 2015; 63%, Shimada et al., 2021a). Following best practices to reduce nightlight would likely improve beach nesting success and use (Salmon, 2005; Siqueira-Silva et al., 2020), while allowing human recreational activities to continue (Weishampel et al., 2016). Our data does justify protecting any historic sea turtle nesting beach in the Red Sea based upon its potential, as current nesting activity could be the result of turtles returning from a hatchling event 20–30 years ago and nesting beaches may not be used each year (Attum & Nagy, 2024). The loss of even a single nesting beach could result in a permanent, irreversible decline of sea turtle populations (McClellan & Read, 2009). Although sea turtles in the Egyptian Red Sea have not been well studied compared to other regions, our knowledge of sea turtle ecology should be increasing given the numerous and ongoing citizen science initiatives in the region (Mancini & Elsadek, 2019).

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SHORT COMMUNICATION

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Daily activity of four elapomorphine snakes in south-eastern Brazil

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During fieldwork to prepare an inventory of the snake assemblage in a Cerrado region of south-eastern Brazil, one of us (AB) recorded the activity patterns of three elapomorphine (Dipsadidae, tribe Elapomorphini) species: *Phalotris lativittatus, Phalotris mertensi* and *Apostolepis dimidiata*. In addition, in this report we include the feeding of the elapomorphine *Elapomorphus quinquelineatus* from the Atlantic Forest which together provides some insights into the daily activity patterns of these four species under natural conditions.

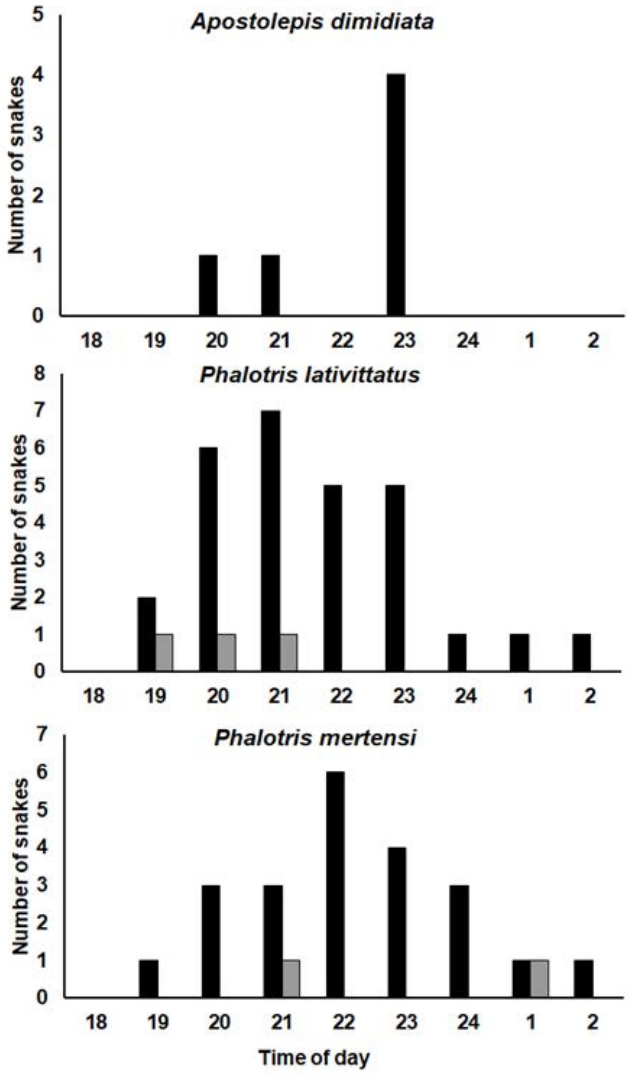

Figure 1. Observed activity patterns of three elapomorphine species in the Cerrado (south-eastern Brazil). Black bars represent adults and grey bars represent newborns (< 200mm).

The study area was in the Cerrado of the municipality of São Simão, state of São Paulo (21° 28' S, 47° 33' W; 650 m a.s.l., range: 530–960 m). This region encompasses three characteristic Cerrado vegetation types: forested savannah (Cerradão), tree savannah (Cerrado sensu stricto) and grassy-woody savannah (Campo sujo) (Veloso, 1992), as well as gallery forests that border streams and rivers. Parts of the municipality are also covered by eucalyptus and sugarcane plantations. Surveys for live or road kill snakes were made from a vehicle travelling at speeds of up to 40 km/h along four roads within the municipality - Chaffy Jorge, Capitão José Luiz de Oliveira e Silva, Conde Francisco Matarazzo and Rodovia Anhanguera. The surveys were undertaken during daylight (07:00 to 11:00 h) and at nighttime (18:30 to 02:00 h) throughout the rainy season from October 2020 to April 2021 and again from October 2022 to March 2023. A total of 5,388 km was sampled during the day and 6,050 km at night. The body size of observed specimens was estimated, with individuals measuring < 200 mm considered newborns.

Active snakes were recorded across all three vegetation types and some disturbed areas and included *P. lativittatus* (n = 30), *P. mertensi* (n = 21) and *A. dimidiata* (n = 6). Almost all specimens of *P. lativittatus* and *A. dimidiata* were found in preserved Cerrado areas (forested or grassy-woody savannah), with only one individual of *P. lativittatus* recorded in a sugarcane plantation. In contrast, individuals of

Figure 2. *Elapomorphus quinquelineatus* swallowing an amphisbaenian *Leposternon microcephalum* at 10:57 h.

P. mertensi were found in preserved Cerrado areas (n = 12), marsh or gallery forests (n = 5), and sugarcane or eucalyptus plantations (n = 5). All species were encountered only during nighttime sampling (19:00 to 02:00 h, Fig. 1).

The feeding record of *E. quinquelineatus* was documented by a trail walker (F.R. Silva, pers. comm.) in a preserved area of the Atlantic Forest at Parque do Atalaia, municipality of Macaé, state of Rio de Janeiro (22° 15' S, 42° 02' W; 230 m a.s.l). The specimen of *E. quinquelineatus* (total length ~500 mm) was found at 10:57 h on 5 August 2023 while feeding on an amphisbaenian *Leposternon microcephalum* on a forest trail. Both the predator and prey were on the surface, and part of the snake's body was covered by leaf litter (Fig. 2). The snake was starting to swallow its prey head-first, with its mouth still biting the amphisbaena's head. We estimate that the ratio of total prey length to predator snout-vent length was at least 0.7.

Elapomorphine snakes have been considered as both diurnal and nocturnal (Marques et al., 2015; 2017; 2019; 2023), but data from direct observations are scarce (Sawaya et al., 2008; França & Braz, 2013; Costa et al., 2010). The data presented here consistently show nocturnal activity for three species (*A. dimidiata, P. lativittatus* and *P. mertensi*). In captivity, some individuals of the elapomorphine *Apostolepis assimilis,* from south-eastern Brazil, exhibit exclusively or predominantly diurnal behaviour, whereas others exhibit intense nocturnal activity (Torello-Viera & Marques, 2017). However, activity patterns may be plastic, varying with environmental conditions (Torello-Viera & Marques, 2017; Abom et al., 2012; Banci et al., 2025).

The intrinsic activity patterns of the *Phalotris* genus remains unknown. However, unlike *Apostolepis*, species of *Phalotris* have always been reported as nocturnal (Marques et al., 2015; Sawaya et al., 2008), which is supported by this study. In contrast, diurnal activity—likely intrinsic at least for *Apostolepis*—has been observed in *Elapomorphus* spp. in nature. Our report of *E. quinquelineatus* feeding in daylight in the Atlantic Forest mirrors a previous report of the species preying on the same amphisbaenian species at around 10:00 h (Caramaschi & Niemeyer, 2012). Although *E. quinquelineatus* has also been observed moving at night (Costa et al., 2010), the two instances of daylight feeding contrast with the predominantly nocturnal activity of elapomorphine species in the Cerrado.

We suggest that the apparent difference in daily activity patterns among free-ranging elapomorphine snakes—*Apostolepis* and *Phalotris* (nocturnal) versus *Elapomorphus* (diurnal)—may be related to their distinct habitats. Although abiotic factors (humidity, temperature and insolation) may influence the time of activity, predation pressure may also play an important role.

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SHORT COMMUNICATION

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A baited pitfall trap for the Madeiran wall lizard *Teira dugesii*

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Pitfall traps are used for presence/absence surveys and for making population estimates of small terrestrial reptiles, mammals and invertebrates (Lettink & Monks, 2016). The traps may be used in different locations simultaneously and will provide comparable results provided sampling design and trapping effort are similar (Cechin & Martins, 2000; Maritz et al., 2007). The trap described here, known locally as the 'lagartixeiro', is used to capture the Madeiran wall lizard *Teira dugesii*, a species endemic to the Madeira Archipelago, including the Selvagens Islands. The species has an average snout-vent length (SVL) of 64 mm (Cook, 1979) and is locally very abundant; its populations may have the greatest mean body mass density (38.34 kg/hectare) of any terrestrial vertebrate (Arbuckle & Arbuckle, 2023).

The pitfall trap consists of a plastic bucket that, to prevent the lizards from escaping, should have a minimum height of 25 cm and minimum diameter at the top of 27 cm. A wire is stretched across the top of the bucket (Fig. 1A) from which a suitable bait is suspended from the centre by a thread. The bait of choice is a small piece of banana but *T. dugesii* is omnivorous so other baits may be used (e.g. tomato, apple). To ensure that any lizard traversing the wire towards the bait falls into the bucket, two tubes made from Spanish cane *Arundo donax* or plastic are threaded onto the wire either side of the bait. These tubes will rotate when a lizard steps onto them causing the lizard to fall into the bucket from which it is unable to escape (Fig. 1A).

Many island lizards climb trees and shrubs. In such cases, the trap is placed next to the tree but it should be placed in the shade in order to prevent the bucket from being heated by the sun. This avoids heat stress and desiccation that could kill the captive lizards (Dodd, 1992; Hobbs & James, 1999). If it is not possible to have the trap in the shade then to reduce the risk to the lizards, the sampling period should be reduced to 30–45 minutes. Regarding the colour of the bucket, it is generally believed that when compared to lighter shades black is more effective (Crawford & Kurta, 2000). For *T. dugesii*, it was observed that the highest capture rates occurred in buckets of blue and black colours, while colours such as red and white resulted in a significantly fewer captures. However, black buckets may reach higher temperatures in the sun, potentially resulting in increased mortalities. Other factors to bear in mind are that the captured lizards are easy prey for predators such as birds, rodents and cats (Wooley et al., 2021), and also that

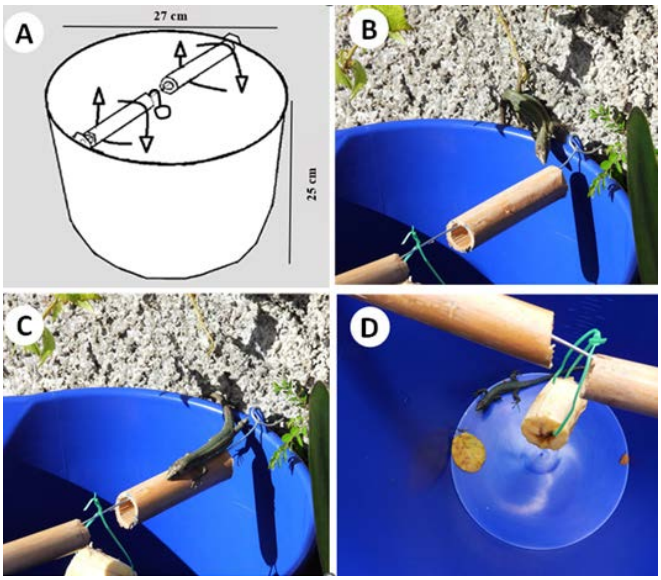

Figure 1. A pitfall trap for capturing *Teira dugesii* - **A.** Representation of the rotation of the tubes in the pitfall trap, **B.–D.** Sequence of an individual *Teira dugesii* falling into the pitfall trap

it is better to avoid trapping in the last hours of sunlight, otherwise after release the lizards may have insufficient warmth to make a quick/safe return to their natural shelters.

The pitfall trap is specific for species that show climbing behaviour, as is the case with many lacertids (Vanhooydonck & Damme, 1999) and in the specific case of *T. dugesii*, it is possible to capture a large number of individuals in a short period of time, e.g. with three traps we captured 96 lizards in one hour in northern Madeira. It is important to note that this trap is suitable for studying diet, as the lizards fall into the bucket before feeding on the bait but the success of the trap depends largely on the lizards being attracted to the food bait, so placing the trap where there are other food sources close by may significantly reduce its effectiveness. A particular advantage is that the trap, unlike more conventional pitfalls, does not disturb the habitat as it is not necessary to dig a hole in which to place the bucket and no drift fence is needed as a guide to the trap (Ellis, 2013; Maritz et al., 2007). Other advantages include ease of construction and low cost (< 5 € per unit).

One disadvantage with the trap that we have noticed is that it may be unsuitable for studying the age structure of

populations, as many juveniles do not approach the trap due to the presence of adult individuals; these are known to be cannibalistic (Cosgrove & Pie, 2025). Furthermore, the trap may be unsuitable for small mainland lizards, which are usually insectivorous in contrast to island species that are more likely to be omnivorous and include fruit in their diets (Valido et al., 2003; Vidal & Sabat, 2010). Consequently, this type of trap is likely more suitable for use on islands than on the mainland.

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SHORT COMMUNICATION

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Range extension of the Persian krait *Bungarus persicus* to south-eastern Iran

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The ecology of venomous snakes is understudied, particularly in the Middle East, despite the region being a global hotspot of species richness for such snakes. Kraits (*Bungarus* spp.) are venomous elapid snakes, crepuscular and nocturnal in habit, that are widely distributed in the south and south-east Asia, with their westernmost limit in Iran (Abtin et al., 2014). The Persian krait *Bungarus persicus* (Fig. 1), is believed to belong to the *Bungarus sindanus* complex, although further molecular studies on Iranian *Bungarus* spp. are needed to clarify the validity of *B. persicus* and evaluate its similarity to congeners (Shahi et al., 2022); this would confirm its status as endemic to Iran. One peculiarity of *B. persicus* is that the holotype has a small loreal scale on each side of the head, which is very unusual for an elapid snake, however, the paratype lacks loreals but both specimens have a characteristic black pigmented spot in the loreal area (Abtin et al., 2014). The related species *B. sindanus* is found up to 900 m above sea level and is distributed across Afghanistan, Pakistan and India (Srinivasulu et al., 2021). There was no record of Persian krait in Iran until 2013 (Abtin et al., 2014) and as yet the species has not been assessed by the IUCN.

After the discovery of the first specimen of *B. persicus* in Hormozgan province on 11 October 2020 in Benk village, Minab county (Arianejad & Ghasemi, 2021), we initiated a study from 2020 to 2023 to gain a deeper understanding of the species' ecology, and in this report give details of the extent of the distribution of *B. persicus* in Kerman and Hormozgan provinces of south-eastern Iran with the help of specimens collected by local people.

The study area was in the Kerman and Hormozgan provinces of south-eastern Iran (Fig. 2) where the main economic activities are smallholder agriculture, traditional ranching and fishing. The area has an average annual precipitation of < 150 mm making it one of the driest habitats for kraits globally. The region has two rainy periods, including January and February with winter rainfall, and late July to October (higher temperature) with monsoon rainfall. It is covered in open woodlands and scrublands and does not include any protected areas.

We asked a few hundred local residents in Minab, Bashagard, Rudan counties in Hormozgan province and Manujan county in Kerman province, whom we met during a Persian Leopard Conservation Project, about where they find kraits in order to document those locations. We trained local residents to catch kraits alive using large bags and a long stick, to avoid killing them if possible, and to record the time of capture (Fig. 1S, in Supplementary Material). Local people kept the live captures and any kraits that had been killed for checking by the senior author, which were then photographed. We documented the locations and altitudes for these snakes (GPS, Garmin 60CSx), as well as the date and time of day of these observation. We prepared a species distribution map of the region using QGIS 3.34.

During 2020–2023, we collected a total of 24 records of *B. persicus*, of these 22 came from Hormozgan province (Minab, n = 8 and Beshagard, n = 14 counties) and two from Kerman province (Manujan county, n = 2) (Figs. 1 & 2, also see Supplementary Material Table 1S for detailed co-ordinates and altitudes. The specimens displayed the darkly pigment spot in the 'loreal area' (Fig. 1) but no actual loreal scales were observed.

Of the 24 *B. persicus* records, 21 were contributed by local residents and 3 were from separate field work. Our findings extend the distribution range of the species west as far as Minab county, located in Hormozgan province; these records of the krait are the westernmost distribution limit of the species worldwide. According to our findings, it is possible that *B. persicus* is also present in the north and west of Hormozgan province or the other counties of Kerman province.

The altitude range of our records was from 342 m a.s.l. (Chah Shahi in Manujan) up to 1,629 m a.s.l. (Chakbor in Bashagard) with an average altitude of 975 m (standard deviation ± 417 m). The altitude distribution of *B. persicus* is notably higher than from similar species. For instance,

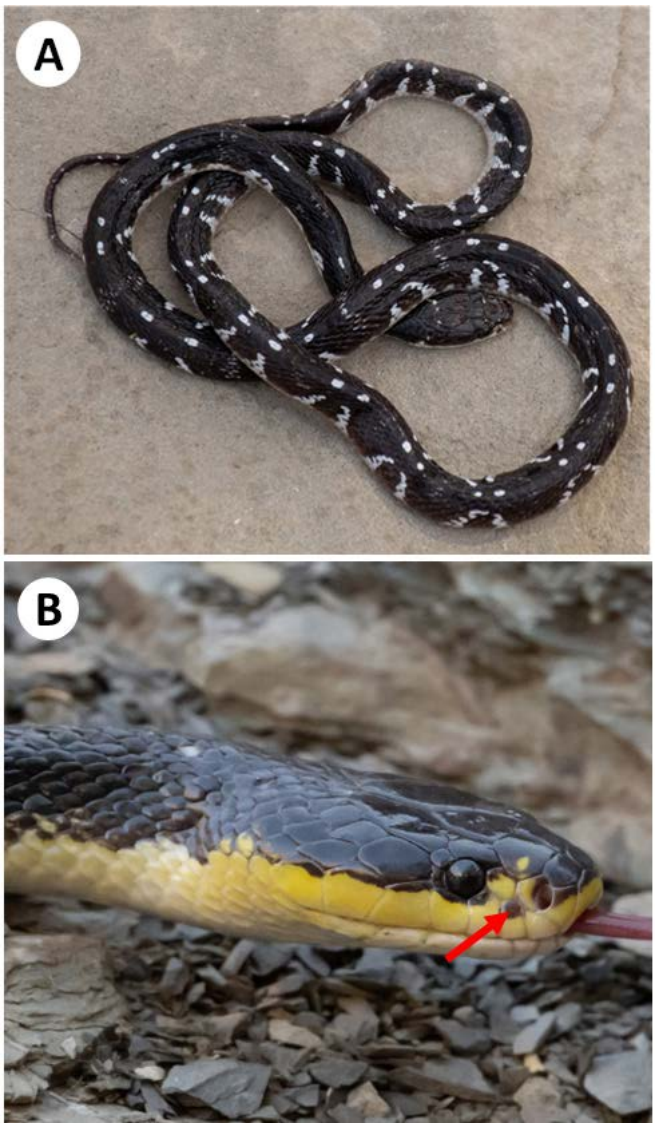

Figure 1. Persian krait *Bungarus persicus* - **A.** Live captured specimen from Bashagard, Hormozgan province, **B.** Lateral view of head, red arrow showing the position of the darkly pigmented spot in the 'loreal area'

the highest record of the species in Iran is 729 m higher than the 900 m a.s.l. published altitude of *B. sindanus* (Srinivasulu et al., 2021), while the highest altitude recorded for *Bungarus walli* is only 150 m a.s.l. (Ghosh et al., 2022).

Our observations on *B. persicus* were all made from May to October (Table 1S in Supplementary Material) suggesting that this is the period in which the species is most active. All records were made at dusk or later and the majority of the records were documented before midnight or at midnight (Table 1S in Supplementary Material), the typical crepuscular and nocturnal behaviour of *Bungarus* spp..

Bungarus persicus has not yet been assessed by the IUCN but it is protected by the Iranian Department of the Environment, and killing it is subject to a fine of 59,219,000 IRR (70 USD). Unfortunately, this fine is imposed even though local people receive no information on snakes and the health infrastructure to treat snakebite victims is lacking. An unknown number of *B. persicus* are killed annually by local people due to fear of snakebite to themselves or livestock. As *B. persicus* was only recently discovered in south-eastern Iran there remains a need for studies on its phylogeny, ecology, human-krait conflict, conservation and antivenom production.

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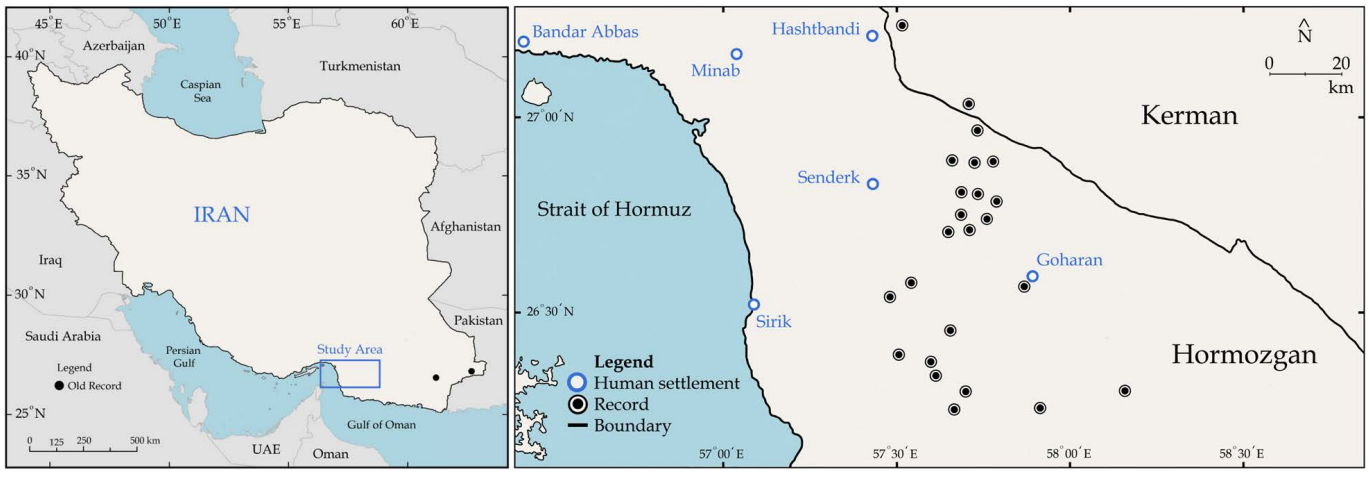

Figure 2. Old records of *Bungarus persicus* in south-eastern Iran (left, from Abtin et al., 2014); new records of *B. persicus*, in Kerman and Hormozgan provinces (right, see Supplementary Material Table 1S for details)

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SHORT COMMUNICATION

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Axanthic-blue colour aberration in the American green treefrog *Hyla cinerea*: clues to the drivers of its spatial distribution

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Deviations from typical and evolutionary-selected colour patterns can occur in non-polymorphic species due to phenotypic expression of recessive genetic mutations, often triggered by evolutionary pressures or even anthropogenic factors (Ellegreen et al., 1997; Jablonski et al., 2014; Masselink & Tanaka, 2021). These chromatic aberrations frequently result in reduced survival and reduced reproductive success due to natural selection (Andrén & Nilson, 1981; Masselink & Tanaka, 2021), making them rare in natural populations (Jablonski et al., 2014; Henle & Dubois, 2017).

In frogs, albinism and leucism are the most extensively documented chromatic aberrations (Jablonski et al., 2014). A less frequently documented aberration is axanthism, which involves the absence of yellow or orange pigmentation (due to a lack of xanthophores), red pigmentation (due to a lack of erythrophores) and reflective or scattering pigmentation (due to a lack of iridophores) (Jablonski et al., 2014; Gould & McHenry, 2024). As a result, axanthic frogs typically exhibit one or a combination of the following colouration patterns: 1) a completely or partially blue body; 2) a grey or unusually dark body—though these cases can be difficult to distinguish from melanism (overproduction of melanin); and 3) a typical body colouration but with dark eyes (Jablonski et al., 2014).

We recently recorded an example of blue axanthism in an American green treefrog *Hyla cinerea* in Illinois (USA). This species, typically characterised by its lime-green dorsal surfaces, is widely distributed across the south-eastern USA. A single case of axanthism has previously been reported for this species (Cain & Utesch, 1976) but involved a non-blue form of this aberration. Consequently, our record represents the first documented case of this aberration for this species in Illinois. To further investigate the spatial distribution of this aberration and provide some, but not exhaustive, insights into the potential drivers of this phenomenon, we searched citizen science data for additional records of blue axanthism in *H. cinerea* and mapped them across the species native range, and in relation to land cover and human population density.

On 13 October 2024, at 15:08 h, we observed a juvenile axanthic-blue *H. cinerea* of unknown sex. It was found resting on a thin leaf at the edge of a clear, spring-fed lentic water body in Union County, southern Illinois (37° 34’29.2” N, 89° 26’22.8” W, WGS84) (Fig. 1A), at an air temperature of about 19 °C. The surrounding habitat consisted of low vegetation, sparse trees and a rocky outcrop (Fig. 1B). The dorsal surfaces of the individual were entirely cerulean blue,

Figure 1. American green treefrogs *Hyla cinerea* observed in southern Illinois (USA) - **A**. An axanthic-blue juvenile, **B.** Lentic water body habitat, and **C.** Typical-coloured juvenile specimen

with some scattered white dots bordered by greyish blue. The tympanic region was greyish blue. It exhibited a lateral white stripe extending from the jaw to the thigh, typical for the species, with some blue pigment extending below this line. The ventral surfaces were not directly inspected to avoid handling the specimen but, apart from the bluish vocal sac, appeared whitish, as is typical for the species. The eye was golden-brown, darker than the typical eye colour for the species. Besides the axanthic individual, three other juvenile *H. cinerea* were observed nearby, all displaying the typical lime-green dorsal colouration (Fig. 1C).

We compiled an additional 62 records (53 single localities) of axanthic-blue American green treefrogs by searching citizen science data from the Aberrantly Blue Frogs project at the online platform iNaturalist (https://www.inaturalist.org/projects/aberrantly-blue-frogs http://www.inaturalist.org) up until 31 December 2024. While we acknowledge that axanthism can manifest as a range of grey to dark body colours or darker eyes, we limited our search exclusively to blue-coloured individuals to ensure that we were examining a singular biological phenomenon (the lack of xanthophores) and avoiding the inclusion of melanism cases. The extent of blue colouration in these axanthic individuals varied widely, from isolated blotches on the typical green background, such as limited to the tympanum (e.g. https://www.inaturalist.org/observations/252558377), to scattered blotches (e.g. https://www.inaturalist.org/observations/252558376), and complete dorsal surface coverage, shown by our record. This approach highlights the valuable contribution of citizen science initiatives in documenting chromatic variation. Additionally, it provides an initial indication of the rarity of this particular aberration in natural populations of *H. cinerea* as records of blue axanthism found on iNaturalist accounted for only 0.1% of the total observations of this species at the time of the search (42,166 observations). This percentage is even likely overestimated, as aberrant blue frogs are probably more prone to be documented on iNaturalist, and some records may be repeated observations of the same individuals.

Through the mapping of citizen science records and the new record from Illinois, it is evident that most observations of axanthic-blue *H. cinerea* are concentrated near the edges of the species range (Fig. 2A). This pattern is only not corroborated in central Texas and southern Florida. Among the states where the species is native, the chromatic aberration has been recorded as follows (numbers of observations in parentheses): Alabama (2), Florida (6), Georgia (1), Illinois (2), Louisiana (2), Maryland (2), North Carolina (10), Oklahoma (1), South Carolina (3), Texas (3) and Virginia (21). However, nine other states within the species native range remain without records of this aberration: Arkansas, Indiana, Kentucky, Missouri, New Jersey, Pennsylvania, Mississippi, Tennessee, and West Virginia (Fig. 2A).

The spatial distribution of blue axanthism in *H. cinerea* may be influenced by several factors that are not mutually exclusive, but here our attention is focused on hypotheses related to natural population dynamics and anthropogenic influences. Regarding natural drivers, the higher frequency of axanthism records from populations located at the

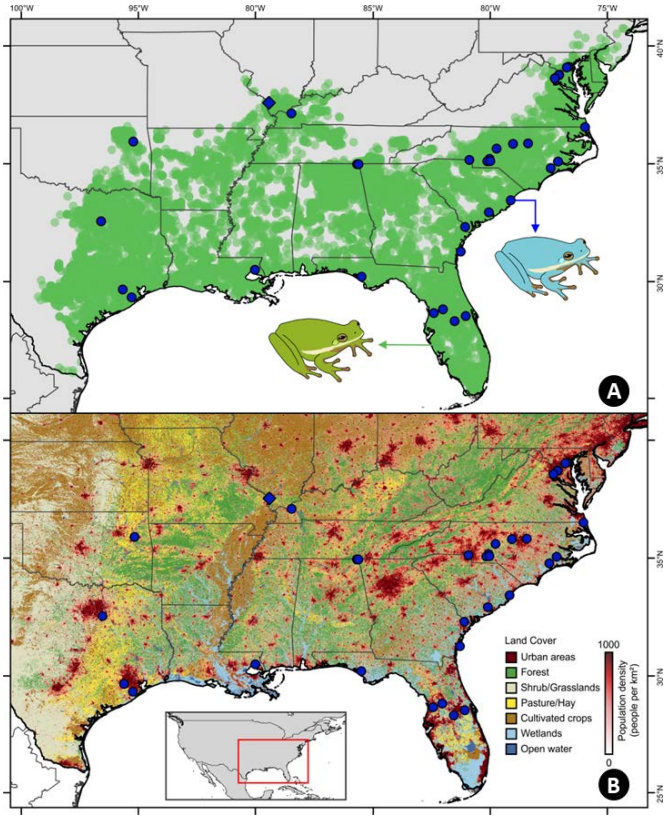

Figure 2. Geographic distribution of axanthic-blue American green treefrogs *Hyla cinerea* across the south-eastern United States (blue symbols), including our new record from southern Illinois (diamond) and citizen science contributions up to 31 December 2024 (iNaturalist.org; circles) - **A.** The distribution of axanthism compared to the species overall range (green symbols) based on all iNaturalist records, and **B.** The distribution of axanthism compared to land cover (sensu US Geological Survey, 2025) and human population density (sensu Pesaresi et al., 2024)

periphery of the species range supports the Centre-Periphery Hypothesis (reviewed by Pironon et al., 2017). This hypothesis posits that peripheral populations often exhibit reduced genetic diversity compared to central populations, due to factors such as restricted gene flow, smaller effective population sizes, lower habitat suitability and founder effects (Brown, 1984; Pironon et al., 2017). These factors can independently or synergistically enhance evolutionary processes such as natural selection and genetic drift, resulting in increased accumulation and fixation of mutations in peripheral regions (Eckert et al., 2008; Pironon et al., 2017). As a result, chromatic aberrations, including axanthism, are expected to be more prevalent in peripheral populations than in central ones. We also cannot rule out the effects of sampling bias in generating this pattern, as the regions with fewer records on iNaturalist are also more central to the species distribution (Fig. 2A). However, the fact that even well-sampled areas within this central region still lack records of this chromatic aberration seems to further support the Centre-Periphery Hypothesis.

Other researchers have proposed that anthropogenic factors, such as pesticide use and other chemical exposures, can increase mutation rates in species, potentially contributing

to an increased occurrence of chromatic aberrations in natural populations (Ellegreen et al., 1997; Jablonski et al., 2014). However, no clear spatial correlation is observed between axanthic-blue cases of *H. cinerea* and agro-pastoral systems (sensu US Geological Survey, 2025), which typically employ large amounts of chemicals in their operations. This is particularly evident as the areas surrounding the largest expanse of cultivated crops and pastures within the species range lack any records of blue axanthism. On the other hand, a spatial correlation is apparent between axanthism cases and human population density (sensu Pesaresi et al., 2024), with a higher numbers of records reported in urban areas (Fig. 2B). Such a correlation is not entirely consistent, as densely populated central regions, such as Atlanta (Georgia), Birmingham (Alabama) and Jackson (Mississippi), as well as peripheral areas like Austin and San Antonio (Texas) and the coastal region of Florida, show no records of blue axanthism in this species (Fig. 2B). Such a correlative inconsistency between anthropogenic influence and blue axanthism in *H. cinerea* suggests that the observed association between higher axanthism case numbers and urban areas is more likely a result of sampling bias or genetic factors rather than conclusive evidence of any harmful effects caused by environmental pollution.

Considering this focus, we postulate that the occurrence of blue axanthism in *H. cinerea* is likely influenced by a combination of natural population dynamics and sampling biases. Although urban regions tend to yield more observations of axanthic blue individuals, this pattern is not consistent across the species range, as several densely populated regions within the species range lack records of this chromatic aberration. Thus, evolutionary forces, particularly those affecting peripheral populations, may play a more significant role in the increased incidence of this chromatic aberration at the edges of the species distribution. Nonetheless, we reinforce that these hypotheses remain highly speculative, and further research is needed as more data become available to allow for robust statistical testing. This would enable more explicit tests to confirm or refute these hypotheses, or propose alternative explanations.

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SHORT COMMUNICATION

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Molecular confirmation of the rhino rat snake *Rhynchophis boulengeri* in southern Vietnam

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In 2019, we made the first record of a rhino rat snake *Rhynchophis boulengeri* (Mocquard, 1897) in southern Vietnam (Nguyen et al., 2020), a single male specimen (ITBCZ 4506) found dead on a road in Song Hinh Protected Forest, Phu Yen Province. This amounted to a 600 km range extension. The morphology of the specimen was broadly congruent with *R. boulengeri*.

In 2021, *R. boulengeri* was split so that populations in Hainan Province, China are now assigned to *Rhynchophis hainanensis* (Peng et al., 2021). Given that there is a substantial distance between southern Vietnam and all other locations where *R. boulengeri* is found and that there are now two species within the genus, there is clearly interest in checking for potential cryptic species. Consequently, we sequenced the cytochrome b (cytb) gene for the specimen collected in Phu Yen Province and calculated uncorrected p-distances between this sequence and other available *Rhynchophis* sequences on GenBank (see Table 1 & Table 2) following methods described in Kane et al. (2023).

Uncorrected p-distances between the sequences obtained from the specimen we collected in Phu Yen Province and *R. boulengeri* from Lao Cai Province more than 1,000 km to the north was 0.79% and up to 1.36% from sequences from specimens with no associated locality data (Table 2). This is typical of within species variation for this gene (e.g. Laopichienpong et al., 2016). Uncorrected p-distances between *R. boulengeri* from Phu Yen Province and *R. hainanensis* ranged from 3.02–3.51%. Furthermore, the specimen from Phu Yen Province has a single loreal scale and black orbital stripe, clearly distinguishing it from *R. hainanensis* (Peng et al., 2021). We conclude that the specimen from Phu Yen Province is indeed *R. boulengeri*.

Table 1. List of taxa of *Rhynchophis* specimens and cytb sequences used in this study

Species	Collection site	Voucher	GenBank	Reference
Rhynchophis boulengeri	Phu Yen, Vietnam	ITBCZ 4506	PV588734	This study
Rhynchophis boulengeri	Lao Cai, Vietnam	ANU2012083	MW495262.1	Peng et al., 2024
Rhynchophis boulengeri	Not reported	MVZ Herp 218698	LC640447.1	Kambayashi et al., 2022
Rhynchophis boulengeri	Not reported	NA	AF471053.1	Lawson et al., 2005
Rhynchophis boulengeri	Guangdong, China	ANU2018176	MW495261.1	Peng et al., 2024
Rhynchophis hainanensis	Hainan, China	ANU20190002	MW495258.1	Peng et al., 2021
Rhynchophis hainanensis	Hainan, China	HNU20190001	MW495259.1	Peng et al., 2022
Rhynchophis hainanensis	Hainan, China	ANU20190003	MW495260.1	Peng et al., 2023

Table 2. Uncorrected p-distances between species of *Rhynchophis* sequences used in this study based on cytb sequences.

	1	2	3	4	5	6	7	8
1. PV588734 *R. boulengeri*								
2. MW495262.1 *R. boulengeri*	0.79%							
3. LC640447.1 *R. boulengeri*	1.36%	0.56%						
4. AF471053.1 *R. boulengeri*	1.36%	0.56%	0.00%					
5. MW495261.1 *R. boulengeri*	0.91%	0.11%	0.68%	0.68%				
6. MW495258.1 *R. hainanensis*	3.51%	2.66%	2.78%	2.78%	2.78%			
7. MW495259.1 *R. hainanensis*	3.02%	2.42%	2.54%	2.54%	2.54%	0.45%		
8. MW495260.1 *R. hainanensis*	3.38%	2.54%	2.65%	2.65%	2.66%	0.34%	0.34%	

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NATURAL HISTORY NOTE

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Predation of an adult Aesculapian snake *Zamenis longissimus* by a western whip snake *Hierophis viridiflavus*

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Ophiophagy is a relatively rare phenomenon in snakes of the Mediterranean regions of Europe, although it has been recorded among both viperid (Freiria et al., 2006) and colubrid snakes (Capula et al., 2014; Di Nicola et al., 2020; Gandini, 2021; Vaccaro et al., 2023). However, no species is exclusively snake-eating (Pleguezuelos & Fahd, 2004; Capula et al., 2014) and most reported cases of ophiophagy are adults feeding on juveniles of other snake species or on adults of much smaller species, e.g. adult western whip snake *Hierophis viridiflavus* consuming the smaller-sized *Vipera aspis* and smaller conspecific (Capula et al., 2014). Cases of adult snakes feeding on adult individuals of approximately the same size are comparatively rare and include an adult *H. viridiflavus* swallowing an adult conspecific in Sicily and an unsuccessful attempt by a western whip snake to predate a similar sized adult *Zamenis lineatus* (Vaccaro et al., 2023). Here we report what we believe to be the first case of an *H. viridiflavus* predating an adult *Zamenis longissimus* of almost similar length.

On 28 June 2015, we observed the case of ophiophagy in a coastal area situated about 20 km north-west of Rome (surroundings of Maccarese) with Mediterranean scrub, pine forest and mixed forest, reed habitats nearby waterbodies. At 10:18 h an adult male *H. viridiflavus*, about 130 cm long, was sighted while ingesting a *Z. longissimus* of almost the same size (Fig. 1). We know the prey to have been an adult due to the size of the visible portion of the prey's body and the colour pattern which in *Z. longissimus* shows a clear ontogenetic shift from a "maculated" to a "uniform" morph in the adult (Di Nicola et al., 2021). The tail of the prey was projecting from the predator's mouth and appeared motionless. Once approached the whip snake escaped quickly, while still ingesting its prey, from an area with bare sandy soil to another with much higher vegetation, keeping the anterior portion of the body in an upright position.

Figure 1. Predation of an adult *Zamenis longissimus* by an adult *Hierophis viridiflavus*

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NATURAL HISTORY NOTE

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Arboreality in Brongersma's worm snake *Amerotyphlops brongersmianus* from south-eastern Brazil

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Arboreality has emerged as a prominent trait in snake evolution, occurring in both ancient lineages (e.g. Boidae, Pythonidae) and more recent radiations (e.g. Viperidae, Elapidae and Colubridae). Although arboreality is widespread, it is unevenly distributed across snake lineages of the world (Pizzatto et al., 2007; Harrington et al., 2018). While scolecophidian snakes are predominantly fossorial or ground-dwelling, they occasionally exhibit arboreal or climbing behaviour (Gaulke, 1995; Das & Wallach, 1998).

Brongersma's worm snake *Amerotyphlops brongersmianus* (Vanzolini, 1976) is a scolecophidian species widely distributed across South America (Graboski et al., 2018; 2023) and in Brazil it occurs in several biomes, including the south and north Atlantic Forest, Cerrado, Amazonian savanna enclaves, and Caatinga, mainly at low elevations (Nogueira et al., 2019). On 12 December 2019, at 22:07 h, an adult *A. brongersmianus* was observed perched on a shrub about 1.73 m above the ground (Fig. 1) in the district of São João do Jabuti, municipality of Guarapari, Espírito Santo, south-eastern Brazil (20° 37'45.76" S, 40° 34'25.45" W; WGS 84; 150 m a.s.l.). The observation followed a period of heavy rainfall the previous day. The snake was briefly captured for photographic documentation and was not collected.

To our knowledge, this is the first observation of *A. brongersmianus* displaying arboreal behaviour. Although at least 23 scolecophidians of different species across various genera have been observed in trees/shrubs (Das & Wallach, 1998). Recorded heights above ground range from 0.5 m to 5 m and include *Anilios nigrescens* at 5 m in a she-oak tree in Australia (Shine & Webb, 1990) and *Ramphotyphlops angusticeps* at 4 m in a palm tree (Das & Wallach, 1998). Scolecophidians have also been observed in man-made structures highlighting their remarkable adaptability to anthropogenic environments. Examples include *Trilepida macrolepis* found 0.6 m up a vertical concrete wall, *Myriopholis macrorhyncha* in the thatched roof of a house, and *Epictia tesselata* within the wall of an old adobe house (Das & Wallach, 1998)

In our observation, the snake's ascent of a shrub may be linked to environmental conditions, particularly the heavy rainfall that occurred the previous day, which may

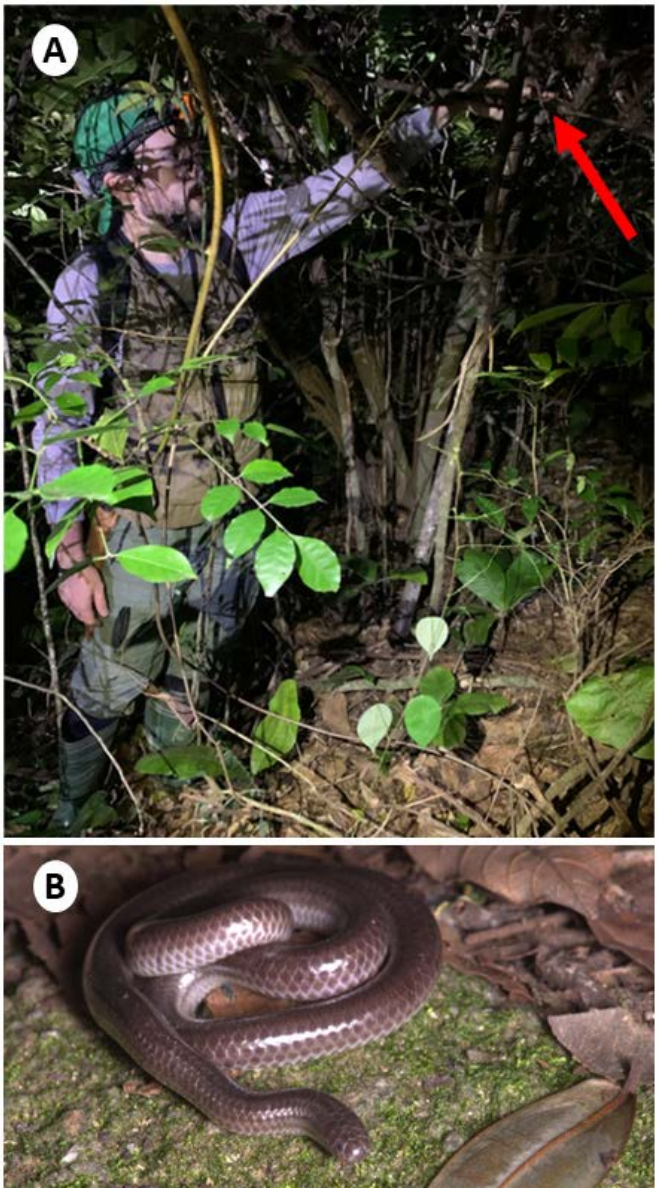

Figure 1. Observation of *Amerotyphlops brongersmianus* in a tall shrub - **A.** Researcher (approximately 1.80 m tall) indicating the location where the snake was found (red arrow), **B.** Photograph of the snake

have prompted the snake to seek refuge above ground. Some authors have suggested that arboreal or climbing behaviour in scolecophidian snakes may occur when they follow chemical cues left by their prey, typically ants and termites (Gehlbach et al., 1971; Webb & Shine, 1992; Torres et al., 2000). However, in this instance, no visible prey trails or ants/termites were observed on the tree or surrounding vegetation, leaving the exact motivation for the snake's behaviour uncertain.

The available evidence suggests that for scolecophidians, arboreality is at least an occasional behaviour. However, it is likely that climbing activity is more widespread than previously acknowledged, with some species potentially relying on arboreal habitats as a significant aspect of their ecological niche - possibly rivalling or even surpassing their presence in subterranean environments. Furthermore, the timing of the observation, at night, aligns with the predominantly nocturnal activity patterns of fossorial snakes, which tend to emerge during the cooler, darker hours to forage or engage in reproductive activities (Parpinelli & Marques, 2008; Khouri et al., 2022). This observation raises important questions about the scolecophidian snake species' ability to adapt to environmental stressors such as heavy rainfalls, as well as its occasional use of arboreal habitats.

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Puff adder *Bitis arietans* mouth gaping behaviour in South Africa

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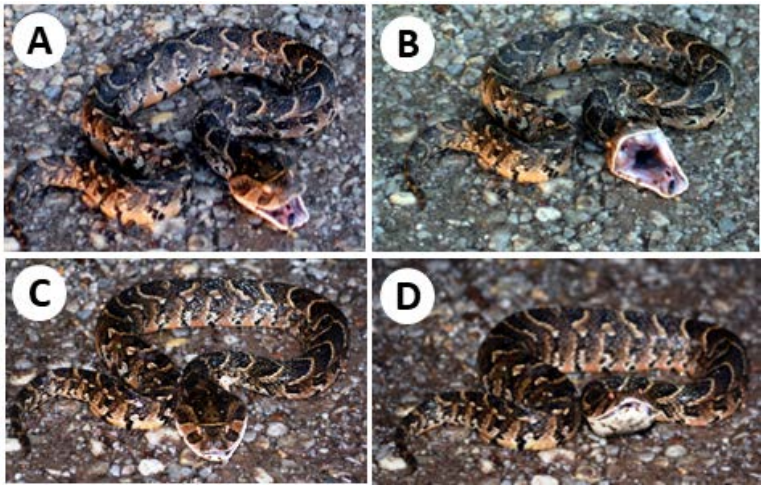

Figure 1. A behavioural sequence during mouth gaping by *Bitis arietans* - **A.** Snake displays gentle body movements, opening and closing its mouth and protruding its tongue, **B.** Snake with mouth open widely, **C.** Assuming a defensive posture with head level to the ground, and **D.** Head raised facing the observer in a defensive position, with mouth slightly open

Vipers have developed a wide range of defensive behaviours, which have evolved to mitigate predation risks across various environments (Campbell & Lamar, 2004). The puff adder *Bitis arietans*, a highly venomous species widely distributed across sub-Saharan Africa, south-western Arabia and Morocco (Mehrtens, 1987), is responsible for a significant number of snakebite-related fatalities in Africa (Barlow et al., 2013). This species employs several antipredator strategies, including camouflage, body inflation accompanied by hissing and whistling, and rapid strikes from a characteristic S-shaped defensive posture (Mallow et al., 2003). Here, we report an observation of mouth-gaping behaviour in *B. arietans*, a defensive display that has been rarely documented in this species.

At 15:08 h on 8 February 2017, an adult *B. arietans* was encountered by Thiago Silva-Soares on an unpaved road near a thicket of small bushes in Stormsrivier, Tsitsikamma National Park, South Africa (33° 58'36" S, 23° 53'38" E, WGS 84; 231 m a.s.l.). On approaching the snake, it exhibited behaviour suggestive of death feigning (Fig. 1A). Over approximately two minutes, the snake exhibited slow body movements, intermittent mouth opening and closing and repeated tongue protrusion. At one point, it opened its mouth widely (mouth-gaping) (Fig. 1B) and remained in this position for about 30 seconds. Subsequently, the snake maintained its mouth slightly open, retracted its tongue and assumed a defensive posture. Initially, its head was level with the ground (Fig. 1C); however, as the observer approached closer, the snake ceased its initial display, raised its head and faced the observer while retreating into a defensive position, consistently keeping its mouth slightly open (Fig. 1D).

To the best of our knowledge, this observation represents the first documented instance of potential death feigning in *B. arietans*, characterised by pronounced mouth-gaping and repeated tongue protrusion. While the mouth-gaping behaviour is clearly evident and aligns with known defensive displays in other snake species, the interpretation of death feigning remains provisional and warrants further investigation.

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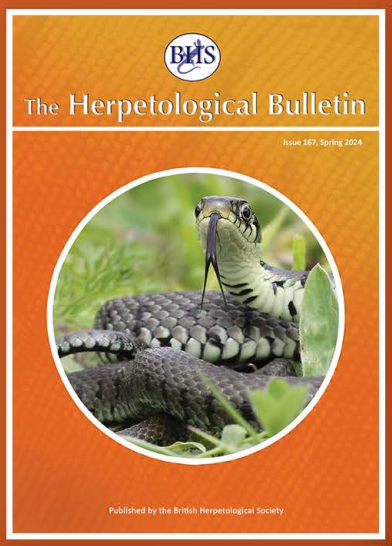
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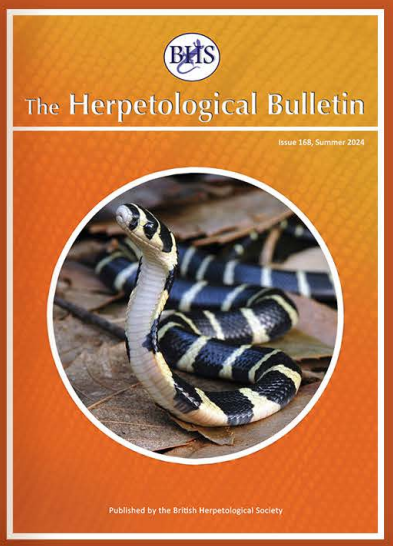
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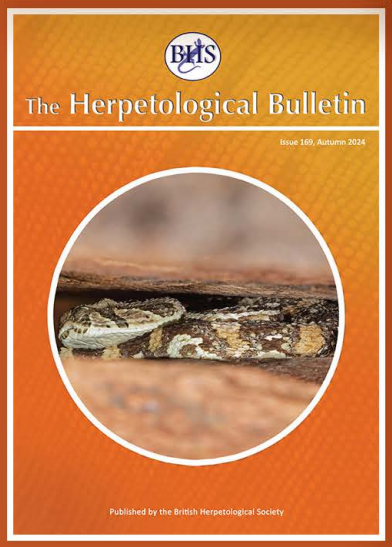
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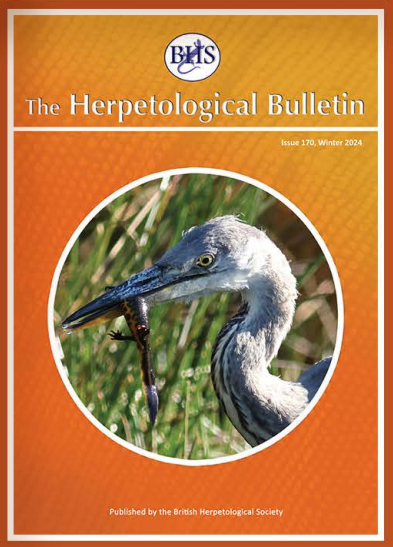
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