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The Herpetological Bulletin is a quarterly publication in English, without page charges to authors. It includes full-length papers, natural history notes, book reviews, and other items of general herpetological interest. Emphasis is placed on natural history and conservation as well as captive care that includes breeding, husbandry, veterinary, and behavioural aspects.

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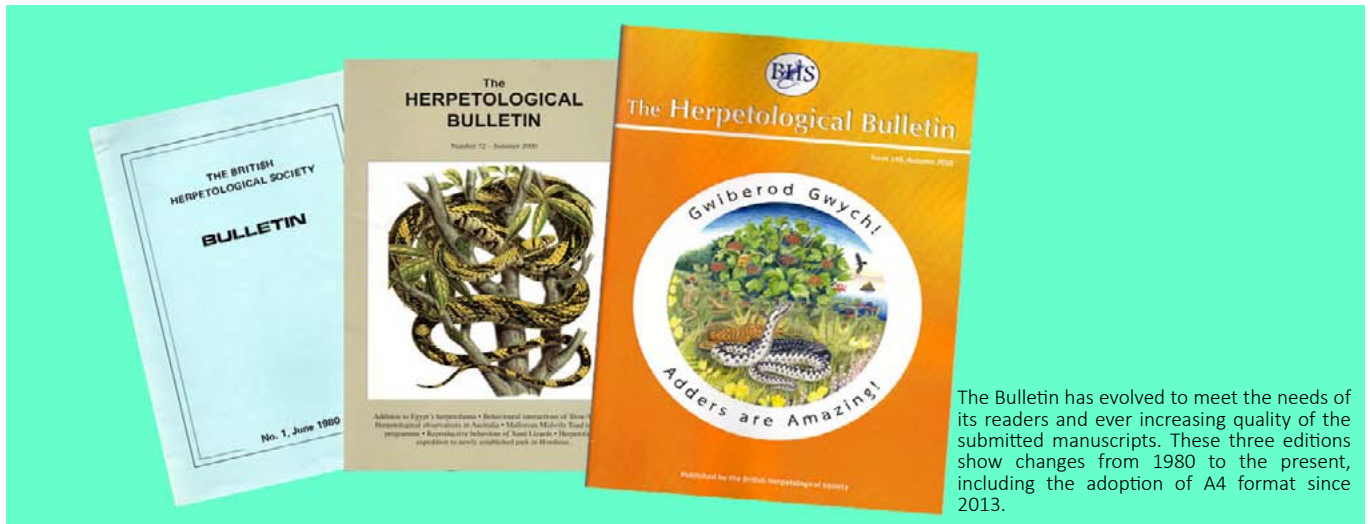
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Note that the views expressed by contributors to *The Herpetological Bulletin* are not necessarily those of the Editor or the British Herpetological Society.

Front Cover: An adult Bahamian pygmy boa (*Tropidophis curtus barbouri*) wrapped into a tight ball (convolving) as a mean of defence. In defence this species may also autohaemorrhage, which is the subject of an article presented on p.39.
Photograph taken by Sebastian Hoefler.

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The Bulletin has evolved to meet the needs of its readers and ever increasing quality of the submitted manuscripts. These three editions show changes from 1980 to the present, including the adoption of A4 format since 2013.

The Bulletin in its 40th year

Welcome to *The Herpetological Bulletin*. This publication is not usually graced with an editorial but as we have been at your service since 1980 and have now reached the 150th issue, it is time to celebrate with some reflection.

It would be old fashioned to describe The British Herpetological Society (BHS) as a learned society but it has a modern role as the hub of a learning community. *The Bulletin* is pivotal to that community by encouraging scientific communications of a high standard from professional and amateur herpetologists alike. *The Bulletin* exists to help authors from around the world share their findings and their enthusiasm for amphibians and reptiles. Aspiring authors, sometimes people for whom English is a second or third language, find that *The Bulletin* has a sympathetic editorial team dedicated to helping them make the most of their publishable material and ensuring that it is readable, interesting, and enhanced with a flash of colour.

The history of *The Bulletin* has been one of gradual development in size, layout, and colour (Fig. 1). In 1980, the Council of the BHS decided that its newsletter should be transformed into *The Bulletin of the British Herpetological Society*, younger sister to the *British Journal of Herpetology*. It was to act as a venue for articles on natural history, captive care and husbandry, as well as news, views and notices from the BHS. This released *The Journal* to focus on more technical and esoteric scientific contributions, in an age that saw a sharp increase in these. The first *Bulletin* was type-set and appeared in only black and white. By issue #19 the first colour photos appeared between the covers, with illustrations of skins from the Northern Territory of Australia. By issue #23 the front cover also had a colour photo. At the same time, the content was changing gradually, with news and views and notices of the BHS moving to *The NatterJack* newsletter, making more room for natural history in *The Bulletin*. Issue #70 of *The Bulletin* saw its name changed to *The*

Herpetological Bulletin (similarly the *Journal* was renamed *The Herpetological Journal*) to reflect a more international outlook. Until 2014 (issue #127) all those working for *The Bulletin* were volunteer members of the BHS but since then a Managing Editor has been contracted to prepare the edited papers for publication, ensure the timely appearance of each issue, and to introduce innovations in layout and design.

Despite its modest beginnings, authors publishing in *The Bulletin* now enjoy some important advantages. These include its attractive A4 appearance and layout, the prompt publication of articles (usually within 3 months of receipt), no publication charges, a PDF of their work which they are free to distribute, and after one year any reader has free access to their work via the BHS website. And for readers, who are often also authors, additional benefits are that *The Bulletin* is issued quarterly and at a modest cost. Online access for a year to PDFs of *The Bulletin* and *NatterJack* is only £20 (or £18 for a student) and that includes membership of the BHS. For the enthusiastic collector, hardcopies of *The Bulletin* can be obtained at a modest extra cost.

The Bulletin has been a great success owing to the many and varied authors who have contributed to it, the hundreds of reviewers who have spent countless hours appraising manuscripts, and the thirteen BHS members who have held editorial responsibility for the final product. The future of *The Bulletin* is secure with the BHS. Innovations in layout and design will continue; for example this issue is the first to have live links to relevant video clips in the PDF files, and, for the foreseeable future, *The Bulletin* will be available both electronically and as hardcopy.

This issue of *The Bulletin* offers the usual wide range of articles, including - the appalling slaughter green toads in Greece to satisfy the tourist trade; the parasitism of amphibians by fly larvae and their predation by leeches; the captivity husbandry of glass frogs; and, the extraordinary defence behaviour of Bahamian dwarf boas. But the issue

starts with something different, a memorial to one of the founders of the BHS, Maxwell Knight. The BHS founders mostly came from scientific/medical backgrounds but Maxwell Knight had a naval education and his interests were particularly wide ranging, as you will read, but most

importantly he was unusually prescient in his concerns for the need to conserve nature. A reminder of these concerns is a fitting subject for the 150th Issue of a *The Bulletin* whose readers are dedicated to the future wellbeing of the creatures they love.

Rick Hodges and Roger Meek
Scientific Editors

Maxwell Knight

Memorial to a herpetologist, naturalist, conservationist and much more

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In 1947, Maxwell Knight (1900-1968) was a founding member of the British Herpetological Society (BHS). For the rest of his life, he attended meetings, served on committees and contributed articles, notes and records to its journal. He was well-known as a naturalist, conservationist, broadcaster and writer but he also had an entirely different and secret career. He was a spy-master, and in later years unfounded assertions about his private life stirred up controversy.



Figure 1. The public face of Maxwell Knight as a naturalist

To mark 50 years since his death, The Maxwell Knight Symposium was held in 2018 under the patronage of the BHS. The account below is based on extracts of the transcripts of talks and submitted notes at the symposium, several of which can be seen on the 'Frightened Face of Nature' (FFON) website (www.ffon.co.uk). These presentations were based on the recollections of eight people who either knew or were influenced by Maxwell Knight (MK): John Cooper, Simon King, John Burton, Norma Chapman, Graham Wellstead, June Chatfield, Mark Rose, and Paul Pearce-Kelly.

As a conservationist MK was a pioneer and contemporary of Peter Scott and his published thoughts are still very relevant. Despite being a spy-master, his greatest fear was not communist Russia but inhumanity to nature; he predicted the wildlife population declines we are experiencing today. This was reinforced recently when an unpublished manuscript, entitled 'The Frightened Face of Nature', was uncovered in Maxwell Knight's cabinet; a very relevant extract that would chime with Greta Thunberg is reproduced in the box opposite.

From Maxwell Knight *The Frightened Face of Nature* Chapter II - The new approach

In considering the future for wildlife it is vital that the man in the street who, in the long run, will have responsibility for ignoring or fighting for it, should have the point put before him fairly and squarely without his being enveloped in a haze of jargon and technicalities. If he does nothing to help those who are fighting his battles for him at the moment, he will have only himself to blame if his children's children curse him for not being better informed, or for being so gutless that he stood by watching the rape of nature – and did nothing.

Maxwell Knight's hope was that the 'progress at any cost approach' would change, and that industrialised nations would stop playing the short-term nature-unfriendly game of habitat destruction so often carried out in the name of progress. Another extract from a chapter of 'The Frightened Face of Nature' illustrates this.

From Maxwell Knight *The Frightened Face of Nature* Chapter III - The age of science

By all means let man use his great powers to invent new devices; let him give of his best to see that all shall benefit from his genius in curing, healing, and housing those in want. But do not suggest that this can only be done by destroying what is fine to look at or listen to, whether in the arts or nature.

If human brains can find means of defying space, improving means of communication and bouncing pictures off satellites, surely he can also discover ways in which these things can be done without destruction - for destruction first is the cry of mad revolution and is the reverse of evolution.

Maxwell Knight's interest in natural history stemmed from his childhood. He was fascinated by all living things but had a particular love of amphibians and reptiles. Brought up in Mitcham, south London, the young MK had access to the local common and its wildlife and this gave him the opportunity to collect and keep amphibians and reptiles.

In his early teens MK's father died and an uncle took on responsibility for him. After an undistinguished schooling, he was sent to sea as a naval cadet, at least acknowledging his liking for the outdoor world. Much of his later life was based in London and it was only in his later years that the natural history led to another career overlapping with his MI5 work. During the war and afterwards, he spent most time living away from London in an MI5 safe-house at Camberley, Surrey, and that gave him more scope to keep pets and to develop a series of radio programmes on natural history, later television too, as well as writing.

In 1984, a book entitled, 'The Man Who Was M' was published by Anthony Masters. This contained allegations about homosexuality, bankruptcy, blackmail and ill-treatment of his wife. The book caused great distress to those who knew MK and many of these assertions were challenged in a critical review of the book by John Cooper (Cooper, 1986). However, what Anthony Masters did bring to light was previously confidential information about MI5 and the part played by MK before, during, and after the Second World War. MK is believed to have been the model for 'M' in Ian Fleming's James Bond novels. The revelations about MK's service in the war were a great surprise to many of those who knew him as they were only familiar with his work as a naturalist. And then, in 2017, a further book was published, 'M Maxwell Knight, MI5's Greatest Spymaster', by best-selling non-fiction author and historian Henry Hemming. This lauded MK as a great patriot but also stated that no evidence could be found for the earlier disparaging claims about MK's private life. For more fascinating details and detailed personal recollections, take a look on the internet at 'Maxwell Knight the naturalist' (Cooper, 2018).

Maxwell Knight was MI5's legendary 'M', a self-taught spymaster who, with the help of young case officers and talented agents, was responsible for counter-subversion and managed successfully to penetrate the British fascist movement and arguably help shorten the Second World War (Andrew, 2009). He was MI5's most gifted agent handler of the inter-war years; his sixth sense for enlisting would-be talented agents and officers and developing formal observation training - based on his natural history studies - still remains a thing of legend within the Security Services. He championed the recruitment of female agents; broke the Woolwich Arsenal spy case; uncovered a plot to prevent the entry of America into the Second World War and suppressed the fifth column of Nazi sympathisers who were prepared to help pave the way for the occupation of Britain. What made him a talented spymaster and what prepared him for the challenges he faced as 'M' were his observational skills - derived from the study of animal behaviour, and his ability to look deep into nature (Knight, 1960). These talents were honed as a child - inspired by his father - and sharpened by the Boy Scout movement. For more fascinating details take a look on the internet at 'Maxwell Knight the spy-master' (King, 2018).

The conservationist and journalist John Burton had experience of MK as a broadcaster. His memories of MK began with listening to him participating in the BBC's natural history radio programmes in the late 1940s and the 1950s, such as The Naturalist, Nature Parliament and Naturalists'

From Maxwell Knight 'Animals and Ourselves' (1962) (Children and Animals)

The great thing to establish, as early as possible, is confidence between children and animals. Nearly all children are interested in and curious about animals; and it is an unusual parent who has not experienced a toddler finding its first earthworm and bringing it in with the question, "What is it?"

Notebook. Then in the 1950s he occasionally found himself participating in programmes presented by MK and so was able to get to know him. On one occasion, in 1959, he spent a day in the field with MK recording material for a programme in The Naturalist series. It was a fine, sunny and very hot day in what was a glorious summer that year. They visited the Plumstead Marshes bordering the River Thames to find and record Britain's bush-crickets and grasshoppers for the programme. It became increasingly overcast with black clouds heralding the arrival of a heavy thunderstorm. MK became anxious to complete their activities before it arrived, apparently because once when he was fishing, his long fishing rod was struck by lightning. Quite understandably, that made him uneasy when lightning threatened. Coincidentally, the Woolwich Arsenal, scene of MK's world-famous break up of a Soviet spy-ring in the 1930s, was sited at the western end of Plumstead Marshes and the programme-making activities described above were in sight of it. John Burton was, of course, unaware then of the part MK had played in that momentous event, but subsequently he imagined that it must have come into MK's mind as they walked along the high outfall sewer bank across the marshes that overlooked the Arsenal.

The closing session of the Commemorative Symposium, 'Newts, Nadders and Neophyte Naturalists', provided entertainment as well as serving as a reminder of the fragility of habitats in Britain, even half a century ago. A team consisting of Simon King, Sarah Pellett, Charles, Rachel and Jonny Foster, Max, Hilda, Margaret and John Cooper re-enacted the 'Surrey Pond Scene' scene, a childhood encounter with youths and newts at a Surrey pond that had been recounted in MK's unpublished book 'The Frightened Face of Nature', found written in his own hand in the filing cabinet - see earlier. The 'Surrey Pond Scene' was beautifully acted out by young naturalists; MK would have approved. Maxwell Knight's name and reputation have been very much resurrected in recent years. Those who attended the Symposium or read this report will, we hope, be encouraged to carry his message forward.

From Maxwell Knight 'Animals and Ourselves' (1962)

If one is blessed with some appreciation of scenery; if one can get delight from the songs of birds; if the sight of a butterfly can give pleasure to the eye; then these are additional reasons for desiring and praying that our legislators should, before it is too late, remember that man and animals are not mutually exclusive - or are they?

ACKNOWLEDGEMENTS

The Maxwell Knight Commemorative Symposium was planned and organised by the British Herpetological Society (BHS) and generously supported by the British Chelonia Group (BCG), the Amateur Entomologists' Society (AES), the Institute of Animal Technology (IAT) and the Frightened Face of Nature (FFON) and others. The day would not have been such a success without the support of the BHS organising committee, Simon Townson, Paul Eversfield and Mark Hollowell. John Pickett who had been so supportive in the planning stages had greatly looked forward to attending and participating but, alas, that was not to be. It was fitting, therefore, that there was a tribute to John at the beginning of the programme.

REFERENCES

- Andrew, C. (2009). *The Defence of the Realm: The Authorised History of MI5*. Allen Lane, 1032 pp.
- Cooper, J. E. (1986). Book review. The Man who was M. The Life of Maxwell Knight by Anthony Masters. *Bulletin of the British Herpetological Society* 17: 46-47.
- Cooper J.E. (2018). *Maxwell Knight the Naturalist*. <https://thefrightenedfaceofnature.com/maxwell-knight-symposium/lectures/introductory-lecture-maxwell-knight-the-naturalist-prof-john-e-cooper/> accessed Oct 2019
- Hemming, H. (2017). *M Maxwell Knight, MI5's Greatest Spymaster*. Preface Publishing, 400 pp.
- King S.H. (2018) *Maxwell Knight the spy-master*. <https://thefrightenedfaceofnature.com/maxwell-knight-symposium/lectures/maxwell-knight-the-spy-master-mr-simon-h-king/> accessed Oct 19
- Knight, M. (1962). *Animals and Ourselves*. Hodder and Stoughton, 160 pp.
- Knight, M. (1960). *The Young Field Naturalist's Guide*. G. Bell & Sons, 144 pp.
- Masters, A. (1984). *The Man Who Was M. The Life of Maxwell Knight*. Basil Blackwell, 212 pp.

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Spatial ecology study reveals nest attendance and habitat preference of banded kraits (*Bungarus fasciatus*)

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ABSTRACT - An understanding of the spatial ecology of species living in and around human-dominated habitats is needed to develop conflict mitigation strategies and predict how organisms cope with ongoing anthropogenic habitat alteration. Here we present the results of a six-month telemetry study in Thailand of the banded krait (*Bungarus fasciatus*), a venomous elapid snake. We quantified home range size and habitat use of three adult kraits (1 male, 2 females) in an agricultural habitat. The kraits travelled an average of 47.20 m ± 23.54 m between shelter sites and occupied home ranges of on average 21.45 ha ± 19.56 ha (95 % dynamic Brownian Bridge Movement Models). They preferred to seek day time refuge amongst less disturbed habitat features, including waterways and rice paddy dykes, while avoiding routinely disturbed monoculture fields. The two female kraits also exhibited reduced movement while simultaneously attending their nests in a shared burrow complex. Fifteen neonates were observed emerging from the nest site. We recommend further investigation of banded krait reproductive ecology and ability to persist in heavily disturbed habitats.

INTRODUCTION

Biodiverse tropical regions continue to undergo rapid habitat loss resulting from deforestation and agricultural expansion (Hughes, 2017; Imai et al., 2018). To conserve biodiversity, biologists and land managers must understand how species fulfil their essential life history activities in today's human-modified landscapes (Hansen & Defries, 2007; Martínez-Abraín & Jiménez, 2015). Human-wildlife conflict is among the leading threats faced by fauna across the globe (Torres et al., 2018) especially for species, such as the banded krait (*Bungarus fasciatus*), able to inflict injury to humans (Amarasinghe et al., 2015; Dickman, 2010; Dunham et al., 2010).

Banded kraits are a large, typically yellow and black banded, elapid snake that are distributed from India in the west to China in the east, and south into the Indonesian archipelago (The Reptile Database, 2017). They are nocturnal and thought to occur in a variety of habitats ranging from tropical evergreen forests to degraded areas near human inhabitation (IUCN, 2013; Purkayastha et al., 2011). Previous research has focused mostly on the potent venom of the banded krait, especially its therapeutic potential and the synthesis of anti-venom (Gomes et al., 2017). Despite the risks to human health posed by banded kraits across a wide geographic distribution (Tongpoo et al., 2018), there have yet to be any studies on the spatial ecology of this species. An understanding of the species spatial requirements, habitat use, and reproductive behaviour may provide valuable insight for assessing threats posed by humans to kraits and vice versa (Maritz et al., 2016). Herein we report the home range area estimates and habitat use of three radio-tracked banded kraits (one male and two females) during

a 6-month period (1 April 2017 – 30 September 2017). The kraits inhabited a riparian belt dominated by rice paddies in north-east Thailand. We also report observations on nest attendance by the two female kraits.

MATERIALS AND METHODS

We used radio-telemetry to track banded kraits in the Transitional Zone of the Sakaerat Biosphere Reserve (SBR), located in Nakhon Ratchasima, Thailand. The dominant land uses in the SBR's Transitional Zone are rice paddies, sugarcane fields, upland agriculture, irrigation canals, and villages. With the help of field technicians from 2015 to 2017, we implemented 354 man-hours of unstandardised night walking and road-cruise surveys to capture kraits throughout the SBR Transitional Zone. We surveyed various targeted locations in the Chiang Sa Tributary. The initial capture sites of the three kraits were located within 100 m of each other, within a retention pond complex characterised by a cluster of three shallow ponds bordered by a semi-natural and annually harvested wood stand, primarily composed of *Eucalyptus camaldulensis*, and spanning approximately 1.16 ha in area (14.53° N, 101.97° E). The ponds were encircled by monocultures of sugarcane (*Saccharum* spp.) and rice (*Oryza sativa*). The average elevation across the study area, determined by creating a Minimum Convex Polygon (MCP) around all plotted krait locations, was 232 m a.s.l., and the dominant land use was irrigated rice (65.43 %), followed by human settlement (8.64 %). During our focal study period (1 April 2017 – 30 September 2017), terraced rice paddies were flooded with shallow water to enable rice growth. During the dry season (January – March) fields were drained, harvested and left fallow for livestock grazing, as is typical in north-east

Thailand (Kanokkanjana & Garivait, 2013).

We assigned each radio-tracked krait an identification code corresponding to the chronological order of capture (e.g. BUFA01, BUFA02, BUFA03). After capture, we anaesthetised the kraits using isoflurane to reduce stress while recording morphometric measurements (Setser, 2007) such as body mass, snout-to-vent length (SVL), tail length (TL), and head dimensions. We used cloacal probing described by Laszlo (1975) to determine sex. A veterinarian from the Nakhon Ratchasima Zoo surgically implanted kraits with VHF radio transmitters, following the methodology outlined by Reinert & Cundall (1982), with one of three transmitter models, (3.8 g SB-2, 9 g SI-2, and the 11 g SI-2 Holohil Inc., Ontario, Canada) depending on their availability. The ratio of transmitter mass to krait mass was 0.007 ± 0.090 % (mean \pm SD), significantly less than the maximum mass to body ratios recommended in telemetry studies of other vertebrates (Aldridge & Brigham, 1988; Knapp & Abarca, 2009).

We quantified spatial results using krait locations collected once daily, between 08:00 h and 18:00 h. So as not to stress the snakes, we avoided pinpoints during early mornings, nights and evenings to ensure that the nocturnal kraits were stationary within their daytime shelters. During the daytime, trackers followed the signal emitted from the internal radio transmitters to each krait's location, using an R410 ATS radio receiver in conjunction with an RA-23K C BA 17 Teleonics antennae. When a krait had moved to a new location from the previous track, trackers approached no closer than 6 m, relying on triangulation to pinpoint the new location using the 'draw line' feature on a Garmin 64S GPS unit. When a krait remained at a new location for at least one successive day, trackers attempted to locate the krait's exact position by approaching within 1 m of the shelter site if the site was accessible by walking across exposed soil, ruling out the possibility of treading on an unseen snake obscured by vegetation.

We estimated home ranges from the day time krait locations using dynamic Brownian and Bridge Movement Models (dBBMM) following the modern methodology used by Karelus et al. (2017), Kranstauber et al. (2012) and Silva et al. (2018) to quantify utilisation distributions (UD) as home ranges from which to quantify habitat use. Unlike other home range estimation methods such as kernel density estimates, which do not account for spatially autocorrelated animal movement data (Kranstauber et al., 2012), dynamic Brownian Bridge movement models incorporate the temporal structure of animal locations to estimate UD based on their movement's trajectory. We set the dBBMM moving window size to 15 (equivalent to 15 days) and a margin size of 3 to detect possible changes in movement between 3-day periods. Dynamic BBMM also requires input of the telemetry location error; we took this to be average GPS accuracy in the field (3 m). We calculated dBBMM UD estimates using Program R (R Core Team, 2017) in R Studio (R Studio Team, 2017), using packages 'BBMM' (Kranstauber et al., 2017), 'ctmm' (Fleming et al., 2017a), 'move' (Kranstauber et al., 2016), 'sp' (Pebesma & Bivand, 2005), 'ggplot2' (Wickham, 2009), 'adehabitatHR' (Calenge et al., 2015), and 'rgdal' (Roger et al., 2017). We designated the 95 % and 99 % dBBMM isopleth contour utilisation distributions to represent areas of 'core

utilisation' and a more generous 'total utilisation' as these larger contour values predict a more accurate area of use without over-smoothing (Walter et al., 2011).

We also report home range sizes using 100 % minimum convex polygons (MCP). Despite their drawbacks, MCPs are easy to conceptualise and have been widely applied to home range estimation in previous reports of snake spatial ecology (Croak et al., 2013; Mohammadi et al., 2014; Stiles et al., 2017; Vanek & Wasko, 2017). As a result of our small sample sizes, the power of robust statistical comparisons was limited, therefore we only report spatial results as descriptive statistics (mean \pm standard error) and limit our reporting to tracked kraits whose home ranges passed bootstrap analyses (i.e. non-asymptotic) as recommended by Marshall et al. (2018). We considered a home range asymptotic when the average home range size derived from 90 % of randomly ordered krait shelter sites were within 10 % of the home range estimate derived from the total shelter sites. Consequently, we were limited to reporting home range estimates only between the months of April – September 2017 as prior to, and following this 6-month period, tracking intervals were infrequent and irregular.

We estimated habitat use and availability on multiple scales, first using the 95 % dBBMM estimates, to compare 'core use' areas to those available within the total 100 % MCPs. Second, we evaluated shelter site preference by using Duncan's Index of Preference to calculate habitat use ratios from habitat at the krait diurnal locations (i.e. shelter sites) compared to the proportion each habitat comprised of the total home range (Duncan, 1983). We defined the areas within the 95 % dBBMM UD as available habitat from which to compare shelter site location habitats. We transformed preference scores, displaying them as log-normalised indices in which preference occurs when score values were greater than 0.3, following Duncan's methodology. We categorised habitats into 10 dominant types within each study area, including: canals, a recently constructed canal, field dykes, field margins, human settlement, tree plantations, ponds, rice, roads, and sugarcane (descriptions in Table 1). We

Table 1. Description of available habitats within krait home ranges

Habitat	Code	Description
Rice	RCE	Seasonally flooded rice (<i>Oryza sativa</i>) paddies harvested 2/year
Sugarcane	SGN	Sugarcane (<i>Saccharum</i> spp.) fields harvested 1/year
Plantation	PLT	Plantation forests of (<i>Eucalyptus camaldulensis</i>), or mixed fruit orchards (> 10 m at minimum width)
Canal	CAN	Irrigation canals and artificially channelized streams (> 3m wide)
New Canal	NCN	Recently constructed irrigation canal, baring exposed, sparsely vegetated banks
Pond	PND	Retention and fishponds
Field Dyke	DKE	Narrow embankments subdividing rice paddies (< 2m wide) and (91 cm \pm 53 cm high)
Field Margin	MGN	Either a depression or embankment linearly dividing habitat types (> 2 m wide)
Settlement	SET	Actively used buildings and surrounding lot
Roads	ROD	Paved or dirt (3 – 5 m in width)

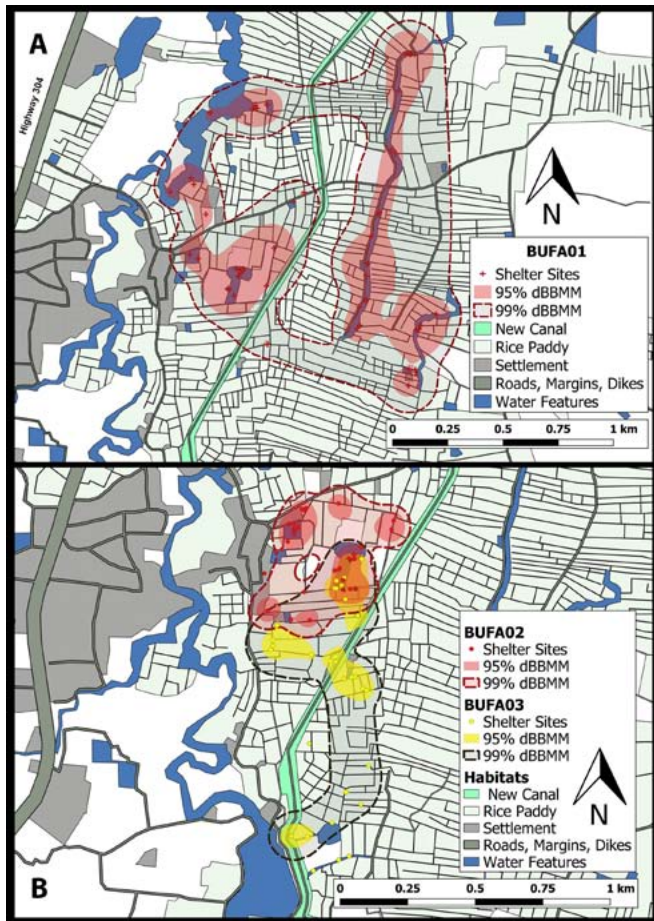


Figure 1. Map of the dynamic Brownian Bridge movement model utilisation distribution for *Bungarus fasciatus*, **A**) The single adult male (BUFA01), **B**) The two adult females (BUFA02 & BUFA03). Different levels of utilisation indicated by 95 % and 99 % contours represent greater and lesser use.

Table 2. Summary of tracking periods and basic biometric attributes of the three radio-tracked banded kraits recorded from their initial capture

Snake ID	Capture date	Last track date	Sex	Mass (g)	SVL (mm)	Transmitter type
BUFA01	2015-08-16	2017-11-22	M	1449	1576	Holohil SI-2: 11g, 33 x 11 mm
BUFA02	2016-11-26	2017-11-30	F	642.8	1341	Holohil SI-2: 9g, 33 x 11 mm
BUFA03	2017-03-14	2017-10-12	F	-	1131	Holohil SB-2: 3.8g, 14x9.5mm

Table 3. Movement and home range size from the simultaneous tracking period (April – September 2017) for each krait, MDF - mean displacement between fixes; MDD - mean daily displacement; MCP - minimum convex polygon calculated from 100 % of locations; ‘Unique shelters’ - the number of individual shelter sites used; ‘Revisited shelters’ - shelter sites used more than once

Snake ID	Fixes	Relocations	MDF (m)	MDD (m)	MCP (ha)	95 % dBMM	99 % dBMM	Unique shelter	Revisited shelters
BUFA01	161	58	221.2	79.69	127.33	49.10	134.88	48	6
BUFA02	161	36	110.35	24.67	13.60	7.10	20.28	34	3
BUFA03	159	46	128.72	37.25	30.85	8.14	30.00	38	5

digitised habitat features from Google Maps (2018) satellite imagery in QGIS (Quantum GIS Development Team, 2018). Indistinguishable habitat patches were subsequently visited in person on the ground for correct classification.

From 04 April 2017 to 01 July 2017, we used time-lapse photography to monitor a shared nesting site containing several burrow entrances used by the two radio-tracked female kraits. This enabled us to confirm nest attendance by the female kraits and photograph hatchling neonates as they emerged from below ground. We positioned two Bushnell field cameras (Trophy Cam HD Essential E3, Model: 119837) with infrared night capability on tripods spaced approximately 2 – 3 m from the focal nesting location. Cameras were programmed to each capture 1 still-image per minute. Each image displayed the ambient temperature and time taken. Field technicians replaced camera batteries and SD cards daily during their regular radio tracks of each krait.

RESULTS

Spatial ecology

We report the home range sizes of three radio-tracked banded kraits from 01 April – 30 September 2017, details of these snakes are shown in Table 2. Based on the 481 unique diurnal fixes, home range estimate means varied between 21.45 (± 19.56 ha) for the 95 % dBMM, 61.72 (± 51.88 ha) for the 99 % dBMM, and 57.26 (± 50.04 ha) for 100 % MCP. There were no instances of active movement or arboreal space use by the kraits during the 481 total diurnal tracks occurring between 08:00 – 18:00 h, throughout the study period.

On average, mean daily displacement (MDD) of kraits was 47.20 ± 23.54 m. BUFA01, the single adult male had the greatest MDD (79.69 m), while the two female kraits moved less than half the daily distance than the single tracked male (Table 3). We failed to make daily diurnal tracking fixes so that we only have on average 26.7 ± 1.6 days tracked/month; consequently, in Table 3 we have included values for mean displacement between fixes (MDF).

Habitat use

Based on the proportion of potential habitat types within each home range estimate, kraits selected permanent water features (ponds and canals) above all else. Field margins and dykes comprised the smallest portion of the land cover in each home range; however, they represented a slightly higher proportion of dBMM estimates, indicating higher selection. Human settlements were used in slightly higher proportion than their availability (3.42 % ± 3.25 % dBMM and 1.36 % ± 0.81 MCP) but this may be the result of their association with adjacent aquaculture ponds (Fig. 1). Rice monoculture

was the most abundant potential habitat across all home range estimates (Fig. 2A). However, rice paddies comprised a smaller portion of the more accurate 95 % dBBMMs (57.47 % \pm 5.23%) than the less accurate 100% MCPs (67.65 % \pm 10.16 %), indicating an overall avoidance of open rice paddies (Fig. 2B).

Preference indices derived from shelter site locations varied across the three individuals (Fig. 2B). Preferred habitats by BUFA01 included field margins, pond banks, canals, and to a lesser extent, the paddy dykes. The two females showed shelter site preference for field margins, paddy dykes, and pond banks. However, the male (BUFA03)

was the only individual to shelter amongst the sparsely vegetated banks of a recently constructed canal (NCN, Fig. 2B), which was available to all three snakes. BUFA03 also had slight preference for roadsides. Agricultural fields were again avoided, with preference scores falling far below the 0.30 preference threshold for each krait (Fig. 2B).

Nest attendance

While attending their nests, the two females simultaneously shared the same burrow complex for 64 days, BUFA02 (14 April – 01 July 2017; 75 days) and BUFA03 (04 April – 17 June 2017; 77 days). Both females were radio-located at their nest site throughout this period except for 8 separate days spent at nearby shelter sites (BUFA02) and 1 day each at 2 separate shelter sites (BUFA03). During the nesting period the MDDs of BUFA02 and BUFA03 were only 13.76 m and 12.56 m respectively, which amounted to a reduction by 58 % and 77 % when compared with the MDDs for the non-nesting period in the dry and rainy season of 2017. Both females moved less frequently during nesting but when they did move the resulting displacements were greater than those seen between shelter sites during non-nesting periods. Frequency of relocations during the nesting period was also reduced for both females. However, MDFs were greater as their infrequent moves between the short-term shelter sites and the nesting site were farther than the mean MDDs during non-nesting periods (Table 3). The dBBMM movement variance did not differ significantly between the two females while nesting (two-sample Kolmogorov Smirnov test $D = 0.108$, $p = 0.59$). The movement variance of the single male, BUFA01, did not change during the nesting period as the snake continued to make frequent, long-distance moves compared to the females (Fig. 3).

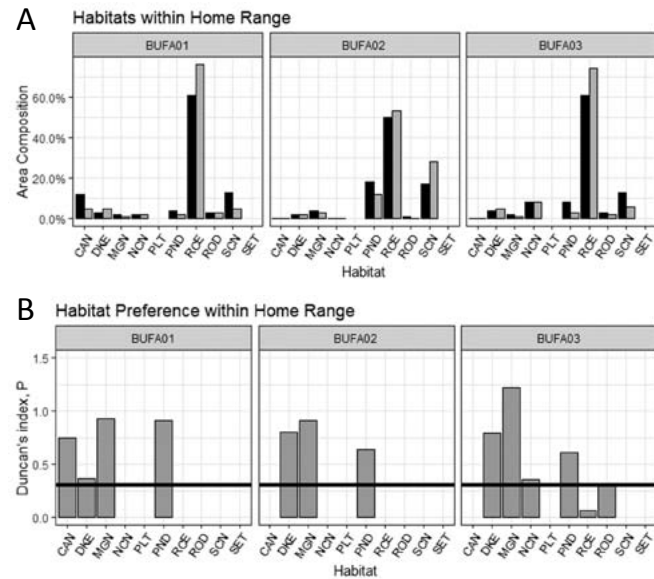


Figure 2. Habitat Preference **A)** Percent composition of 95 % dBBMM (black) and 100 % MCP (grey) home range estimates, **B)** Habitat preference indices for kraits using log-normalised Duncan's Index. Preferences calculated from 95 % dBBMM utilisation distribution. Habitat codes: CAN- canal, DKE- paddy dyke, MGN- field margin, NCN - new canal, PLT - plantation forest, PND - pond, RCE - rice paddies, ROD - road, SCN - sugarcane fields, SET - settlement. Preference occurs above 0.30 (indicated by line).

Neonate emergence

At the nesting site, we observed 15 neonates emerge from three separate burrow entrances within an approximately 2 m long strip of raised field margin, measuring approximately 1.4 m wide and 1.2 m high, separating a sugarcane and

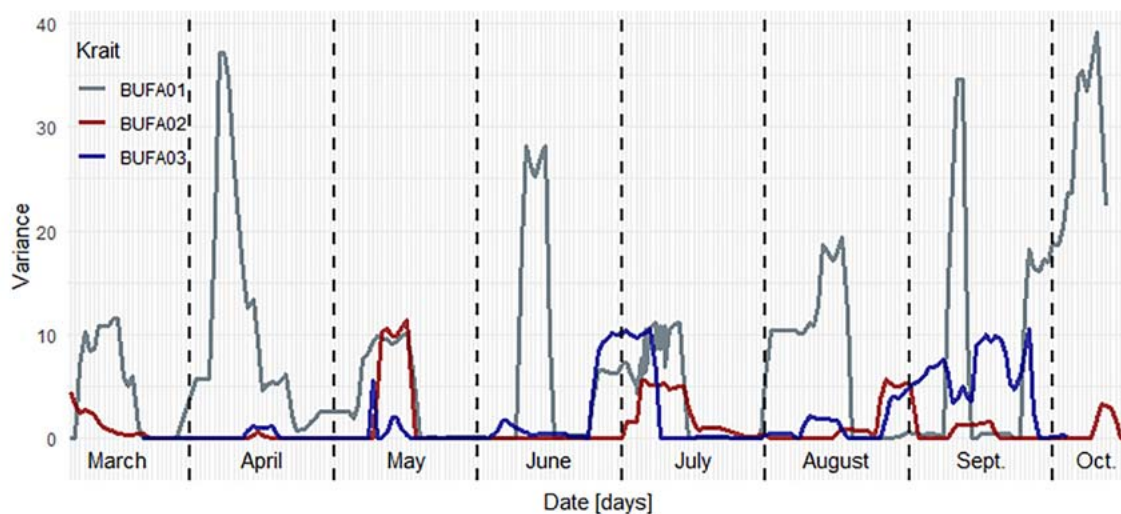


Figure 3. Variance estimation (σ^2m) over time for the three kraits showed a subtle drop in movement activity during the nesting period for the two females (BUFA02 & BUFA03) between April – June. However, the single adult male (BUFA01) continued to make major movements throughout the same period.

Table 4. Field camera records of neonate *B. fasciatus* emerging from a shared nesting chamber and some basic biometric attributes of the captured individuals. The field cameras were set to record 1 frame/minute.

Neonate ID	Emergence from nest		No. photo frames	Temp. (°C)	Sex	Mass (g)	SVL (cm)
	Date	Time					
1	24 June	01:10	3	22	-	-	-
2		02:23	8	22	-	-	-
3	25 - 26 June	21:59	NA	25	Male	332	15.9
4		02:05	NA	25	Male	342	16
5		02:51	NA	25	Male	-	14.7
6	28 - 29 June	21:12	9	26	-	-	-
7	30 June	00:25	6	25	-	-	-
8	06 - 07 July	20:51	1	26	-	-	-
9		23:48	3	22	-	-	-
10		00:05	2	25	-	-	-
11		00:08	6	25	-	-	-
12		01:42	2	24	-	-	-
13		03:30	14	24	-	-	-
14		19:19	1	23	-	-	-
15	07 July	20:24	8	22	-	-	-

cassava field. The first two emerged on the night of 23 June 2017, followed by 3 individuals the following night and 2 individuals on subsequent nights (28 - 30 June). A second batch of 6 individuals emerged on the night of 06 July 2017 accompanied by 2 more the following night. The first 7 neonates to emerge likely belonged to BUFA03, the first female to arrive at the nest site on 04 April 2017 and the last 8 individuals to BUFA02, who arrived on 14 April 2017. Our field cameras, set to capture 1 image at the same time every minute, recorded in situ neonates on a total of 55 photo-frames, with a mean of 4.58 frames (i.e. minutes) to disperse from the nest site and immediate surrounding field margin. Temperature stamps on emergence frames ranged from 23° – 26° C. We monitored the nest site with field personnel on the night of 25 June 2017 and managed to physically capture and make measurements of three individuals as they emerged from a nest burrow (Table 4).

DISCUSSION

Our study demonstrates that banded kraits have relatively large home ranges and preferred micro-habitats such as waterways, rice paddy dykes, and field margins when inhabiting farmland. Undisturbed micro-habitats may be a key factor in the continued persistence of kraits by facilitating important life history events (sheltering, foraging, mating, and nesting). Our observations on krait nesting activity also highlight the need for further investigation into elapid reproductive biology. Nest attendance while sharing space by our two females may provide preliminary evidence of parental care and sociality in banded kraits.

However, any conclusions drawn from our study are limited as observations were based on a sample of only three snakes. Despite extensive night surveys, we were only able

to capture four adult individuals to radio-track (1 krait/118 survey man-hours) and maintained only 3 individuals long enough to report meaningful observations.

Parental care in snakes has been documented from viviparous species of Viperidae and Pythonidae (Alexander, 2018; Brashears & Denardo, 2012) and may be a more prevalent trait among temperate snake species (Butler et al., 1995; Clark et al., 2012; Halliwell et al., 2018; Hill III et al., 2006; Muellman et al., 2018). In elapid snakes, reports of nest attendance or other parental behaviour is limited to female king cobras (*Ophiophagus hannah*) guarding the nests they build from vegetation on the forest floor (Dolia, 2018; Whitaker et al., 2013) and Indochinese spitting cobras (*Naja siamensis*) guarding egg clutches for the complete egg incubation period in Thailand (*B. Nadolski* personal communication, 2019). Previous observations of the nesting behaviour of kraits have been limited to captive specimens of *Bungarus flaviceps* (Chanhome, 2014). Interestingly, the two female kraits in our study shared the same burrow complex while nesting. We are unsure to what extent they shared space below ground; however, both snakes were observed on camera entering and exiting the same set of burrow entrances. As far as we are aware, there are no previous reports of communal nesting or maternal sociality in wild elapid snakes.

Four habitat features (ponds, margins, canals, dykes) had the highest preference scores (Fig. 2B), despite accounting for only 16.15 % of land area across the three snake's study areas. In a separate study, an individual Malayan krait (*Bungarus candidus*) in an upland agricultural area in the SBR also showed strong preference for a vegetated field margin (Knierim et al., 2018). Similarly, king cobras (*Ophiophagus hannah*) in the SBR prefer irrigation canals and field margins in agricultural landscapes (Marshall et al., 2018); canals may

serve as movement corridors for snakes through unfavourable landscapes (Whitaker & Shine, 2000).

Rice paddy dykes and termite mounds have been shown to serve as biodiversity reservoirs for micro fauna and arthropods in rice dominated agricultural systems of north-east Thailand (Choosai et al., 2009; Ichihara et al., 2014).

Paddy dykes are also important micro-habitat features for some vertebrates, hosting higher abundances of field paddy rats (*Rattus argentiventer*) in Indonesia (Brown et al., 2001), and tree frogs (*Dryophytes japonicus* & *Dryophytes suweonensis*) in South Korea (Groffen et al., 2018). Surveys of rice fields for *R. argentiventer* burrow entrances by Brown et al. (2001), identified field dykes and canals as the two micro-habitats having the highest densities of rat burrows. Both rodents and amphibians may serve as potential prey items for banded kraits (Chan-ard et al., 2015). Furthermore, the burrows excavated by rodents provided kraits in our study with structural refuges for sheltering and nesting in an exposed landscape that is subject to frequent disturbance from farming activities.

Ultimately, the preference for field margin and water features in agricultural habitats is likely related to the disturbance levels or availability of prey and shelter sites. Habitat use by snakes is often explained by multiple factors (Heard et al., 2004). Therefore, assessments of micro-habitat characteristics at known krait shelter sites should test hypotheses to further our inquiry. Future work should also assess the micro-habitat features surrounding krait's diurnal retreats, mating, and nesting sites. Animal burrows can be a limited resource, providing shared refuge for an array of species in otherwise harsh environments (Hofstede & Dziminski, 2017; Pike & Mitchell, 2013), and we therefore expect them to be a key enabling feature in the persistence of snakes and other taxa in agricultural habitats.

Our conclusions on krait spatial ecology and habitat selection are limited and largely unsupported quantitatively due to our difficulty in radio tracking an adequate sample size of kraits. Future research on banded krait ecology should attempt to radio track a larger sample to give quantitative assessments of spatial and habitat requirements. Banded kraits are known to occur in different habitats than those in our study, including coastal wetlands and major cities, (Kurniawan et al., 2018; Purkayastha et al., 2011). Their use of these habitats is also worthy of future study.

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REFERENCES

- Aldridge, H.D.J.N. & Brigham, R.M. (1988). Load carrying and maneuverability in an insectivorous bat: a test of the 5% "rule" of radio-telemetry. *Journal of Mammalogy* 69: 379-382.
- Alexander, G.J. (2018). Reproductive biology and maternal care of neonates in southern African pythons (*Python natalensis*). *Journal of Zoology* 305: 141-48.
- Amarasinghe, A.A.T., Madawala, M.B., Karunarathna, D.M.S.S., Manolis, S.C., Silva, A. & Sommerlad, R. (2015). Human-crocodile conflict and conservation implications of saltwater crocodiles *Crocodylus porosus* (Reptilia: Crocodylia) in Sri Lanka. *Journal of Threatened Taxa* 7: 7111-7130.
- Barnes, C.H., Strine, C.T., Suwanwaree, P. & Hill III, J.G. (2017). Movement and home range of green pit vipers (*Trimeresurus* Spp.) in a rural landscape in north-east Thailand. *The Herpetological Bulletin* 142: 19-28.
- Berger-Tal, O., Polak, T., Oron, A., Lubin, Y., Kotler, B.P. & Saltz, D. (2011). Integrating animal behavior and conservation biology: a conceptual framework. *Behavioral Ecology* 22: 236-39.
- Brashears, J. & Denardo, D.F. (2012). Do brooding pythons recognize their clutches? Investigating external cues for offspring recognition in the children's python, *Antaresia childreni*. *Ethology* 118: 793-98.
- Brown, P.R., Singleton, G.R. & Sudarmaji (2001). Habitat use and movements of the rice-field rat, *Rattus argentiventer*, in West Java, Indonesia. *Mammalia* 65:151-166.
- Butler, J.A., Hull, T.W. & Franz, R. (1995). Neonate aggregations and maternal attendance of young in the eastern diamondback rattlesnake, *Crotalus adamanteus*. *Copeia* 1995: 196-98.
- Chan-ard, T., Nabhitabhata, J. & Parr, J.W. (2015). *A Field Guide to the Reptiles of Thailand*. Oxford University Press, 314 pp.
- Chanhome, L. (2014). Reproduction of the red-headed krait (*Bungarus flaviceps*) in captivity. *Tropical Natural History* 13: 59-63.
- Choosai, C., Mathieu, J., Hanboonsong, Y. & Jouquet, P. (2009). Termite mounds and dykes are biodiversity refuges in paddy fields in north-eastern Thailand. *Environmental Conservation* 36: 71.
- Clark, R.W., Brown, W.S., Stechert, R. & Greene, H.W. (2012). Cryptic sociality in rattlesnakes (*Crotalus horridus*) detected by kinship analysis. *Biology Letters* 8: 523-525.
- Dickman, A.J. (2010). Complexities of conflict: the importance of considering social factors for effectively resolving human - wildlife conflict. *Animal Conservation* 13: 458-66.
- Dolia, J. (2018). Notes on the distribution and natural history of the king cobra (*Ophiophagus hannah* Cantor, 1836) from the Kumaon Hills of Nepal. *Herpetology Notes* 11: 217-22.
- Duncan, P. (1983). Determinants of the use of habitat by horses in a Mediterranean wetland. *The Journal of Animal Ecology* 93-109.
- Dunham, K.M., Ghiurghi, A., Cumbi, R. & Urbano, F. (2010). Human-wildlife conflict in Mozambique: a national perspective, with emphasis on wildlife attacks on humans. *Oryx* 44: 185-193.
- Frédéric, A., Eva, H.D., Stibig, H., Mayaux, P., Gallego, J., Richards, T. & Malingreau, J. (2002). Determination of deforestation rates of the world's humid tropical forests.

- Science* 297: 999-1002.
- Gomes, A., Saha, P.P., Bhattacharya, S., Ghosh, S., & Gomes, A. (2017). Therapeutic potential of krait venom. *Toxicon* 131: 48-53.
- Groffen, J., Borzée, A. & Jang, Y. (2018). Preference for natural borders in rice paddies by two treefrog species. *Animal Cells and Systems* 22: 205-11.
- Halliwell, B., Uller, T., Holland, B.R. & While, G.M. (2018). Reptiles. *Nature Communications* 8: 1-8.
- Hansen, A.J. & Defries, R. (2018). Ecological mechanisms linking protected areas to surrounding lands. *Ecological Applications* 17: 974-88.
- Hart, K.M., Cherkiss, M.S., Smith, B.J., Mazzotti, F.J., Fujisaki, I., Snow, R.W. & Dorcas, M.E. (2015). Home range, habitat use, and movement patterns of non-native Burmese pythons in Everglades National Park, Florida, USA. *Animal Biotelemetry* 3: 1-13.
- Heard, G.W., Black, D. & Robertson, P. (2004). Habitat use by the inland carpet python (*Morelia spilota metcalfei*: Pythonidae): seasonal relationships with habitat structure and prey distribution in a rural landscape. *Austral Ecology* 29: 446-460.
- Hill III, J.G., Chanhom, L., Artchawakon, T., Thirakhump, K. & Voris, H.K. (2006). Nest attendance by a female malayan pit viper (*Calloselasma rhodostoma*) in northeast Thailand. *The Natural History Journal of Chulalongkorn University* 6: 57-66.
- Hofstede, L. & Dziminski, M.A. (2017). Greater bilby burrows: important structures for a range of species in an arid environment. *Australian Mammalogy* 39: 227-37.
- Hughes, A. (2017). Understanding the drivers of southeast Asian biodiversity loss. *Ecosphere* 8: 1-33.
- Ichihara, M., Matsuno, K., Inagaki, H., Saiki, C., Mizumoto, S., Yamaguchi, S., Yamashita, M. & Sawada, H. (2014). Creation of paddy levees to enhance the ecosystem service of weed seed predation by crickets. *Landscape and Ecological Engineering* 11: 227-233.
- Imai, N., Furukawa, T., Tsujino, R., Kitamura, S. & Yumoto, T. (2018). Factors affecting forest area change in southeast Asia during 1980-2010. *PLoS ONE*: 1-14.
- International Union for Conservation of Nature. (2013). IUCN Red List of Threatened Species. <http://www.iucnredlist.org> (accessed April 2019)
- Kanokkanjana, K. & Garivait, S. (2013). Alternative rice straw management practices to reduce field open burning in Thailand. *International Journal of Environmental Science and Development* 4: 119-23.
- Karelus, D.L., McCown, J.W., Scheick, B.K. & Oli, M.K. (2018). Micro-habitat features influencing habitat use by Florida black bears. *Global Ecology and Conservation* 13(2017): e00367.
- Knapp, C.R. & Abarca, J.G. (2009). Effects of radio transmitter burdening on locomotor ability and survival of iguana hatchlings. *Herpetologica* 65: 363-372.
- Knierim, T.K., Marshall, B.M., Hayes, L., Suwanwaree, P. & Strine, C.T. (2018). The movements and habitat preferences of a Malayan Krait (*Bungarus candidus*) in an agrarian landscape. *The Herpetological Bulletin* 143: 30-33.
- Kranstauber, B., Kays, R., Lapoint, S.D., Wikelski, M. & Safi, K. (2012). A dynamic Brownian Bridge Movement Model to estimate utilization distributions for heterogeneous animal movement. *Journal of Animal Ecology* 81: 738-746.
- Kranstauber B., Smolla, M. & Scharf, A.K. move: visualizing and analyzing animal track data. R package version 3.0.1. 2017: <http://CRAN.R-project.org/package=move>.
- Kurniawan, N., Firdaus, A.S., Nugraha, F.A.D., Maulidi, A. & Kurianto, A.S. (2018). Fishermen's perspective on herpetofauna: a case study from Kuala Tungkal. *The Journal of Tropical Life Science* 8: 1-5.
- Laszlo, J. (1975). Probing as a practical method of sex recognition in snakes. *International Zoo Yearbook* 15: 178-79.
- Loughran, Caleb L., Beck, Daniel D. & Weaver, R.E. (2015). Use of communal shedding sites by the northern pacific rattlesnake (*Crotalus oreganus oreganus*) in central Washington State. *Northwestern Naturalist* 96: 156-60.
- Luo, Y., Fu, H. & Traore, S. (2014). Biodiversity conservation in rice paddies in China: toward ecological sustainability. *Sustainability* 6: 6107-24.
- Maritz, B., Penner, J., Martins, M., Crnobrnja-Isailović, J., Spear, S., Alencar, L.R.V., Sigala-Rodriguez, J., Messenger, K., Clark, R.W., Soorae, P., Luiselli, L., Jenkins, C., & Greene, H.W. (2016). Identifying global priorities for the conservation of vipers. *Biological Conservation* 204: 94-102.
- Marshall, B.M., Strine, C.T., Jones, M.D., Artchawakon, T., Silva, I., Suwanwaree, P. & Goode, M. (2018). Space fit for a king: spatial ecology of king cobras (*Ophiophagus hannah*) in Sakaerat Biosphere Reserve, north-eastern Thailand. *Amphibia-Reptilia* 40: 163-178.
- Marshall, B.M., Strine, C.T., Jones, M.D., Theodorou, A., Amber, E., Waengsothorn, S., Suwanwaree, P. & Goode, M. (2018). Hits close to home: repeated persecution of king cobras (*Ophiophagus hannah*) in northeastern Thailand. *Tropical Conservation Science* 11: 1-14.
- Martínez-Abraín, A. & Jiménez, J. (2015). Anthropogenic areas as incidental substitutes for original habitat. *Conservation Biology* 30: 593-98.
- Mohammadi, S., Kluever, B.M., Tamashiro, T., Amano, Y. & Hill III, J.G. (2014). Spatial and thermal observations of a Malayan krait (*Bungarus candidus*) from Thailand. *Tropical Natural History* 14: 21-26.
- Muellman, P.J., Cunha, O.D. & Montgomery, C.E. (2018). *Crotalus horridus* (Timber Rattlesnake) Maternal scent trailing by neonates. *Northeastern Naturalist* 25: 50-55.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853-58.
- Pe, T., Myint, T., Htut, A., Htut, T., Myint, A.A. & Aung, N.N. (1997). Envenoming by Chinese krait (*Bungarus multicinctus*) and banded krait (*B. fasciatus*) in Myanmar. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 91: 686-688.
- Pike, D.A. & Mitchell, J. C. (2013). Burrow-dwelling ecosystem engineers provide thermal refugia throughout the landscape. *Animal Conservation* 16: 694-703.
- Purkayasth, J., Das, M. & Sengupta, S. (2011). Urban herpetofauna: a case study in Guwahati City of Assam, India. *Herpetology Notes* 4: 195-202.
- Reinert, H. & Cundall, D. (1982). An improved surgical implantation method for radio-tracking snakes. *Copeia*

- 1082: 702-5.
- Rosa, I.M.D., Smith, M.J., Wearn, O.R., Purves, D., Ewers, R.M., Rosa, I.M.D., Smith, M.J., Wearn, O.R., Purves, D. & Ewers, R.M. (2016). The environmental legacy of modern tropical deforestation. *Current Biology* 26: 2161-66.
- Row, J. & Blouin-Demers, G. (2006). Kernels are not accurate estimators of home-range size for herpetofauna. *Copeia* 4: 797-802.
- Seaman, D.E. & Powell, R.A. (1996). An evaluation of the accuracy of kernel density estimators for home range analysis. *Ecology* 77: 2075-85.
- Setser, K. (2007). Use of anesthesia increases precision of snake length measurements. *Herpetological Review* 38: 409-11.
- Silva, I., Crane, M., Suwanwaree, P., Strine, C. & Goode, M. (2018). Using dynamic Brownian Bridge Movement Models to identify home range size and movement patterns in king cobras. *PloS one* 13(9): p.e0203449.
- Strine, C., Silva, I., Barnes, C.H., Marshall, B.M., Artchawakom, T., Hill, J. & Suwanwaree, P. (2018). Spatial ecology of a small arboreal ambush predator, *Trimeresurus macrops* Kramer, 1977, in northeast Thailand. *Amphibia-Reptilia*: 1-11.
- The Reptile Database. (2017). *Bungarus fasciatus*. <http://www.reptile-database.org> (accessed December 2017)
- Tongpoo, A., Sriapha, C., Pradoo, A., Udomsubpayakul, U., Srisuma, S., Wananukul, W. & Trakulsrichai, S. (2018). Krait envenomation in Thailand. *Therapeutics and Clinical Risk Management* 14: 711.
- Torres, D.F., Oliveira, E.S. & Alves, Rômulo R.N. (2018). Understanding human-wildlife conflicts and their implications. *Ethnozoology*: 421-445.
- Trimble, M.J. & Van Aarde, R.J. (2012). Geographical and taxonomic biases in research on biodiversity in human-modified landscapes. *Ecosphere* 3: 1-16.
- Vanek, J.P. & Wasko, D.K. (2017). Spatial ecology of the eastern hog-nosed snake (*Heterodon platirhinos*) at the northeastern limit of its range. *Herpetological Conservation and Biology* 12: 109-18.
- Walter, W.D., Fischer, J.W., Baruch-Mordo, S. & VerCauteren, K.C. (2011). What is the proper method to delineate home range of an animal using today's advanced GPS telemetry systems: the initial step. *Modern telemetry*. IntechOpen.
- Whitaker, N., Shankar, P.G., & Whitaker, R. (2013). Nesting ecology of the king cobra (*Ophiophagus hannah*) in India. *Hamadryad* 36: 101-7.
- Whitaker, P.B. & Shine, R. (2000). Sources of mortality of large elapid snakes in an agricultural landscape. *Journal of Herpetology* 34: 121-128.
- Wood, C., Qiao, Y., Li, P., Ding, P., Lu, B., & Xi, Y. (2010). Implications of rice agriculture for wild birds in China. *Waterbirds* 33: 30-43.

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Captive husbandry and breeding of the reticulated glass frog, *Hyalinobatrachium valerioi* (Anura: Centrolenidae)

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ABSTRACT - The reticulated glass frog (*Hyalinobatrachium valerioi*) from Central and South America is a species that has been widely collected from the wild for the pet trade. An ex situ population of this frog was recently established at Slimbridge Wetland Centre, UK, in the hope of learning more about the husbandry and biology of this species. We report on the captive husbandry of adults and the successful breeding and rearing of larvae to metamorphosis. Clutch size was 28 eggs. The eggs hatched at 15-18 days after laying and tadpoles reached a total length of 34 mm. The first individuals started metamorphosis 147 days after hatching at water temperatures of 22-28 °C. *Hyalinobatrachium valerioi* is still being collected from the wild for the international pet trade and to our knowledge this is the first documented breeding of this species in the UK. The husbandry methods described here could be used to breed this and other *Hyalinobatrachium* species in captivity and so reduce collecting from wild populations.

INTRODUCTION

The glass frog genus *Hyalinobatrachium* currently comprises 32 described species (Frost, 2018). They are widely distributed across Central and South America from tropical Mexico to south-eastern Brazil and Argentina with an isolated population in Tobago, West Indies (Ruiz-Carranza & Lynch, 1991). *Hyalinobatrachium valerioi* (Dunn, 1931), commonly known as the reticulated glass frog or La Palma glass frog, occurs from central Costa Rica to the Pacific slope of Ecuador; western slope of the western Andes in the Magdalena Valley, Colombia, from sea level to 1500 m elevation (Dunn, 1931; Taylor, 1951; Taylor, 1958; Starrett & Savage, 1973; Ruiz-Carranza & Lynch, 1991; Frank & Ramus, 1995). *Hyalinobatrachium valerioi* occurs in lowland moist and wet forest as well as premontane wet forest and rainforest (Savage, 2002). The species is assessed as of Least Concern by the IUCN (Solís et al., 2008).

Hyalinobatrachium valerioi is a nocturnal frog (Guyer & Donnelly, 2005) and males maintain territories by emitting a call from under leaves during the wet season (Savage, 2002). Male frogs defend their territories aggressively, even fight with rival males, and emit specific calls when another male enters their territory (Savage, 2002). During mating, the female lays a clutch of approximately 35 eggs. The typical oviposition site is the underside of a leaf above running water (Savage, 2002). The female leaves the eggs once they have been fertilised. The male then defends the eggs by attacking any intruding males that come near to the eggs. The male also guard against intruding wasps that have been observed feeding on the eggs and embryos of the frog (McDiarmid, 1978). The male continues to vocalise to attract more females to lay eggs on the same leaf, up to seven different clutches having been seen on one leaf under the protection of a guarding male (Savage, 2002). The male

hydrates the eggs by sitting on them and emptying its bladder (Savage, 2002). Although *H. valerioi* has been kept and bred in captivity the methods used to breed them and rear the tadpole to metamorphosis have not been documented in detail.

METHODS AND RESULTS

Captive and breeding conditions

Nine captive-bred *H. valerioi* were obtained in September 2016 from three private collections in Europe. These comprised two adult males, four adult females and three juveniles (two males, one female). The largest female measured 19 mm and the largest male was 18 mm. On arrival, one female was obviously gravid as eggs could be seen through the ventral surface, during quarantine she reabsorbed the eggs.

The group was initially housed in a 40 x 45 x 38 cm glass vivarium with a 15 x 45 cm mesh ventilation section at the top of the tank with front opening access in our quarantine facility. Despite the fact that this species hides under leaves in the rainforest we provided full spectrum UV lighting as it is likely that the frogs would get some exposure to UV-B radiation naturally through reflection.

UV-B provision was considered particularly important for the healthy development of the juveniles in the group as it is required for vitamin D3 biosynthesis (e.g. Michaels et al., 2015; Tapley et al., 2015) that plays an important role in calcium metabolism, muscle development, organ formation, muscle contraction as well as immune and nervous system functions (Whitaker & Wright, 2001). We used an Arcadia 7 % D3 Compact Bulb (Arcadia Products plc, Redhill, UK) which sat directly on the mesh top of the vivarium. UV index was measured weekly with a Solarmeter 6.5 UV index meter (Solartech Inc., Harrison Township, MI); UVI gradients were measured through the mesh and ranged 0-3 UVI at the level

of the leaves the frogs were under or on top of. While frogs were in quarantine the enclosure was simply furnished with potted plants such as *Philodendron hederaceum* and *Spathiphyllum cochlearispathum*, and damp paper towel was used as a substrate, changed weekly. Two small water dishes were provided and these were changed daily and filled with aged tap water, to reduce chlorine content, the tap water at Slimbridge is generally hard with GH: 6-8 °d (general hardness). The diet for the frogs consisted of crickets (*Gryllus assimilis* and *Gryllus bimaculatus*) and fruit flies (*Drosophila melanogaster*). Frogs were fed once a week with all food dusted with the dietary supplement Nutrobal (Vetark Ltd., Winchester, UK).

After 6 months, in March 2017, the quarantine period was complete and the frogs were moved into a bespoke glass vivarium 50 x 65 x 65 cm (Figs. 1A and 1B) (Custom aquaria, Rushden, UK). The tank was designed so that seasonal fluctuations from Costa Rica (Table 1) could be mimicked to best reflect the cycle of the animals in the wild for breeding, without having to disturb them and move them to rain chambers. The enclosure design facilitated seasonal flooding using a rain shower head as well as a misting nozzle in case different types of rainfall (drops rather than mists) triggered breeding during the rainy season. The enclosure had a false bottom which allowed water depth to be adjusted according to seasonality. A waterfall was created to circulate the water, flowing down rocks at the back of the tank to the stream from a height of 15 cm, this gave the stream a gentle flow using an EDEN 109 submersible pump (PfG GmbH, Hörstelm Germany) which was situated in the false bottom reservoir. Water temperature ranged from 19-23 °C. The substrate of the stream and banks was gravel; the edges of the artificial stream bank were covered in soil, moss and leaf-litter. Plants were provided and planted into the soil around the edge of the water on the banks so that leaves were overhanging the stream; these provided resting sites as well as potential oviposition sites. Only plants from the natural range of *H. valerioi* were provided and these included *S. cochlearispathum*, *Philodendron laciniatum*, *P. hederaceum*, *P. erubescens* and *Monstera acuminata*. We used the same UVB emitting lighting array described earlier, but also introduced a 50 W basking light (Arcadia Products plc, Redhill, UK) next to the UV light and a GroBeam 1500 ND Natural Daylight LED (Tropical Marine Centre Ltd, Rickmansworth, UK) which was

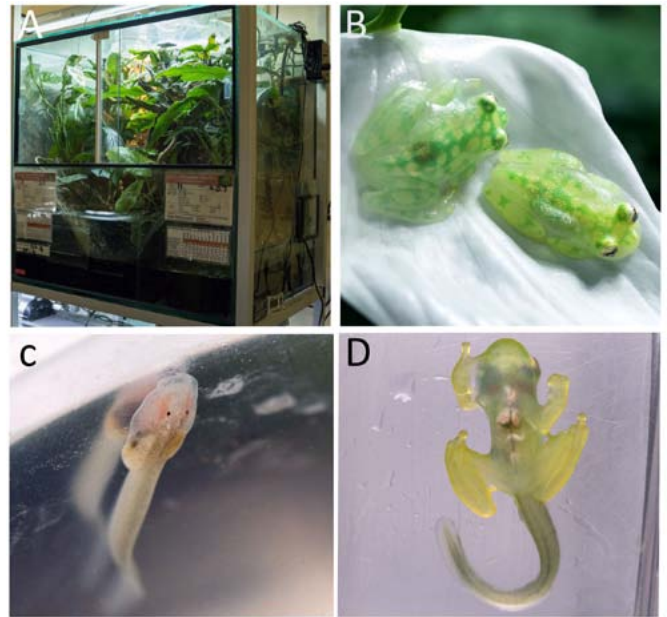


Figure 1. A) Breeding enclosure for *H. valerioi*; B) Adult *H. valerioi*; C) Tadpole of *H. valerioi* and D) *H. valerioi* near completion of metamorphosis

controlled by an AquaRay SmartControl 8 (Tropical Marine Centre Ltd, Rickmansworth, UK). Photoperiod was adjusted monthly in accordance to climate charts (Weather to travel, 2019) using data from Costa Rica on the season’s day length and sunlight hours (Table 1).

Temperature was controlled by HabiStat Digital Temperature Thermostat (HabiStat corporation, Switzerland), Day/Night that was plugged into a 60W, 59 x 28 cm HabiStat High Power Mat Adhesive. This allowed us to drop temperatures at night and change temperature in accordance with the parameter chart (Table 1). The misting water was provided with locally collected rain water and humidity increased by the use of a misting system through two nozzles in the vivarium that were controlled by an automatic timer, allowing for an adjustable misting period. Misting water was 19-23 °C with following parameters: pH 6.8-7, KH: 3-6 °d (carbonate hardness), and GH: 8 °d. Although we tried to follow the temperature regime in Table 1, it was sometimes difficult due to extremes of ambient temperature. Therefore, summer night time temperatures

Table 1. Environmental parameters from Costa Rica that were used as a base line for husbandry parameters in this study (Weather to Travel, 2019)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Max day temperature °C	23	24	25	25	25	25	24	24	24	24	24	23
Min night temperature °C	15	15	16	16	17	17	17	17	17	17	16	16
Hours of day light	12	12	12	12	13	13	13	12	12	12	12	12
Hours of sunshine	7	7	7	7	7	7	6	6	6	6	6	6
UV index (maximum)	11	11+	11+	11+	11+	11+	11+	11+	11+	11+	11	10
Monthly rainfall (mm)	6	7	9	56	261	296	205	282	372	343	155	36
Days with some rainfall	12	9	8	10	19	24	23	24	24	23	18	16

were often higher than the suggested ambient temperature. The ambient daytime temperature of the enclosure from October- February was 23-24 °C with night-time drops as low as 17 °C. From March-April day time temperatures were increased to 24-26 °C and at this point feeding was increased to twice a week to facilitate egg development. The mister was switched off from December – March to simulate the dry season, at this time the enclosure was hand sprayed with an atomiser twice a day mainly for the plants. In March, the mister was switched back on and was set to run daily for the 30 second periods, late morning, early evening and again at night. In early April, the timer was increased to mist for 60 seconds at each period.

Observations on breeding success and tadpoles rearing

The first clutch of eggs was discovered on 22 April 2018 under a leaf of *P. erubescens*, the clutch contained 28 eggs. As there was a male guarding the eggs, we decided to leave them in situ rather than remove them and risk disturbing the guarding male. During this time, a plastic tub with water collected from the enclosure was placed directly under the eggs so that tadpoles hatching from the nest could be caught in the water below. The first tadpole hatched 15 days after oviposition on 7 May 2018. At this time, the leaf with the eggs on it was moved directly above a tadpole rearing enclosure that had been established in advance with aged, oxygenated tap water. This allowed the tadpoles to drop into the rearing enclosure naturally rather than transferring the tadpoles once they had all hatched. Out of the 28 eggs, one egg was infertile and one tadpole failed to hatch.

A second clutch was found on 31 May 2018, but these eggs were all infertile. The initial tadpole rearing enclosure was 15 x 20 x 13 cm and contained 3 litres of water. A mix of gravel, sand and leaf litter was used as a substrate, simulating the known microhabitats of the tadpoles at the bottom of streams (Rada et al., 2007). This allowed tadpoles to dig into the substrate. A few areas of the enclosure floor were left bare. Tadpoles were provided with oak (*Quercus robur*) leaves as the leaf litter, which we anticipated would release antioxidants into the water which could potentially aid their development (Szuroczi et al., 2016). An aerator was installed immediately, as the tadpoles would usually live in well oxygenated water. The temperature ranged from 22-28 °C, but was usually maintained at the top end of this range (25-28 °C), in the hope of speeding up larval development. The reason for trying this was that it is well documented that some centrolenid frogs have prolonged larval development, which can be in excess of five months (Hoffmann, 2004; Rada et al., 2007) with tadpoles reaching stage 41 in up to 265 days (Hoffmann, 2010). Tadpoles, particularly of stream breeding species, can be very sensitive to water quality and the longer the larval development, the greater the risk of human error and / or technical malfunctions of equipment, which could lead to the death of tadpoles.

Tadpoles were fed on a mix of tropical fish flake and Repashy Superpig (Repashy Ventures Inc, Oceanside, CA), once a day in the morning once the lights were on. On 10 June 2018, tadpoles were moved into a larger tank 25 x 60 x 25 cm with 28 litres of water. All furnishings remained the same but an

additional internal filter was installed to process nitrogenous waste. A water change of 25 % was carried out weekly. High ambient temperatures led to excessive evaporation, so that some of the water changes were simply top ups. Water was tested once a month before a water change and nitrogenous waste (nitrite, nitrate and ammonia), which is toxic to aquatic organisms, was measured. Nitrite remained below 0.3 mg/l, nitrate remained below 100 mg/l, and ammonia was never at detectable levels. When the tadpoles were moved, only 15 remained. Water parameters ranged from - pH: 6.8-7, KH: 3-6 °d, GH: 8 °d during larval development.

The first tadpoles emerged from the water on 30 September 2018, 147 days after hatching (Fig.1C). They were moved into a 15 x 20 x 13 cm enclosure suitable for metamorphs. This enclosure contained shallow water and plastic plants for metamorphs to climb out onto. Once fully metamorphosed, with resorption of the tail that took 8-10 days (Fig.1D), they were transferred to a tub measuring 15 x 20 x 13 cm. The substrate was gravel covered with moss and live plants (*P. erubescens* and *M. acuminata*). A small water dish was also provided. Metamorphs began feeding once they had absorbed their tail and were fed on the same diet as the adults.

DISCUSSION

The rearing of tadpoles to froglets, in this case, suggests that metamorphosis can be faster than previously documented (five months – Hoffmann, 2004; Rada et al., 2007; Hoffmann, 2010) if temperature ranges are kept towards the top end of the tadpoles' thermal tolerance. Rearing tadpoles in this way could be used to influence captive breeding programmes by reducing the cost and time of managing tadpoles which are often more sensitive to suboptimal husbandry than adults.

These observations refer to a single breeding event, consequently further research in captivity of tadpole development times and hatch rate success in the presence of adults is warranted. Parental attendance has led to greater egg survivorship in glass frogs (Lehtinen et al., 2014).

The global trade in amphibians and the lack of associated biosecurity may facilitate the spread of amphibian pathogens (Garner et al., 2009; Martel et al., 2014). A recent proposal to include all centrolenid frogs on appendix II of CITES has been submitted due to the concern that international trade may threaten some species (CITES, 2019). *Hyalinobatrachium valerioi* is still being collected from the wild for the international pet trade and to our knowledge this is the first documented breeding of this species in the UK. The husbandry methods described here could be used to breed this and other *Hyalinobatrachium* species in captivity and so reduce collecting from wild populations.

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REFERENCES

- CITES (2019). Proposals for amendment of Appendices I and II. <https://cites.org/eng/cop/18/prop/index.php> Accessed 02 February 2019.
- Dunn, E.R. (1931). New frogs from Panama and Costa Rica. *Occasional Papers of the Boston Society of Natural History* 5: 385-401.
- Frank, N., & Ramus, E. (1995). *Complete Guide to Scientific and Common Names of Amphibians and Reptiles of the World*. Pottsville, Pennsylvania: N. G. Publishing Inc. 377 pp.
- Frost, D. R. (2018). Amphibian Species of the World: an Online Reference (version 6.0). American Museum of Natural History, New York, USA. <http://research.amnh.org/herpetology/amphibia/index.html> (accessed February 2019)
- Garner, T.W.J., Stephen, I., Wombwell, E. & Fisher, M.C. (2009). The amphibian trade: Bans or best practice. *Ecohealth* 6: 148-152.
- Guyer, C. & Donnelly, M. A. (2005). *Amphibians and Reptiles of La Selva, Costa Rica and the Caribbean Slope: A Comprehensive Guide*. University of California Press, Berkeley. 367 pp.
- Hoffmann, H. (2010). The glass frog tadpoles of Costa Rica (Anura: Centrolenidae): A study of morphology. *Abhandlungen der Senckenberg Gesellschaft für Naturforschung* 567: 46-51.
- Hoffmann, H. (2004). Description of the previously unknown tadpole of *Hyalinobatrachium pulveratum* (Anura: Centrolenidae). *Revista de Biología Tropical* 52: 219-228.
- Lehtinen, R.M., Green, S.E. & Pringle, J.L. (2014). Impacts of paternal care and seasonal change on offspring survival: A multiseason experimental study of a Caribbean frog. *International Journal of Behavioural Biology Ethology* 120: 400-409.
- Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M.C., Farrer, R.A., Schmidt, B.R., Tobler, U., Goka, K. & Lips, K.R. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346: 630-631.
- McDiarmid, R. W. (1978). Evolution of parental care in frogs. In *The Development of Behavior: Comparative and Evolutionary Aspects*, pp. 127-147. G. M. Burghardt and M. Bekoff (Eds.). Garland STPM Press, New York, USA.
- Michaels, C.J., Antwis, R.E. & Preziosi, R.F. (2015). Impacts of UVB provision and dietary calcium content on serum vitamin D3, growth rates, skeletal structure and coloration in captive oriental fire-bellied toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition* 99: 391-403.
- Rada, M., Sánchez-Pacheco, S.J. & Velásquez-Álvarez A.A. (2007). Some considerations on the ex situ management and care of glass frog egg masses and tadpoles (Anura: Centrolenidae). *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 31: 167-170.
- Ruiz-Carranza, P.M. & Lynch, J.D. (1991). Ranas centrolenidae de Colombia I. Propuesta de una nueva clasificación generica. *Lozania, Bogotá* 57: 1-30.
- Savage, J.M. (2002). *The Amphibians and Reptiles of Costa Rica*. University of Chicago Press, Chicago and London. 516 pp.
- Solís, F., Ibáñez, R., Chaves, G., Savage, J., Bolaños, F., Kubicki, B., Jaramillo, C., Fuenmayor, Q., Coloma, L.A., Ron, S.R., Cisneros-Heredia, D., Bolívar, W. & Almendáriz, A. (2008). *Hyalinobatrachium valerioi*. The IUCN Red List of Threatened Species 2008: e.T55036A11243874. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T55036A11243874.en> (accessed January 2019)
- Starrett, P. H. Savage, J.M. (1973). The systematic status and distribution of Costa Rican glass-frogs, genus *Centrolenella* (Family Centrolenidae), with description of a new species. *Bulletin of the Southern California Academy of Sciences* 72: 57-78.
- Szuroczki, D., Koprivnikar, J. & Baker, L.R. (2016) Dietary antioxidants enhance immunocompetence in larval amphibians. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* 201: 182-188.
- Tapley, B. (2009). Aspects of captive husbandry of Taylor's bug-eyed frog, *Theloderma stellatum* (Taylor, 1962). *Herpetological Bulletin* 108: 31-33.
- Tapley, B., Rendle, M., Baines, F.M., Goetz, M., Bradfield, K.S., Rood, D., Lopez, J., Garcia, G. & Routh, A. (2015). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for pre-release health screening. *Zoo Biology* 34: 46-52.
- Taylor, E.H. (1951). Two new genera and a new family of tropical American frogs. *Proceedings of the Biological Society of Washington* 64: 33-40.
- Taylor, E.H. (1958). Notes on Costa Rican Centrolenidae with descriptions of new forms. *University of Kansas Science Bulletin* 39: 41-68.
- Weather to Travel (2019) <https://www.weather2travel.com/climate-guides/> (accessed February 2019)
- Wright, K.M. & Whitaker, B.R. (2001). *Amphibian Medicine and Captive Husbandry*. Krieger Publishing Company. 499 pp.

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The use of visible implant elastomer to permanently identify caecilians (Amphibia: Gymnophiona)

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ABSTRACT - Identifying individual animals is important for studying populations and for the optimal management of individual animals in captivity. In the absence of natural markings that discriminate individuals, such identification may require animals to be marked by researchers. Amphibians are challenging subjects to mark due to their small size and sensitive, permeable and frequently shed skin. Visible Implant Elastomer (VIE) has been widely used to mark amphibians, but no long-term study has validated this technique in caecilian amphibians. We anaesthetised and attempted to VIE mark seven *Herpele squalostoma* and one *Microcaecilia unicolor* held at ZSL London Zoo. No specimens suffered ill effects of anaesthesia or VIE injection, but mean persistence of marks was 191 days in *H. squalostoma* suggesting that this marking technique is not suitable for identifying individuals of this species in the long-term. We were unable to inject VIE into the *M. unicolor* and/or the elastomer was not visible through the darkly pigmented skin. Further research is required to develop methods for long-term marking of a diversity of caecilians.

INTRODUCTION

Identifying individual animals facilitates the estimation of population size, and understanding population dynamics, home range, longevity and numerous other life history, ecological and biological characteristics (Donnelly et al., 1994). The identification of individuals is also important in the management of captive animals in laboratory, zoo and private settings. Furthermore, it may help understand and police the illegal wildlife trade (Buhlmann & Tuberville, 1998). Individuals of some amphibian species have distinctive naturally occurring markings that may aid in discriminating individuals and in their reidentification over time. Identifications based on photographic records of natural markings are minimally invasive and have been used to identify individual amphibians in all three extant amphibian orders (Hagström, 1973; Bailey, 2004; Kramer et al., 2001; Bradfield, 2004; Kenyon et al., 2009). However, many amphibians do not have distinctive or temporally stable individual markings (Wengert & Gabriel, 2006; Kraus & Allison, 2009), and in such cases more invasive marking techniques may need to be adopted (see review by Ferner, 2007). Amphibians are particularly challenging candidates to mark due to their relatively small size, the permeability and sensitivity of their frequently shed skin and their often complex life cycles (Heemeyer et al., 2007).

Caecilians (Gymnophiona) are elongate limbless amphibians (see Wilkinson, 2012 for an introduction). They can be difficult to study because most species burrow in soil (Gower & Wilkinson, 2005) and their limblessness and fossoriality means that they present fewer options for marking, and marks that have proven useful in other (anuran

and caudatan) amphibians may be shed or may impair marked animals. Some caecilian species have markings that have been suitable for the generation of individual photographic identifications (Kramer et al., 2001) and the natural variation in annulation patterns in *Dermophis mexicanus* (Duméril & Bibron, 1841) have been used to distinguish between members of a small population of captive animals (Wright & Minott, 1999).

Previous work has shown that some marking techniques may be appropriate for identifying individual caecilians. These include the use of Panjet (Wright Health Group Ltd., Dundee; Measey et al., 2001; Measey & Di Bernardo, 2003; Measey et al., 2003); freeze branding (Measey et al., 2001); soft visible implant alphanumeric tags (Measey et al., 2001; Measey et al., 2003; Gower et al., 2006) and visible implant elastomer (VIE; Measey et al., 2001). However, the long-term stability and visibility of any of these identifiers has not been determined for periods greater than 15 weeks (Measey et al., 2001; Measey & Di Bernardo, 2003; Measey et al., 2003) and the longer-term, viability of the markings is unknown.

Most field applications of individual identification require a longer persistence of marks and so further investigation to identify viable marking techniques for caecilians is required. Moreover, the morphology, ecology and life history of caecilians varies widely among species (e.g. Taylor, 1968; Wilkinson & Nussbaum, 2006; Gower & Wilkinson, 2005; San Mauro et al., 2014), and so an expansion of marking trials to more taxa is also necessary in order to better design appropriate marking techniques for Gymnophiona.

VIE is a liquid polymer that solidifies when mixed with a curing agent. The polymer is coloured and fluoresces under black light (UVA light) and can be injected superficially

into animals to create individual marks for identification. VIE is commonly used to mark amphibians and has gained popularity in recent years. Bailey (2004) reported a 100 % VIE mark retention rate in 36 marked salamanders (*Eurycea bislineata*) over 44 weeks. A 100 % VIE retention rate was also reported for laboratory housed *E. bislineata* over 15 weeks (Marold, 2001). Other studies have questioned the reliability of VIE for marking amphibians; Brannelly et al. (2013) reported that VIE tag movement occurred within one week in 50 % of the tags implanted into the toad *Nectophrynoides asperginis*, and VIE tag movement and loss was reported in a study by Brannelly et al. (2014) evaluating marking techniques for the tree frog *Litoria verreauxii alpina*.

Very few population parameter estimates for caecilians have been made and there have been limited attempts to test field methods representing barriers to further research and caecilian conservation (Gower & Wilkinson, 2005). Maintaining caecilians in captivity provides an opportunity to study caecilians and develop and validate methods that can be used to understand and conserve them (Wake, 1994; O'Reilly, 1996; Wilkinson et al., 2013; Maddock et al., 2014; Tapley et al., 2014, 2018; Rendle et al., 2014). To test the application of VIE as a method to permanently identify individual caecilians, we attempted to mark captive *Herpele squalostoma* (Stutchbury, 1836) and *Microcaecilia unicolor* (Duméril, 1863) with VIE and to determine how long marks remain visible.

MATERIALS AND METHODS

Ethics

This study was compliant with the BHS Ethics Policy (British Herpetological Society, 2017). Ethical approval to mark caecilians with VIE using the described methods was granted by the ZSL ethics committee (Project ZDZ17). The methods used to mark caecilians here, including anaesthesia and recovery did not, in this context, require a Home Office License as a) VIE is a routine marking technique for amphibians, and even though it has been little used in caecilians it is the most routine marking type for use in this group, and b) animals were marked opportunistically as part of a routine veterinary health examination for which they needed to be restrained and anaesthetised.

Study species

The Congo caecilian (*H. squalostoma*; Family Herpelidae) is a burrowing caecilian from lowland forests in south-eastern Nigeria, Cameroon, south-western Central African Republic, mainland Equatorial Guinea, Gabon, Congo, western Democratic Republic of Congo, and Bioko Island in Equatorial Guinea (IUCN SSC Amphibian Specialist Group, 2018). The species is oviparous (Kouete et al., 2013) and exhibits maternal dermatophagy; young receive extended parental care and have specialised deciduous teeth that they use to remove and eat the stratum corneum of maternal skin (Kouete et al., 2012). The black micro caecilian (*M. unicolor*; Family Siphonopidae) is a poorly known species (Bittencourt-Silva & Wilkinson, 2018) that is likely a dedicated burrower (Wilkinson et al., 2013, Bardua et al., 2019) and is known

with certainty only from French Guiana (Wilkinson & Kok, 2010). Reproductive mode is oviparity (San Mauro et al. 2014) and, based on other siphonopids, can be inferred to involve direct development (i.e. no larval stage) and maternal dermatophagy (Wilkinson et al., 2008, 2013).

Husbandry

In 2008, ZSL London Zoo acquired *H. squalostoma* via donation, the animals were long-term wild collected captives that had been imported directly to the UK by a licensed importer. A further four juveniles were loaned to ZSL London Zoo in October 2014. These individuals were legally collected as eggs by Marcel Koute from Nkong in the central region of Cameroon in June 2013 and hatchlings raised by MW. *Microcaecilia unicolor* specimens were legally collected from the Core Mountains at Camp Patawa between 2008 and 2010 by the authors (DG & MW) and transferred to ZSL London Zoo in 2013. Both *H. squalostoma* and *M. unicolor* were maintained at ZSL London Zoo as part of a collaborative project with the Natural History Museum's Herpetology Research Group aimed at refining methods for caecilian husbandry, developing and validating field methods, and discovering aspects of life history and behaviour.

Microcaecilia unicolor and *H. squalostoma* of unknown sex were housed in a dedicated, climate-controlled facility. Room temperature ranged from 24–27 °C. *Herpele squalostoma* were maintained in two groups in separate enclosures and *M. unicolor* were housed individually. All enclosures were glass and custom-made (56 x 56 x 35 cm) with slanted bottoms to create a humidity gradient. Part of the lid consisted of a fine mesh for ventilation. Specimens were provided with a 15 cm deep layer of Megazorb (Northern Crop Driers (UK) Ltd.) substrate (Tapley et al., 2014) a waste product from the paper making industry which contains unbleached, wood derived cellulosic fibre and inorganic pigment (Kaolin and calcium carbonate), which is sold for equine husbandry. Dry Megazorb was soaked in water for 24 hrs until saturated. Specimens were fed three times per week with live worms (*Lumbricus* sp. and *Eisenia* sp.). *Herpele squalostoma* were occasionally also offered freshly killed crickets (*Gryllus bimaculatus* and *G. assimilis*) left on the surface of the substrate.

Marking

To observe and monitor any potential detrimental effects of marking, *H. squalostoma* were marked in two batches, seven months apart. We also attempted to mark a single *M. unicolor*. Caecilians were anaesthetised by a ZSL veterinarian for a routine veterinary health examination. Caecilians were anaesthetised in either buffered tricaine methanesulfonate 1g/L (PHARMAQ Ltd., Hampshire, UK) or 4 % isoflurane (Zoetis Inc., New Jersey, USA) in oxygen in a plastic bag (details to presented elsewhere) in order to prevent injury during the marking process because unanaesthetised caecilians are extremely difficult to manually restrain. VIE elastomer and a curing agent (Northwest Marine Technology Inc., Shaw Island, Washington, USA), were prepared following the manufacturer's guidelines and mixed in a 10:1 ratio. Using an insulin syringe and needle (BD U-100 Insulin 0.3 mL /

cc), approximately 0.05 ml of the prepared elastomer was injected subcutaneously into the dorsal surface one third of the distance between head and terminus. Each caecilian was marked with a different coloured VIE. Elastomers were implanted between annular grooves. The needle was inserted perpendicular to the long axis of the body with the needle tip pointing towards the vertebrae, then rotated to a near-parallel orientation to the skin surface and advanced at a low angle of insertion underneath the skin for c. 8–10 mm. Even pressure was then applied to the plunger of the syringe to extrude VIE while the needle was slowly withdrawn, creating a linear mark. Pressure was removed from the plunger c. 2–3 mm from the injection site so that the trail of VIE stopped well before the injection aperture; failing to do so can result in the solidified mark being extruded through the aperture.

During the procedure, animals were laid out on an absorptive disposable bed pad soaked with amphibian Ringer’s solution (Wright & Whitaker, 2001), and frequently rinsed with the same solution in order to avoid dehydration and damage to the skin. Animals recovered from anaesthesia in a container of shallow amphibian Ringer’s solution and were not returned to their enclosures until they had a normal righting reflex and exhibited a normal response to aversive stimuli (a gentle pinch). Post-recovery, the marked caecilians were weighed (to the nearest 0.1 g), using Pesola spring scales before being released back into their original enclosures where they were housed in two groups. Animals were subsequently periodically checked for the presence of the VIE marks and were weighed each time they were checked. They were checked infrequently in order to minimise disturbance to the animals and to the substrate, including any possible burrow structures. The last date that a marking was recorded as being visible was used as the minimum estimate of mark retention.

RESULTS

All *H. squalostoma* were successfully marked (Figs. 1A & B), although the marking in one animal was not visible the day after marking. We were unable to mark the single *M. unicolor* because we were unable to get the needle to form a channel into which VIE could be freely injected. Attempts to mark further *M. unicolor* were not made as the method was deemed non-viable in this species based on the initial trial.

In *H. squalostoma*, VIE mark retention ranged from 0–422 days (Table 1). On average, marks remained visible for 191 days with a standard deviation of 169.9 days. Mean average

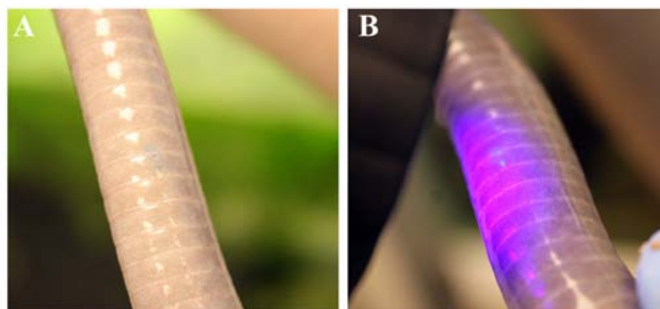


Figure 1. **A)** VIE marking in *H. squalostoma* in ambient light, **B)** VIE marking in *H. squalostoma* under black light

Table 1. VIE mark retention and weight change in *H. squalostoma*

Specimen ID	Colour of marking	Minimum duration mark visible (days)	Weight change over marking period (g)
ZRS16-08880	Orange	422	+35.9
ZRS16-08881	Pink	197	-5.1
ZRS16-08882	Green	42	+0.6
ZRS16-08883	Cherry red	422	+30.6
ZRS15-08651	Red	128	+26.6
ZRS15-08652	Green	0	-2.0
ZRS15-08653	Blue	128	+20.8

body weight change between being marked and the date that the mark was last observed was +15.3 g, and although both weight gains and losses were measured, losses were fewer and generally smaller. No animal showed any clinical signs associated with marking. The single *M. unicolor* also recovered well from the failed marking attempt (where the needle punctured the skin but no VIE was deposited) with no clinical signs of ill health observed.

DISCUSSION

Our data suggest that VIE tags might not be appropriate for marking *H. squalostoma* when individuals need to be identified in long-term studies. The colour of the mark could have been a factor but as only one animal was marked with each colour it is not possible to associate VIE colour and mark retention. In *H. squalostoma*, mark retention was highly variable between individuals. Furthermore, marks were not always easily visible, even when illuminated under a black light and many marks were found only after repeatedly inspecting the animal. This marking technique would be impractical for field use because anaesthesia is required for implantation, and ideally animals should be monitored for 24 hours after implantation to assess anaesthetic recovery status and mark retention (given that one mark was immediately lost in one of our marked individuals). Moreover, due to the difficulty in finding marks known to be present, differentiation between marked and unmarked animals may be problematic in the field even where marks persist. However, this could be ameliorated if all caecilians were marked in exactly the same location of the body.

The VIE marks in *H. squalostoma* did not migrate as reported in some marking trials of other amphibians (e.g. Brannelly et al., 2013, 2014). It is unclear how caecilians lost the marks in this study; no marks were found on a postmortem examination of an animal that died of natural causes over a year after the mark was last observed. This might indicate ejection, absorption, or micro-fragmentation of the VIE tag.

We were unsuccessful in our single attempt to mark a *M. unicolor*. Both *H. squalostoma* and *M. unicolor* have dermal scales in annular folds (Nieden, 1912; Taylor, 1968; Zylberberg & Wake, 1990), but squamation is much less extensive anteriorly in *H. squalostoma*, and this might be

causally related to the difficulty of inserting the elastomer in *M. unicolor*. Ichthyophiidae and Rhinatrematidae also exhibit extensive squamation (Colbert, 1955; Zylberberg et al., 1980), consequently VIE marking would likely be difficult in these taxa. The skin of *M. unicolor* is much more darkly pigmented than the skin of *H. squalostoma*, this could have obscured the visibility of any traces of VIE that might have been injected. We also found it difficult to track the applicator needle at a very shallow depth under the skin in this species.

While our sample size was small and limited to two species representing two of the ten caecilian families; this is the first attempt to validate long-term VIE marking in caecilian amphibians. This method is viable for shorter term studies of at least *H. squalostoma*, given that most animals retained marks and none showed any ill effects of having been anaesthetised and marked. However, its utility for the long-term study of any caecilian species is at best uncertain but warrants further research.

Dark skin pigmentation and possibly other morphological features, such as squamation, may preclude the efficient use of subcutaneous marking techniques in some caecilians. Thus alternative techniques should be developed to permanently identify individual *H. squalostoma* and *M. unicolor* for long-term studies. Natural variation in annulation patterns might be useful in this respect as they have in *D. mexicanus* (Wright & Minott, 1999).

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REFERENCES

Bailey, L.L. (2004). Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias. *Herpetological Review* 35: 38-41.

Bittencourt-Silva, G. B. & Wilkinson, M. (2018). First record of predation on the caecilian *Microcaecilia unicolor* (Duméril, 1863). *Herpetology Notes* 11: 641-644.

Bradfield, K. (2004) *Photographic identification of individual Archey's frogs, Leiopelma archeyi, from natural markings*. DOC Science Internal Series 191. New Zealand Department of Conservation, Wellington, 36 pp.

Brannelly, L.A., Chatfield, M.W. & Richards-Zawacki, C. (2013). Visual implant elastomer (VIE) tags are an unreliable method of identification in adult anurans. *Herpetological Journal* 23: 125-129.

Brannelly, L.A., Berger, L. & Skerratt, L.F. (2014). Comparison of three widely used marking techniques for adult anuran species *Litoria verreauxii alpina*. *Herpetological Conservation and Biology* 9:428-435.

British Herpetological Society (2017). British Herpetological Society: Ethical policy and guidelines. *The Herpetological Bulletin* 141: 46-48.

Buhlmann, K. & Tuberville, T.D. (1998). Use of passive integrated transponder (PIT) tags for marking small freshwater turtles. *Chelonian Conservation and Biology* 3: 102-104.

Colbert, E.H. (1955). Scales in the Permian amphibian *Trimerorhachis*. *American Museum Novitates* 1740: 1-17.

Donnelly, M.A., Guyer, C., Juterbock, E.J. & Alford, R.A. (1994). Techniques for marking amphibians. In: *Measuring and monitoring biological diversity: standard methods for amphibians*, pp. 277-284. Heyer, W. R. (Ed.). Washington DC: Smithsonian Institution Press.

Duméril, A.H.A. (1863). Catalogue méthodique de la collection des batraciens du Muséum D'Historie Naturelle de Paris. *Mémoires de la Société Impériale des Sciences Naturelles de Cherbourg* 9: 295-321.

Duméril, A.M.C. & Bibron, G. (1841). *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles*. Volume 8. Paris: Librairie Encyclopedique de Roret, 430 pp.

Ferner, J.W. (2007). *A review of marking and individual recognition techniques for amphibians and reptiles*. Salt Lake City: Society for the Study of Amphibians and Reptiles, 72 pp.

Fry, D.B. (1915). Herpetological notes. *Proceedings of the Royal Society of Queensland* 27: 60-95.

Gower, D.J. & Wilkinson, M. (2005). The conservation biology of caecilians. *Conservation Biology* 19: 45-55.

Gower, D.J., Oommen, O.V. & Wilkinson, M. (2006). Marking amphibians with alpha numeric fluorescent tags—caecilians lead the way. *Herpetological Review* 37: 302-303.

Green, J. (1818). Descriptions of several species of North American Amphibia, accompanied with observations. *Journal of the Academy of Natural Sciences of Philadelphia* 1: 348-359.

Hagström, T. (1973). Identification of newt specimens (Urodela, *Triturus*) by recording the belly pattern and a description of photographic equipment for such registrations. *British Journal of Herpetology* 4: 321-326.

Heemeyer, J.L., Homyack, J.A. & Haas, C.A. (2007). Retention and readability of visible implant elastomer marks in eastern red-backed salamanders (*Plethodon cinereus*). *Herpetological Review* 38: 425-428.

IUCN SSC Amphibian Specialist Group (2018). *Herpele squalostoma*. The IUCN Red List of Threatened Species 2018: e.T59565A16958011. <http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T59565A16958011.en>. Downloaded on 24 July 2019.

Kenyon, N., Philott, A.D. & Alford, R. (2009). Evaluation of the

- photographic identification method (PIM) as a tool to identify adult *Litoria genimaculata* (Anura: Hylidae). *Herpetological Conservation and Biology* 4: 403-410.
- Kouete, M. T., Wilkinson, M. & Gower, D. J. (2012). First reproductive observations for *Herpele Peters*, 1880 (Amphibia: Gymnophiona: Herpelidae): evidence of extended parental care and maternal dermatophagy in *H. squalostoma* (Stutchbury, 1836). ISRN Zoology 269690. doi:10.5402/2012/269690
- Kouete, M.T., Ndeme, E.S. & Gower, D.J. (2013). Further observations of reproduction and confirmation of oviparity in *Herpele squalostoma* (Stutchbury, 1836) (Amphibia: Gymnophiona: Herpelidae). *Herpetology Notes* 6: 583-586.
- Kramer, A., Kupfer, A. & Himstedt, W. (2001). Haltung und Zucht der thailändischen Blindwühle *Ichthyophis kohtaoensis* (Amphibia: Gymnophiona: Ichthyophiidae). *Salamandra* 37: 1-10.
- Kraus, F. & Allison, A. (2009). A remarkable ontogenetic change in color pattern in a new species of *Oreophryne* (Anura: Microhylidae) from Papua New Guinea. *Copeia* 2009: 690-697.
- Maddock, S.T., Lewis, C.J., Wilkinson, M., Day, J.J., Morel, C. & Kouete, M.T., & Gower, D.J. (2014). Non-lethal DNA sampling for caecilian amphibians. *The Herpetological Journal* 24: 255-260.
- Measey, G.J., Gower, D.J., Oommen, O.V. & Wilkinson, M. (2001). Permanent marking of a fossorial caecilian, *Gegeneophis ramswamii* (Amphibia: Gymnophiona: Caeciliidae). *Journal of South Asian Natural History* 5: 141-147.
- Measey, G.J. & Di-Bernardo, M. (2003). Estimating juvenile abundance in a population of the semiaquatic caecilian, *Chthonerpeton indistinctum* (Amphibia: Gymnophiona: Typhlonectidae), in southern Brazil. *Journal of Herpetology* 37: 371-374.
- Measey, G.J., Gower, D.J., Oommen, O.V. & Wilkinson, M. (2003). A mark-recapture study of the caecilian amphibian *Gegeneophis ramswamii* (Amphibia: Gymnophiona: Caeciliidae) in southern India. *Journal of Zoology* 261: 129-133.
- Marold, M.A.R. (2001). Evaluating visual implant elastomer polymer for marking small, stream-dwelling salamanders. *Herpetological Review* 32: 91-92.
- Nieden, F. (1912). Übersicht über die afrikanischen *Schleichenlurche* (Amphibia: Apoda). *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 1912: 186-214.
- O'Reilly, J.C. (1996). Keeping caecilians in captivity. *Advances in Herpetoculture* 1: 39-45.
- Poynton, J.C., Howell, K.M., Clarke, B.T. & Lovett, J.C. (1999). "1998" A critically endangered new species of *Nectophrynoides* (Anura, Bufonidae) from the Kihansi Gorge, Udzungwa Mountains, Tanzania. *African Journal of Herpetology* 47: 59-67.
- Rendle, M.E., Tapley, B., Perkins, M., Bittencourt-Silva, G., Gower, D.J. & Wilkinson, M. (2015). Itraconazole treatment of *Batrachochytrium dendrobatidis* (Bd) infection in captive caecilians (Amphibia: Gymnophiona) and the first case of Bd in a wild neotropical caecilian. *Journal of Zoo and Aquarium Research* 3: 137-140.
- San Mauro, D., Gower, D.J., Müller, H., Loader, S.P., Zardoya, R., Nussbaum, R.A., Wilkinson, M. (2014). Life-history evolution and mitogenomic phylogeny of caecilian amphibians. *Molecular Phylogenetics and Evolution* 73: 177-189.
- Stutchbury, S. (1836). "1834". Description of a new species of the genus *chameleon*. *Transactions of the Linnean Society. London* 17: 361-362.
- Tapley, B., Bryant, Z., Grant, S., Kother, G., Feltrer, Y., Masters, N., Strike, T., Gill, I., Wilkinson, M. & Gower, D.J. (2014). Towards evidence based husbandry for caecilian amphibians: substrate preference in *Geotrypetes seraphini* (Amphibia: Gymnophiona: Dermophiidae). *Herpetological Bulletin* 129: 15-18.
- Tapley, B., Michaels, C.J., Gower, D.J. & Wilkinson, M. (2018). Filling in the blanks, the role of caecilians in Zoo collections. *BIAZA News Summer Edition* : 26.
- Wake, M.H. (1994). Caecilians (Amphibia: Gymnophiona) in captivity. In: *Captivity and Management and Conservation of Reptiles and Amphibians*, pp. 223-228. Murphy, J.B., Alder, K. & Collins, J.T. (Eds.). London, UK: Society for the study of Reptiles and Amphibians.
- Wengert, G.M. & Gabriel, M.W. (2006). Using chin spots patterns to identify individual mountain yellow-legged frogs. *Northwestern Naturalist* 87: 192.
- Wilkinson, M. (2012). Quick guide: Caecilians. *Current Biology* 22: R668-669.
- Wilkinson, M. & Kok, P.J.R. (2010). A new species of *Microcaecilia* (Amphibia: Gymnophiona: Caeciliidae) from Guyana. *Zootaxa* 2719: 35-40
- Wilkinson, M. & Nussbaum, R. A. (2006). Caecilian phylogeny and classification. In: *Reproductive Biology and Phylogeny of Amphibia, Volume 3. Gymnophiona*, pp. 39-78. J.-M. Exbrayat (Ed.) Science Publishers Inc.
- Wilkinson, M., Kupfer, A., Marques-Porto, R., Jeffkins, H., Antoniazzi, M. M. & Jared, C. (2008). One hundred million years of skin feeding? Extended parental care in a Neotropical caecilian (Amphibia: Gymnophiona). *Biology Letters* 4: 358-361.
- Wilkinson, M., Sherratt, E., Starace, F. & Gower, D.J. (2013). A new species of skin-feeding caecilian and the first report of reproductive mode in *Microcaecilia* (Amphibia: Gymnophiona: Siphonopidae). *PLoS ONE* 8: e57756.
- Wright, K.M. & Minott, T. (1999). Individual identification of captive Mexican caecilians (*Dermophis mexicanus*). *Herpetological Review* 30: 32-33.
- Wright, K.M. & Whitaker, B.R. (2001). *Amphibian Medicine and Captive Husbandry*. Malabar: Krieger. 570 pp.
- Zylberberg, L., Castanet, J. & De Ricqlès, A. (1980). Structure of the dermal scales in *Gymnophiona* (Amphibia). *Journal of Morphology* 165: 41-54.
- Zylberberg, L. & Wake, M.H. (1990). Structure of the scales of *Dermophis* and *Microcaecilia* (Amphibia: Gymnophiona) and a comparison to dermal ossifications of other vertebrates. *Journal of Morphology* 206: 25-43.

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Variable plastron coloration of the eastern painted turtles *Chrysemys picta picta* in a single locality of south-eastern Massachusetts, USA

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Body coloration of animals is a complex morphological trait produced by biochemical and biophysical interactions under genetic control, which can be triggered by environmental influences (Brejcha et al., 2019, Rowe et al., 2006). Conspicuous body colours may signal vital information both between and within species, including sexual selection, mate recognition, conspecific identification, kin recognition, and predator deterrence (McGaugh, 2008) while pigmentation resulting in background-matching helps camouflage (McGaugh, 2008, Reinke et al., 2017, Rowe et al., 2014). Histological and physiological processes of colour production as well as environmental determinants of colour variations have been substantially explored among emydid turtles (Brejcha et al., 2019, McGaugh, 2008, Rowe et al., 2014). These turtles develop variable colours by assimilating dissolved substances from their aquatic environments (Rowe et al., 2016, Rowe et al., 2013). Mostly limited to anecdotal accounts, such observations are rarely documented in the scientific literature.

The colour variations among emydids occur particularly on their limbs, head, carapace, and plastron. Here, we documented the presence of multiple plastron colour variations in the eastern painted turtles, *Chrysemys picta picta* (Schneider, 1783). This species is among the most common and widely distributed chelonian of North America, ranging from southern Canada to northern Mexico, and occupies a diverse range of freshwater and wetland habitats such as ponds, lakes, rivers, streams, freshwater marshes, forested and scrub swamps (Ernst, 1971; Ernst & Lovich, 2009).

At Mass Audubon's Tidmarsh Wildlife Sanctuary, Plymouth Massachusetts, USA, we documented different plastron colorations among different individuals of *C. p. picta*. Yellow and red stripes on legs, and the prominent yellow spot behind the eye, were the identification features we used. Turtles were captured on 24 June 2018 and on four occasions between 7-26 June 2019 via large Promar Collapsible Minnow Traps deployed at a coastal, low-gradient headwater wetland (41.90117°S, -70.57131°W, WGS 84, 3.96 m elev.) where the substrate was a thick layer of decomposing vegetation, peat, and silt. The study site was formally a cranberry farm, now restored into a wetland-aquatic habitat complex.

We captured three turtles with black plastrons where

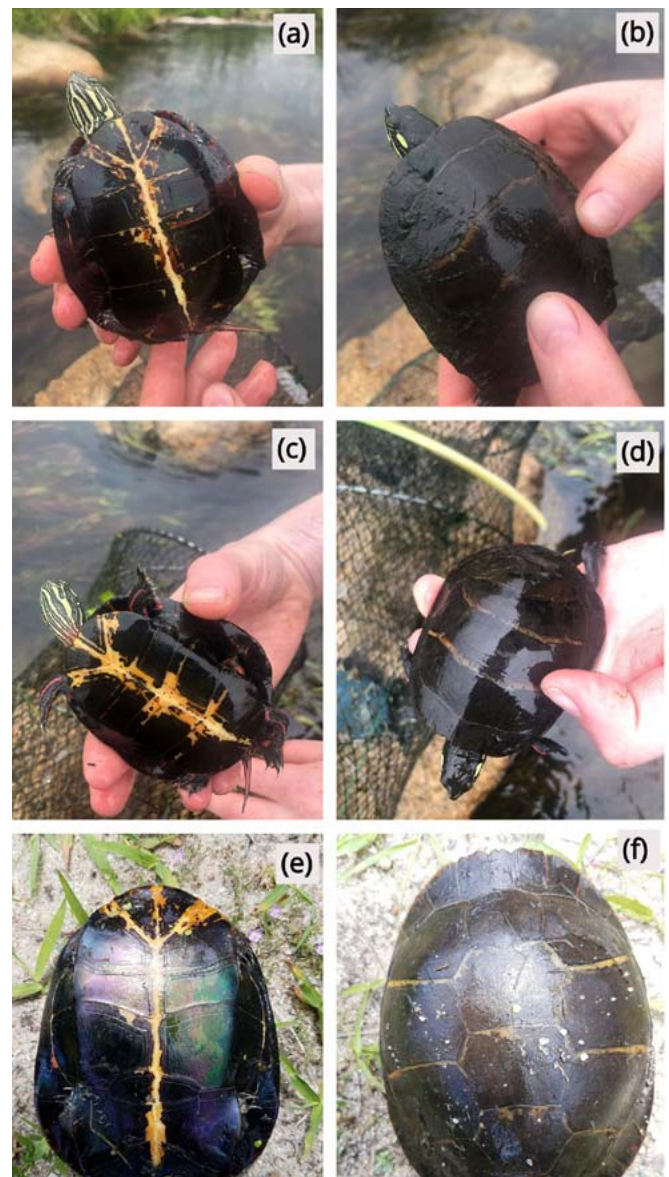


Figure 1. Plastrons and carapaces of three *C. p. picta*, captured at Mass Audubon's Wildlife Sanctuary. Each row of the panel represents the same individual. The plastrons of all these individuals are predominantly black.

the typical plain-yellow coloration was only visible along the plastron sutures (Fig. 1). The black colour extended

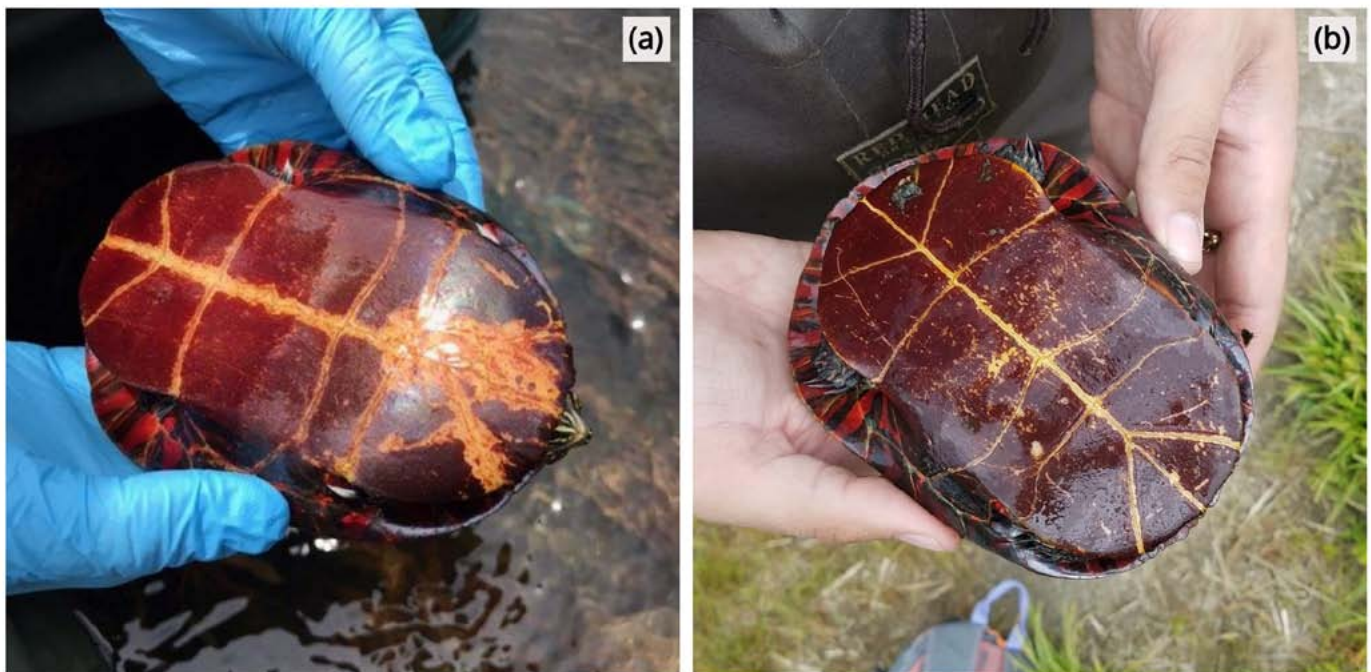


Figure 2. Two individual *C. p. picta* with predominantly reddish-brown plastrons, captured at Mass Audubon's Wildlife Sanctuary

throughout the plastron (except sutures), up to the plastron edge and the hinges. This coloration is likely superficial as evident by its flaky appearance. In some individuals, the plastrons appeared iridescent. Three more turtles had reddish-brown plastrons (Fig. 2) while another had a bright red plastron. Unlike the black plastrons, the reddish-brown and bright red colorations were deeply pigmented into the scutes. The carapace, head, and limbs of turtles with black plastrons were comparatively darker while the red-yellow stripes and spots appeared much brighter. During the two-year period, we captured over 50 painted turtles in the same area; no other turtles captured had colour aberrations.

In general, eastern painted turtles have plain yellow plastrons and olive green or black carapaces (Ernst & Lovich, 2009, Powell et al., 2016). Throughout their range, substantial variations have been observed in plastral background colour, ranging from beige to orange or red, as well as different patterns such as dark spots or blotches of variable sizes and shapes (Cohen, 1992, Hulse et al., 2001). Similarly, light-to-dark hue variations in carapace have also been observed among painted turtles. Although plastral colour variations are not unknown, the presence of multiple colour morphs in the same locality is noteworthy. Colour variations among eastern painted turtles are attributed to among-subspecies phenotypic differences and geographic variations, thus, conservative colorations are generally expected for a subspecies occupying a certain habitat or a watershed (Ernst et al., 2015; Groves, 1983). Turtle integument colour arises from differential arrangements of chromatophores, variable concentration of melanosomes, or melanin deposition in keratinocytes (Rowe et al., 2013). While our observation on integument darkening is probably attributable to the latter two mechanisms, diffusion of tannins and irons could have resulted in the black plastrons. Higher concentrations of dissolved tannins and iron have also been documented in our

study region (Burns, 2017; National Water Quality Monitoring Council, 2019). Exoskeletal absorption of minerals has been documented for other emydids (Lovich et al., 1990).

The wetland we surveyed is rich in tannin sources such as peat deposits and partially-decomposed vegetation (Burns, 2017). Iron mining and refining, a likely source of dissolved iron, was prominent in our study region in the early-industrial era. The wetland surveyed was an irrigation reservoir where iron and tannins could have accumulated within the substrates. Recent restoration interventions and dam removal may have re-suspended these solutes into the water column, which may then have been assimilated into the turtle integument. Dietary carotenoids could have resulted in both red-brown plastral colorations and brighter stripes and spots (Reinke et al., 2017, Steffen et al., 2019).

In the future, surveying similar habitats within the painted-turtle range will reveal geographical patterns and relative frequencies of these colour anomalies.

Conspicuous coloration of the head and limbs may serve as ornamental advertisement or honest signaling of high fitness in mate selection (Polo-Cavia et al., 2013, Steffen et al., 2019, Steffen et al., 2015). However, since painted turtles are not sexually dichromatic, the conspicuous colours we documented are an unlikely driver of mate choice. Painted turtles are quasi-social species, thus, these colorations may help conspecific recognition (Steffen et al., 2019). Plastral and carapace darkening may improve camouflage by increasing background-colour convergence, which could reduce predation rates (Rowe et al., 2016; Steffen et al., 2015). We detected several turtle predators in our study area: red foxes, coyotes, raccoons, crows, bullfrogs, and common garter snakes. Reversible melanisation for both background-colour convergence (Rowe et al., 2016) and efficient thermoregulation have been observed among emydid turtles (Cao et al., 2019; Rowe et al., 2009). In northern latitudes,

such as our study site with cooler spring air temperatures and a shorter growing season, darker integument may help achieve more favourable body temperatures and hence boost metabolism.

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REFERENCES

- Brejcha, J., Bataller, J.V., Bosakova, Z., Geryk, J., Havlikova, M., Kleisner, K., Marsik, P., Font, E. (2019). Body coloration and mechanisms of colour production in Archelosauria: The case of deirocheline turtles. *Royal Society Open Science* 6: 190319 <https://doi.org/10.1101/556670>.
- Burns, M. 2017. A Rapid Assessment for Cranberry Farm Wetland Restoration Potential in Southeastern and Cape Cod Massachusetts. *Masters Thesis*, Antioch University New England, 97 pp.
- Cao, D., Ge, Y., Wei, Y., Duan, H. & Gong, S. (2019). Observations on carapace color change in the juvenile big-headed turtle (*Platysternon megacephalum*). *PeerJ* 7:e7331.
- Cohen, M. (1992). The Painted Turtle, *Chrysemys picta*. *Tortuga Gazette* 28: 1-3.
- Ernst, C.H. (1971). *Chrysemys picta*. *Catalogue of American Amphibians and Reptiles* 106: 1-4.
- Ernst, C.H. & Lovich, J.E. (2009). *Turtles of the United States and Canada*. Baltimore: John Hopkins University Press, 840 pp.
- Ernst, C.H., Orr, J.M., Laemmerzahl, A.F. & Creque, T.R. (2015). Variation and zoogeography of the turtle *Chrysemys picta* in Virginia, USA. *Herpetological Bulletin* 130: 9-15.
- Groves, J.D. (1983). Taxonomic Status and Zoogeography of the Painted Turtle *Chrysemys picta* (Testudines: Emydidae), in Maryland. *American Midland Naturalist* 109: 274-279.
- Hulse, A.C., McCoy, C.J. & Censky, E.J. (2001). *Amphibians and Reptiles of Pennsylvania and the Northeast*. Ithaca: Cornell University Press, 419 pp.
- Lovich, J., McCoy, C. & Garstka, W. (1990). The development and significance of melanism in the slider turtle. In *Life History and Ecology of the Slider Turtle*, 233-254pp. Gibbons J.W. (Ed.). Washington DC: Smithsonian Institution Press.
- McGaugh, S.E. (2008). Color variation among habitat types in the spiny softshell turtles (Trionychidae: Apalone) of Cuatrociénegas, Coahuila, Mexico. *Journal of Herpetology* 42: 347-354.
- National Water Quality Monitorig Concl. (2019). Water Quality Portal. United States Geological Survey and United States Environmental Protection Agency, <https://www.epa.gov/waterdata/water-quality-data-wqx>.
- Polo-Cavia, N., López, P., Martín, J. (2013). Head coloration reflects health state in the red-eared slider *Trachemys scripta elegans*. *Behavioral Ecology and Sociobiology* 67: 153-162.
- Powell R., Conant R. & Collins J.T. (2016). *Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America*. Boston: Houghton Mifflin Harcourt, 494 pp.
- Reinke, B.A., Calsbeek, R. & Stuart-Fox, D. (2017). A test of an antipredatory function of conspicuous plastron coloration in hatchling turtles. *Evolutionary Ecology* 31: 463-476.
- Rowe, J.W., Bunce, C.F. & Clark, D.L. (2014). Spectral reflectance and substrate color-induced melanization in immature and adult Midland painted turtles (*Chrysemys picta marginata*). *Amphibia-Reptilia* 35: 149-159.
- Rowe, J.W., Clark, D.L., Mortensen, R.A., Commissaris, C.V., Wittle, L.W. & Tucker, J.K. (2016). Thermal and substrate color-induced melanization in laboratory reared red-eared sliders (*Trachemys scripta elegans*). *Journal of Thermal Biology* 61: 125-132.
- Rowe, J.W., Clark, D.L., Price, M. & Tucker, J.K. (2009). Reversible melanization following substrate color reversal in midland painted turtles (*Chrysemys picta marginata*) and red-eared sliders (*Trachemys scripta elegans*). *Journal of Herpetology* 43: 402-409.
- Rowe, J.W., Clark, D.L., Ryan, C. & Tucker, J.K. (2006). Effect of substrate color on pigmentation in Midland painted turtles (*Chrysemys picta marginata*) and red-eared slider turtles (*Trachemys scripta elegans*). *Journal of Herpetology* 40: 358-364.
- Rowe, J.W., Clark, D.L., Shaw, D.M. & Wittle, L.W. (2013). Histological basis of substrate color-induced melanization and reversal of melanization in Painted Turtles (*Chrysemys picta marginata*). *Chelonian Conservation and Biology* 12: 246-251.
- Steffen, J.E., Hultberg, J., Drozda, S. (2019). The effect of dietary carotenoid increase on painted turtle spot and stripe color. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 229: 10-17.
- Steffen, J.E., Learn, K.M., Drumheller, J.S., Boback, S.M. & McGraw, K.J. (2015). Carotenoid composition of colorful body stripes and patches in the painted turtle (*Chrysemys picta*) and red-eared slider (*Trachemys scripta*). *Chelonian Conservation and Biology* 14: 56-63.

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Confirmation of *Naja oxiana* in Himachal Pradesh, India

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The Central Asian cobra *Naja oxiana* (Eichwald, 1831), also called the Caspian, Oxus, or Russian cobra, is a species of venomous snake in the family Elapidae. *Naja oxiana* exhibits considerable ontogenetic variation in its pattern; however, juveniles tend to be pale, with a faded appearance.

Juveniles also have noticeable dark and light cross-bands of approximately equal width around the body. Adults are completely light to chocolate brown or yellowish, with some specimens retaining traces of juvenile banding, especially the first few dark ventral bands (Wüster, 1998).

Naja oxiana was originally considered a subspecies of the Indian, spectacled, Asian or binocellate cobra *Naja naja* (Wüster & Thorpe, 1992) and little was known about its distribution in India (Mahajan & Agrawal, 1976). Whilst *N. naja* is confidently recorded throughout the most of India, with the exception of the north-eastern states, the distribution of *N. oxiana* is still in need of confirmation (Whitaker & Captain, 2008). The older literature frequently used the names *Naja n. oxiana* or *N. naja* for several species of Asiatic cobras prior to their reclassification (Wüster, 1998), and included north-west India for the distribution of *N. oxiana*, but with no specific locations (Biswas & Sanyal, 1977). The frequent absence of hood marks in many *N. naja* from northern India often led to these specimens being misidentified as *N. oxiana*, further adding to the confusion (Wüster, 1998). In Wüster & Thorpe's (1992) review of published records of *N. oxiana* from northern India, the only records considered reliable were from Jammu (Murthy & Sharma, 1976; Murthy et al., 1979), and from Solan District, Himachal Pradesh (Mahajan & Agrawal, 1976). Reports from elsewhere in India appear to be due to misidentification of the melanistic morph of *N. naja* (Saikia et al., 2007). The status of the species in India has been all the more mysterious since there have been no confirmed records from India since the 1970s.

Here, we report new observations of *N. oxiana* (Fig. 1) from Himachal Pradesh in 2017 and 2018. Two live *N. oxiana* specimens were encountered in the area surrounding Majoga, in the district of Chamba (Fig. 2) during a research expedition undertaken as part of the Bangor University Biodiversity Informatics and Technology Exchange for the Management of Snakebite (BITES) project in 2017. Two further *N. oxiana* specimens were encountered between June and September 2018 during a collaborative research expedition by BITES,



Figure 1. Female *N. oxiana* displaying juvenile hood markings

Simultala Conservationists (Foundation for Wildlife) and Captive & Field Herpetology teams. All individuals were found on grassy / rocky slopes with sparse mixed alpine shrub and tree cover with occasional large rocks, boulders and rock faces, or on agricultural land and pasture. The habitat in which *Naja naja* can be found in includes agricultural fields around villages at lower elevations (<900 m), up into pine forests. This habitat differs from *N. oxiana* which appears to frequent higher altitude and more open, rocky habitats, which consist of hemp (*Cannabis sativa*) cover along with some, less dense, pine/tree cover.

Identification was confirmed using morphological characters from Wüster (1998) and phylogenetic analysis. Morphological data are provided in Table 1. Interestingly, the ventral and subcaudal scale counts of the new Himachal Pradesh specimens are slightly below the range previously reported for *N. oxiana* (ventral scales 199 – 207 in males and 191 – 210 in females, subcaudal scales 66 – 71 in males and 62 – 70 in females), and within the range of corresponding counts in *N. naja*. However, the low dorsal scale row counts around the neck and the pattern are characteristic of *N. oxiana*.

For 2017 samples, the evolutionary history was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992) in MEGA7 (Kumar, 2016). The analysis involved 20 nucleotide sequences comprising 421 base pairs of 16S large subunit ribosomal DNA,



Figure 2. *Naja oxiana* Indian distribution (Yellow dots = *N. oxiana* observation locations, red star = Chamba, orange diamond = Majoga,)

Table 1. Sample data and morphological characteristics of *N. oxiana* from Himachal Pradesh, India

Species	<i>Naja oxiana</i>	<i>Naja oxiana</i>	<i>Naja oxiana</i>	<i>Naja oxiana</i>
Reference number	17.V18	17.V19	18.16	18.51
Date	21.09.2017	22.09.2017	11.07.2018	16.08.2018
Time (GMT + 5.5 h)	Not recorded	Not recorded	21:56	22:13
Altitude (m a.s.l.)	1900	2100	2021	1565
Morphology				
Sex	Female	Female	Female	Female
Snout-vent length (cm)	99	98	91	106
Tail length (cm)	14	18.5	18.3	21.5
Visible neck banding	Present	Absent	Present	Absent
Hood markings	Present	Absent	Present	Absent
Cuneate scale	Absent (The left 3rd and 4th infralabial scales were damaged)	1 cuneate scale on the left side between 3rd and 4th infralabials	1 cuneate scale between 3rd and 4th infralabial on both sides	Not known
Scalation				
Scale rows at mid-body	21	21	21	21
Scale rows around neck	23	23	23	22
Scale rows before vent (one head length ahead of vent)	16	15	15	15
Ventrals ¹	189	190	189	189
Subcaudals (all paired)	37 (Tail tip missing)	57	60	61
First V of first dark band (ventral scale no.)	6th	6th	6th	5th
Last V of first dark band (ventral scale no.)	8th	9th	9th	8th

¹ Recorded using the Dowling method (Dowling, 1950)

including three sequences of *N. oxiana* and one of *N. naja* generated specifically for this study. Other sequences were downloaded from GenBank. All positions with less than 90 % site coverage were eliminated. The tree with the highest log likelihood (-1114.13) is shown in Figure 3. A discrete Gamma

distribution was used to model evolutionary rate differences among sites (5 categories, parameter = 0.1132). All novel sequences used in this study are deposited on GenBank, with the accession numbers: MN548728-30 and MN648780.

These observations confirm the continued presence of

N. oxiana in northern India, and represent a species range extension of approximately 120 km east-north-east and 220 km north-west from the two previous Indian records from Jammu and Solan District in Himachal Pradesh, respectively, and an elevation increase of 100 m from 2000 m a.s.l. (Wüster, 1998) to 2100 m a.s.l.

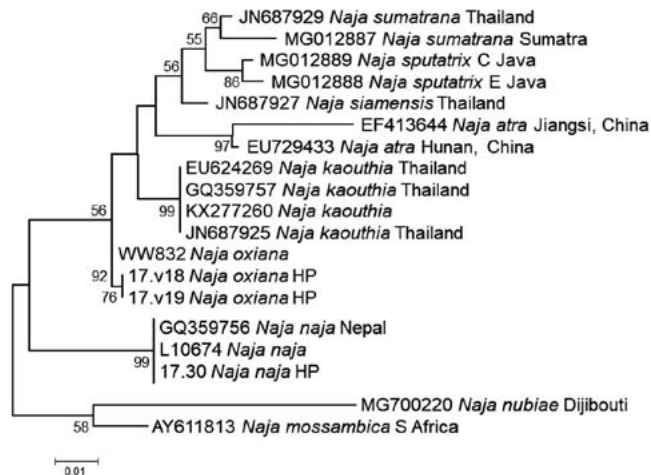


Figure 3. *Naja oxiana* molecular phylogenetic analysis by Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches (only when >50 %). Tips are labelled with associated GenBank Accession numbers. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

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REFERENCES

- Biswas, S. & Sanyal, D.P. (1977). Fauna of Rajasthan, India, Reptilia. *Records of the Zoological Survey of India* 73: 247-269.
- Dowling, H.G. (1951). A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97-99.
- Eichwald, E. (1831). Zoologia Specialis Quam Expositis Animalibus tum Vivis, tum Fossilibus Potissimum Rossiae in Universum, et Poloniae in Species, In *Usum Lectionum Publicarum in Universitate Caesareae Vilnensi Habendarum*. Part 3, Josephi Zawadski, Vilnius. 314 pp.
- Kumar S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.
- Mahajan, K.K. & Agrawal, H.P. (1976). Report on a collection of reptile fauna from Simla Hills, Himachal Pradesh. *Zoological Survey of India* 71: 301-312.
- Murthy, T.S.N. & Sharma, B.D. (1976). A contribution to the herpetology of Jammu and Kashmir. *British Journal of Herpetology* 5: 533-538.
- Murthy, T.S.N., Sharma, B.D. & Sharma, T. (1979). Second report on the herpetofauna of Jammu and Kashmir, India. *Snake* 11: 234-241.
- Saikia, U., Sharma, D.K., & Sharma, R.M. 2007. Checklist of the reptilian fauna of Himachal Pradesh, India. *Reptile Report* 8: 6-9.
- Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* 9: 678-687.
- Whitaker, R. & Captain, A. (2008). *Snakes of India: The Field Guide*. Draco Books, Chennai, India. 385 pp.
- Wüster, W. & Thorpe, R.S. (1992). Asiatic cobras: Population systematics of the *Naja naja* species complex (Serpentes: Elapidae) in India and central Asia. *Herpetologica* 48: 69-85.
- Wüster, W. (1998). The cobras of the genus *Naja* in India. *Hamadryad* 23: 15-32.

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Tail bifurcation in two species of *Desmognathus* salamander (Caudata: Plethodontidae) in south-eastern Kentucky, USA

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For centuries, amphibian malformations have been documented globally and in veritable detail (Vallisneri, 1706; Bourne, 1884; Ouellet, 2000; Haas et al., 2018), particularly in anurans (Lannoo, 2008). Although, rarely have malformations been documented in caudates (for a review of the literature, see Henle et al., 2012), primarily manifesting as post-larval tail malformations (i.e. bifurcation and duplication). Furthermore, among the caudate families in which malformations have been reported, the Plethodontidae - the largest caudate family - has been apparently underrepresented, with only three of the > 470 species documented to exhibit malformations (Henle et al., 2012; Hartzell, 2017). Previously, tail bifurcation within the genus *Desmognathus* (Caudata: Plethodontidae) has only been reported in *D. fuscus* (Rafinesque, 1820) (Hartzell, 2017). We report here tail bifurcation in natural populations of *D. ochrophaeus* Cope 1859 and *D. monticola* Dunn 1916. To our knowledge, these observations represent the first reports of tail bifurcation in either species.

On 7 June 2017, a post-larval *D. ochrophaeus* exhibiting tail bifurcation (Fig. 1a) was observed in a headwater stream that originates and flows through an old-growth forest. The bifurcation occurred at approximately $\frac{1}{4}$ length of the tail from the cloaca. On 19 May 2018, an additional post-larval tail bifurcation was observed in *D. monticola* (Fig. 1b). This bifurcation appeared similar to the malformation previously observed in *D. ochrophaeus*, occurring at approximately $\frac{1}{4}$ to $\frac{1}{2}$ length of the tail from the cloaca. Both salamanders were hand-captured in the riparian area of a small, forested headwater stream (37.078398° N, -82.994013° W, WGS 84, 355 m elev.) at Lilley Cornett Woods Appalachian Ecological Research Station (Letcher Co., Kentucky, U.S.A.).

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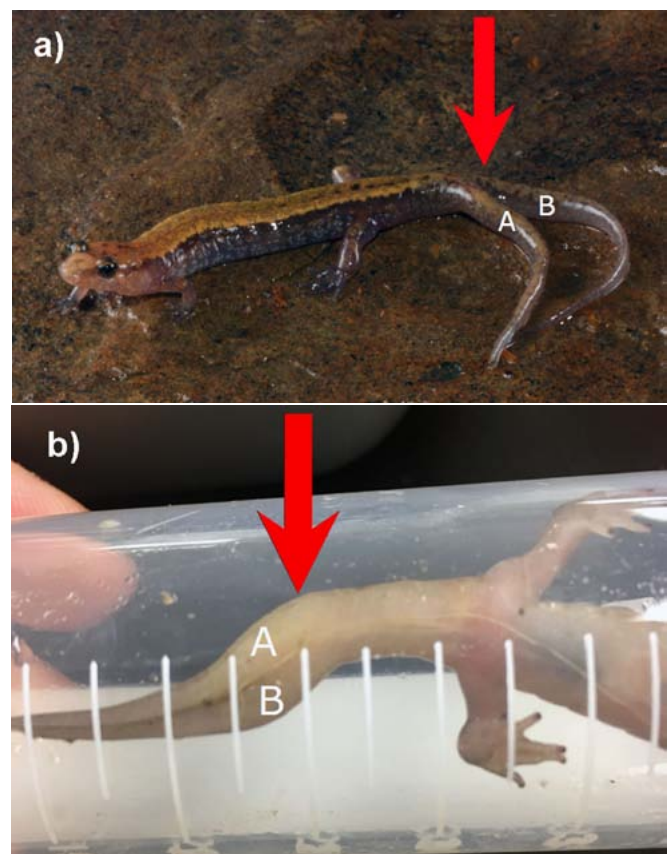


Figure 1. Tail bifurcation in two species of salamander captured in a headwater stream at Lilley Cornett Woods Appalachian Ecological Research Station in Letcher County, Kentucky, U.S.A. **a)** Dorsal view of the tail of an adult *D. ochrophaeus*, and **b)** ventral view of the tail of an adult *D. monticola* to show tail bifurcation. Red arrows indicate the point of bifurcation. Beyond point of bifurcation, individual tail segments are labelled 'A' and 'B'.

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REFERENCES

- Bourne, A.G. (1884). On certain abnormalities in the common frog (*Rana temporaria*). *Quarterly Journal of Microscopical Science* 2: 83–88.
- Haas, S.E., Reeves, M.K., Pinkney, A.E. & Johnson, P.T.J. (2018). Continental-extent patterns in amphibian malformations linked to parasites, chemical contaminants, and their

- interactions. *Global Change Biology* 24: e275–e288.
- Hartzell, S.M. (2017). Tail bifurcation in a Northern dusky salamander, *Desmognathus fuscus* (Caudata: Plethodontidae). *Herpetology Notes* 10: 181–182.
- Henle, K., Mester, B., Lengyel, S. & Puky, M. (2012). A review of a rare type of anomaly in amphibians, tail duplication and bifurcation, with description of three new cases in European species (*Triturus dobrogicus*, *Triturus carnifex*, and *Hyla arborea*). *Journal of Herpetology* 46: 451–455.
- Lannoo, M. (2008). *Malformed Frogs: the Collapse of Aquatic Ecosystems*. University of California Press, West Sussex, U.K.
- Ouellet, M. (2000). Amphibian deformities: Current state of knowledge. In *Ecotoxicology of Amphibians and Reptiles*, pp. 617–646. Sparling, D., Linder, G. & Bishop, C. (Eds.). Pesacola, FL: SETAC Press.
- Vallisneri, V.L. (1706). *Galleria di Minerva Overo Notizie Universali di Quanto e Stato Scritto da Letterati di Europa*. Volume 3. Venice.

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Bufo bufo (Common toad): Predation by the medicinal leech (*Hiruda medicinalis*)

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Interactions between amphibians and invertebrate predators and parasites are important aspects of amphibian ecology. Presented here are observations of medicinal leechs (*Hiruda medicinalis*) feeding on common toads (*Bufo bufo*) that had congregated for spawning in a pond in the New Forest, southern England. A medicinal leech was observed to be attached to the head of a male toad that was in amplexus with a female. A further ten *H. medicinalis* were recorded in the shallow (< 30 cm deep) flooded grasses at the pond margin. Eight were free swimming and two were attached to the female of a second pair of toads that were in amplexus, one to the head of the female and one to the rear leg of the female (Fig. 1). Water temperature was 15 °C, air temperature was 15-16 °C. Most leeches were about 15-17 cm long but several were only about half that length. A count of emergent toad heads reached 51 and observation of the shallow marginal areas indicated that three to four times that number of toads were submerged and passive in or on the vegetation.

Over the following week, three further daytime visits and one nighttime visit was made by the author and members of a local natural history group (Table 1). In contrast to their behaviour during the day, all of the *H. medicinalis* observed after dark were encountered in a passive state on the bottom, but made off slowly after a brief period of illumination.

The period of *H. medicinalis* activity matched the period of toad breeding and no further *H. medicinalis* were recorded during eleven subsequent daytime visits that were made by the author over the following seven months; a period in which the pond reduced greatly in area from a winter maximum of approximately 1,800m² of open water to less than 10m² in late summer. Water temperature is reported to be a major factor in leech activity (Elliott & Kutschera, 2011) and unusually warm February temperatures in 2019 may have accounted for the observations on this occasion.

Lower water temperatures at night time may explain the lack of nocturnal activity by the leeches. The large size of *H. medicinalis* relative to its prey may result in amphibian mortality due to loss of blood (Kutschera et al., 2010). One *H. medicinalis* was attached to a dead female, but dropped off shortly after the female was recovered with no observable marks suggesting that the female was already dead when the leech encountered it. The similar species *H. verbana* has been reported to feed on dead toads (Merilä & Sterner, 2002). Amphibians are targeted by *H. medicinalis* during



Figure 1. A pair of *B. bufo* in amplexus with two *H. medicinalis* (white arrows) attached to the female

Table 1. Details of *H. medicinalis* observations. Ta air temperature, Tw water temperature, *night time survey

Date	Total	Passive	Swimming	Attached	T _a (°C) T _w (°C)
23/2/19	11	0	8	3	15-16° 15°
25/2/19	11	0	9	2	not recorded
26/2/19	1	0	0	1	not recorded
26/2/19*	8	7	0	1	3.8° 9.3°

the spring when their concentrated numbers provide a source of food for the emerging leeches (Merilä & Sterner, 2002). However, *H. medicinalis* is now uncommon, and even protected under the Countryside & Wildlife Act 1981 (Elliott & Kutschera, 2011), and so consequently unlikely to have a major impact on amphibian populations. Eviscerated toad carcasses on the pond margin constitute ample evidence for toad mortality resulting from other predators. Common frogs (*Rana temporaria*) and smooth newts (*Lissotriton vulgaris*) also occur at the site and may constitute an additional food source for the leeches as they have been at other sites (Frazer, 1983; Elliott & Kutschera, 2011). Leeches are attracted to vibrations (Kutschera et al., 2010), therefore it is

possible that the intense, short duration activity of explosive toad breeding may put them at greater risk of predation than the smaller newts that breed over a more extended period. Both the toads and leeches are recolonisers of this site which was drained during the 1950's before being reinstated in the 1990's.

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REFERENCES

Elliott, J. M. & Kutschera, U. (2011). Medicinal leeches: historical use, ecology, genetics and conservation. *Freshwater Reviews* 4: 21-41.

Frazer, D. (1983). *Reptiles and Amphibians in Britain*. London. Bloomsbury, 256 pp.

Kutschera, U., Roth, M. & Ewert, J.P. (2010). Feeding on bufoid toads and occurrence of hyperparasitism in a population of the medicinal leech (*Hirudo verbana* Carena 1820). *Research Journal of Fisheries and Hydrobiology* 5: 9-13.

Merilä, J. & Sterner, M. (2002). Medicinal leeches (*Hirudo medicinalis*) attacking and killing adult amphibians. *Annals Zoologica Fenniscandia* 39: 343-346.

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Mass extermination of green toad metamorphs (*Bufo viridis*) in Greece

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On 14 May 2018, during observations in Laganas (37°43'43.1" N, 20° 52'26.2" E, Zakynthos, Greece) on the Ionian coast, aggregations of freshly metamorphosed toads were found on the sandy shores and shallows at the estuary of a small watercourse (Fig. 1A, B). Based on morphological characteristics, individuals were identified as green toads *Bufo viridis*, sensu lato in view of the sympatric occurrence of *B. variabilis* and *B. viridis* in parts of Greece and the unsettled systematic status of the former taxon (Stöck et al., 2006). In the course of a thorough inspection of the site, several thousand dead individuals of this species were found, along with numerous living toadlets (Fig. 1C). The cause of death was poisoning by stabilised chlorine granules, usually used for swimming pool maintenance, which most probably had been poured directly onto the largest aggregations of these amphibians. Granules had also been poured precisely along the margins between the banks and watercourse, so that the toads would not be able to move away from the watercourse (Fig. 1D). The mouth of the watercourse is used intensively by tourists as a place for walks along the sea and to hotels. Thus, in our opinion, this action was deliberate and most probably done by the staff of neighbouring hotels.

The green toad, categorised in the National Red Data Books of Greece as a species of least-concern, is also one of seven amphibian species in Greece protected under national legislation (protected under Presidential Decree 67/1981) (Sotiropoulos & Lymberakis, 2015), as well as being protected by international law: the Bern Convention (Annex II) and the Habitats Directive of the European Union (Annex IV). Except in the northern parts of its range this is a common or numerous species; however, the basic trend for its populations has been defined as decreasing (amphibiaweb.org, 2019). Among the threats to amphibians in Greece, next to climate change, human activity has been cited, which includes agriculture, pollution, progressive residential development, and the negative impact of tourism (Sotiropoulos & Lymberakis 2015).

The current example is clearly a case of persecution and may result from an aversion to amphibians and/or the treatment amphibians as if they were pests. In a study conducted by Prokop et al. (2016), almost 31 % of high school students declared their willingness, upon encountering a frog in a garden, to remove it, while 6 % were willing to kill it. Aversion to, and fear of, amphibians is deeply rooted

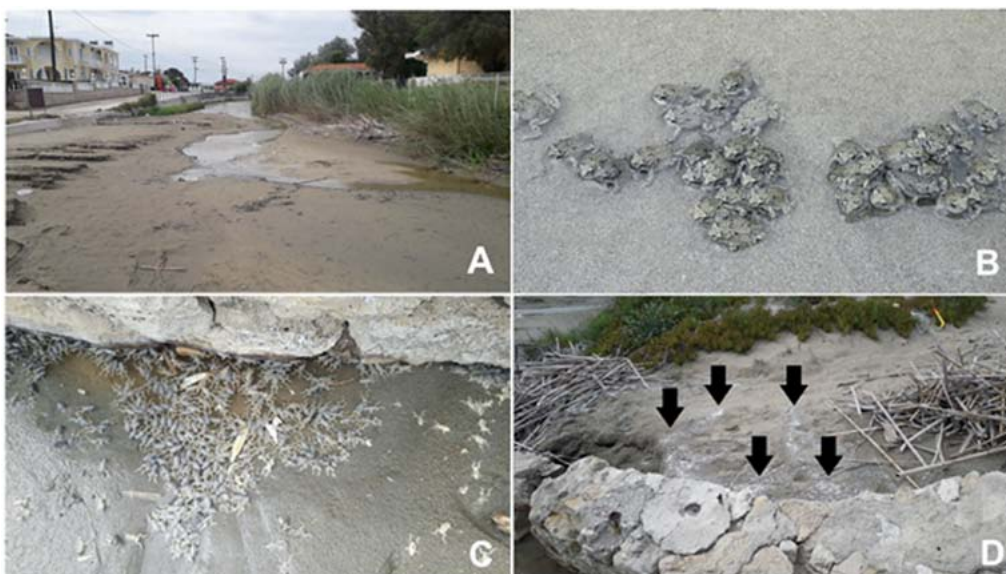


Figure 1. **A)** Site of aggregations of green toad *B. viridis* toadlets in the mouth of a watercourse, leading to the Ionian Sea in Laganas, **B)** Live green toad toadlets, **C)** Photo depicting individuals killed with chlorine granules (about 432 dead individuals) **D)** The banks of the stream sprinkled with chlorine granules (indicated by arrows), most likely to prevent the movement of toadlets to areas adjacent to the river

in the human psyche, and these animals very often elicit disgust in people (Frynta et al., 2019). As Frynta et al. (2019) concludes, 'species with a round body shape, short forelegs, small eyes, warts, pink and grey coloration, or dark and dull coloration were perceived as disgusting or ugly'. These features undoubtedly match many amphibian species, including juvenile green toads. Most probably those people who persecute amphibians might view them much more favourably if they were aware of their ecological importance (Prokop & Fančovičová, 2012).

Actions such as the one we have described here should be openly and firmly condemned. What is more, this is a surprising example of how deeply the transformation of space in the interests of tourism affects nature; not only does it destroy habitats, but animals and plants that do not fit into the idealised space of an 'all-inclusive' hotel are ruthlessly eliminated. It seems probable that if the tourists who are patronising these hotels were made aware of what is being done to the local wildlife for their 'benefit' there would be very strong objections. Local wildlife groups should be encouraged to take appropriate action against what is clearly a wildlife crime.

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REFERENCES

- Frynta, D., Peléšková, Š., Rádlová, S., Janovcová, M. & Landová, E. (2019). Human evaluation of amphibian species: a comparison of disgust and beauty. *The Science of Nature* 106: 41. doi.org/10.1007/s00114-019-1635-8.
- Prokop, P. & Fančovičová, J. (2012). Tolerance of amphibians in Slovakian people: A comparison of pond owners and non-owners. *Anthrozoös* 25: 277-288.
- Prokop, P., Medina-Jerez, W., Coleman, J., Fančovičová, J., Özel, M. & Fedor, P. (2016). Tolerance of frogs among high school students: Influences of disgust and culture. *Eurasian Journal of Mathematics, Science, and Technology Education* 12: 1499-1505.
- Sotiropoulos, K., Lymberakis, P. (2015). Decline and conservation of amphibians in Greece. In: *Amphibian Biology, Volume 11, Part 4: Status of Conservation and Decline of Amphibians: Eastern Hemisphere: Southern Europe & Turkey*. Heatwole, H., Wilkinson, J.W. (Eds). Pelagic Publishing, 362 pp.
- Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebajeva, T., Eremchenko, V., Macey, J.R., Papenfuss, T.J. & Wake, D. B. (2006). Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. *Molecular Phylogenetics and Evolution* 41: 663-689.

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Cataract and lens opacification in a wild toad *Incilius leucomyos* (Anura: Bufonidae)

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The toad *Incilius leucomyos* (McCranie & Wilson, 2000) is a little known endemic species that occurs in disjunct populations on the Atlantic slope of north-central Honduras at 0 - 1,600 m asl (Köhler, 2011). The natural habitats of this species are lowland and premontane broad-leaf wet forest; interposed by streams in which they reproduce. The IUCN Redlist classifies *I. leucomyos* as Endangered (Wilson & Cruz, 2004) owing to its restricted ecological distribution and the rising threats of deforestation and land-use change in Honduras (Wilson & McCranie, 2004). The following note reports the case of an in situ adult *I. leucomyos* that was blind in one eye. Diagnosed pragmatically as a unilateral cataract, this observation is noteworthy as few accounts are available for ocular abnormalities in wild amphibians.

On the 2nd September 2015, 20.14 h, a sub-adult *I. leucomyos* was encountered in a privately protected area of premontane rainforest at Omega Tours Eco-Jungle Lodge, in the region of Pico Bonito National Park, La Cieba, Honduras (GPS [DMM] 15.731744, -86.730381). The individual was one of numerous (mostly adult) toads located active and calling on the surface of leaf-litter besides a trickling stream in closed canopy forest – presumably aggregating for reproduction. Upon observing one individual by flashlight, it was immediately apparent that the pupil of the left eye was cloudy and opaque, which under artificial light reflected back a smoky bluish-white (Fig. 1). The left eye appeared more dilated than the right, and opacity seemed concentrated on the inner lens, instead of across the outer cornea or in the

aqueous chamber. The reactions of the toad when inspected suggest that the left eye was partially or completely blind; though, despite its ailment, the individual appeared to be in good health and behaving normally. Identification of the individual and other surrounding toads as *I. leucomyos*, was established by the presence of orange flecking on the dorsal flanks and toes and black-spot patterning on the dorsum. These basic descriptive features in combination with morphology (Köhler, 2011) differentiate *I. leucomyos* from its widespread and generalist congener *Incilius valliceps*; both species occur in the region of Pico Bonito National Park (McCranie & Solis, 2014). The individual was photographed but its gender was not determined.

Cataract is a clouding of the eye's lens that can cause visual impairment and blindness. It can affect one or both eyes (unilateral or bilateral), and each case can develop differently with regards to size, morphology and degree of opacity. It can range from a small white dot on the anterior capsule to total opacity of the lens (Kalantan, 2012). Anurans are highly dependent upon vision for food acquisition and predator avoidance, and consequently any impairment to the optical or neural tissues of the eye, may reduce individual survival rates (Fite et al., 1998). Perhaps for that reason, cataracts are a rarely documented ailment in wild anurans.

Most literature refers to amphibian ocular abnormalities in veterinary cases or optical studies of captive frogs and toads (Shilton et al., 2001; Kilburn et al., 2019; Rosenthal et al., 2008). Corneal lesions, scarring, fibrosis, opacity, keratitis,



Figure 1. Photographs taken in the field of a sub-adult of the toad *I. leucomyos*, showing a unilateral cataract and lens opacity in the left eye

lipidosis and interstitial cholesterol or lipid keratophagy, are all eye conditions typical of older captive amphibians fed high lipid-rich insect diets (Shilton et al., 2001; Lock, 2017; Russel et al., 1990). Dietary derived ocular impairment seem unlikely to progress under natural circumstances, as a wild amphibian's prey is not cholesterol rich (Lock, 2017) and amphibians might not survive long once developing visual impairment. Moreover, these conditions differ diagnostically from cataracts of the eye lens, in that they instead cause opacity in the outer layer of the cornea. For that reason, the information and visual inspection of this *I. leucomyos* case concludes unilateral cataract as the most probable diagnosis, as no evidence suggested corneal lipid deposition or otherwise damage to the cornea (Shilton et al., 2001; Lock, 2017). Prior studies associate cataracts in amphibians with old age (Rosenthal et al., 2008; Kilburn et al., 2019), but they might also occur due to trauma or radiation exposure (Popov et al., 1963; Worgul & Rothstein, 1975; Hayden et al., 1980; Worgul et al., 1982) and develop during larval stages. Given the individual was not fully-grown or of old age, perhaps the cataract development was of genetic origin during an earlier developmental stage (Shiels & Hejtmancik, 2007).

The cause and severity of the cataract in this case are only subjects of speculation, as it was not possible to collect the specimen for detail ophthalmological investigation. Nevertheless, this observation of *I. leucomyos* attests to the occasional presence and survival of individuals with ocular anomalies in nature.

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REFERENCES

- Fite, K.V., Blaustein, A., Bengston, L. & Hewitt, H.E. (1998). Evidence of Retinal Light Damage in *Rana cascadae*: A Declining Amphibian Species. *Copeia* 1998: 906-914.
- Hayden, J.H., Rothstein, H., Worgul, B.V. & Merriam, Jr. G.R. (1980). Hypophysectomy exerts a radioprotective effect on frog lens. *Experientia* 36: 116-8. doi: 10.1007/bf02004009
- Kalantan, H. (2012). Posterior polar cataract: A review. *Saudi Journal of Ophthalmology: Official Journal of the Saudi Ophthalmological Society* 26 (1): 41-49. doi:10.1016/j.sjopt.2011.05.001
- Kilburn, J.J., Bronson, E., Shaw, G.C., Labelle, P. & Weigt, A. (2019). Phacoemulsification in an American Toad (*Anaxyrus americanus*). *Journal of Herpetological Medicine and Surgery* 29 (1-2): 17-20.
- Köhler, G. (2011). *Amphibians of Central America*. Herpeton, Verlag Elke Köhler, Offenbach, Germany, 379 pp.
- Lock, B. (2017). Corneal Lipidosis / Xanthomatosis in Amphibians. *Veterinary Information Network, Inc.* <https://veterinarypartner.vin.com/doc/?id=7996856&pid=19239> (accessed July 2019)
- McCranie, J. R. & Wilson, L.D. (2000). A new species of high-crested toad of the *Bufo valliceps* group from north-central Honduras. *Journal of Herpetology* 34: 21-31.
- McCranie, J.R. & Solis, J.M. (2013). Additions to the amphibians and reptiles of Parque Nacional Pico Bonito, Honduras, with an updated nomenclatural list. *Herpetology Notes* 6: 239-243.
- Popov, V.V., Goliochenkov, V.A. & Farberov, A.I. 1963. Two components in the development of ray cataract in frogs. *Nature* 199: 1109-1110.
- Rosenthal, K.L., Forbes, N.A., Frye, F.L. & Lewbart, G.A. (2008). *Rapid Review of Exotic Animal Medicine and Husbandry: Pet Mammals, Birds, Reptiles, Amphibians and Fish*. CRC Press: Manson Publishing/The Veterinary Press. London, UK, 320 pp.
- Russell, W., Edwards, D., Stair, E., & Hubner, D. (1990). Corneal lipidosis, disseminated xanthomatosis, and hypercholesterolemia in Cuban tree frogs (*Osteopilus septentrionalis*). *Journal of Zoo and Wildlife Medicine* 21: 99-104.
- Shiels, A. & Hejtmancik, J.F. (2007). Genetic origins of cataract. *Archives of Ophthalmology* 125:165-173. doi:10.1001/archophth.125.2.165
- Shilton, C., Smith, D., Crawshaw, G., Valdes, E., Keller, C., Maguire, G., Connelly, P. & Atkinson, J. (2001). Corneal lipid deposition in Cuban tree frogs (*Osteopilus septentrionalis*) and its relationship to serum lipids: An experimental study. *Journal of Zoo and Wildlife Medicine* 32: 305-19.
- Williams, D.L & Whitaker, B.R. (1994). The amphibian eye: A clinical review. *Journal of Zoo and Wildlife Medicine* 25: 18-28.
- Wilson, L. D., & McCranie, J. R. (2004). The conservation status of the herpetofauna of Honduras. *Amphibian & Reptile Conservation* 3: 6-33. doi:10.1514/journal.arc.0000012
- Wilson, L.D. & Cruz, G. (2004). *Incilius leucomyos*. The IUCN Red List of Threatened Species 2004: e.T54690A11175905. <http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T54690A11175905.en>. (accessed October 2019).
- Worgul, B.V. & Rothstein, H. (1975). Radiation Cataract and Mitosis. *Ophthalmic Research* 7: 21-32. doi: 10.1159/000264731
- Worgul, B.V., Rothstein, H., Medvedovsky C., Merriam, Jr. G.R. & Wilson, M.A. (1982). Radiation cataractogenesis in the amphibian lens. *Ophthalmic Research* 14: 73-82. doi: 10.1159/000265177

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Myiasis in three species of *Bokermannohyla* (Hylidae, Anura)

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Myiasis is an ectoparasitic infection of animal tissue by the larvae of flies in the families Sarcophagidae, Calliphoridae, and Chloropidae (Marcondes, 2009). In amphibians, the deposition of larvae occurs through natural orifices, healthy skin and exposed wounds, leading to the death of the host (Reichenbach-Klinke & Elkan, 1965; Bolek & Coggins, 2002; Bolek & Janovy, 2004). In the present study, cases of myiasis are presented in three species of tree frog, *Bokermannohyla hylax* (Heyer, 1985), *Bokermannohyla caramaschii* (Napoli, 2005) and *Bokermannohyla luctuosa* (Pombal & Haddad, 1993), from different areas inserted in the morphoclimatic domain of the Atlantic Forest of eastern Brazil.

The first *B. hylax* specimen observed and filmed (not collected) was on 8 November 2014, 11.30h at the Parque Estadual da Serra do Mar, Núcleo Santa Virgínia (23° 20' 37.10" S, 45° 8' 19.29" W), municipality of São Luís do Paraitinga, State São Paulo. *Bokermannohyla caramaschii* was subsequently collected in February 2015, 20.30h at the Reserva Biológica Augusto Ruschi (19° 54' S, 40° 33' W) in the municipality of Santa Teresa, State Espírito Santo and *B. luctuosa* was collected in December 2015, 18.30h at the Parque Estadual de Campinhos (25° 02' S, 49° 05' W) in the municipality of Tunas do Sul, State Paraná. The specimens were collected manually and stored in plastic bags. Both the hosts and the fly larvae were fixed in 70 % ethyl alcohol.

The mechanism of *B. hylax* larval deposition adopted by the fly was observed and filmed (YouTube, 2019). First, the fly rests on the host's head and moves laterally toward the loreal region of the frog. It then deposits the larvae on the back of the jaw. During the process the frog attempts to get rid of the larva, trying to remove it with repeated movements of the hands and sometimes endeavours to escape by jumping. The fly may repeat this procedure on several occasions, depositing one larva at a time. The larva migrates to the tympanum and probably penetrates and moves into the internal gular region of the host (Fig. 1 A-D).

In all cases the anurans were found on the ground and showed slow, unbalanced movements. A few hours after capture, the gular region of the specimens was ruptured by the exit of the fly larvae, leading to death of the species

(observed in *B. caramaschii* and *B. luctuosa*). With the aid of stereomicroscopy (Leica EZ4[®]), it was possible to observe that the anurans had a small perforation (hole) in the tympanic membrane (Fig. 1 E-H).

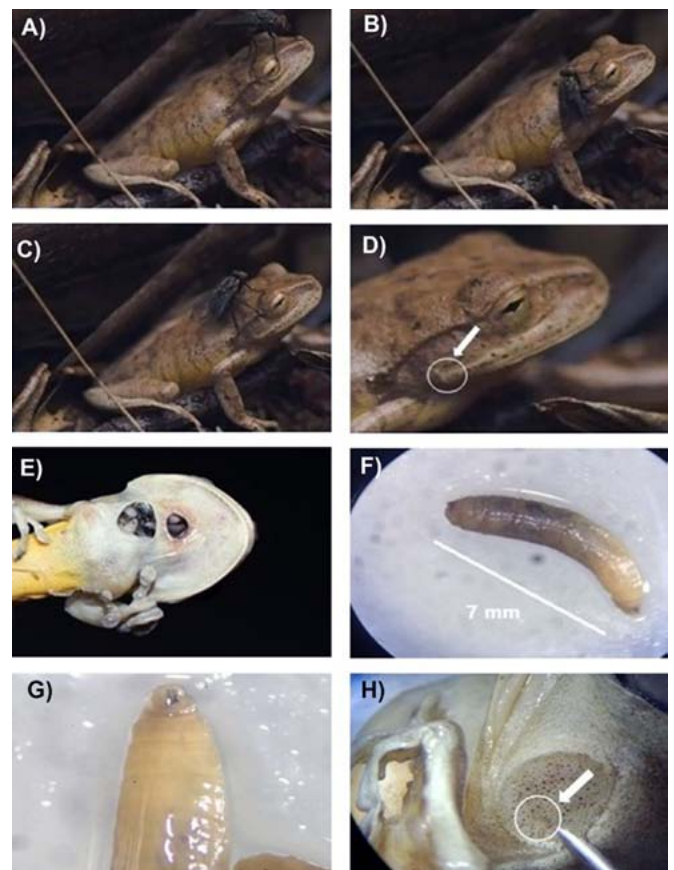


Figure 1. Fly landing and depositing a larva on the host *B. hylax*; **A)** Fly landing on host's head ; **B)** Fly landing and depositing a larva near the host tympanum; **C)** Fly returning to host's head ; **D)** Arrow pointing to the larva (body size ~ 0.5 mm); **E)** Developed larvae breaking the skin of the host while it is still alive (host *B. caramaschii*); **F)** Body size of larva (7.5 mm) after removal from host (*B. luctuosa*); **G)** Details of the mouth of the larva; and **H)** Tympanum of the host pierced by the larva (host *B. luctuosa*)

Müller et al. (2015) reported the presence of perforations caused by sarcophagid larvae along the body of individuals of the genus *Leptodactylus*. Oliveira et al. (2012), observed lesions above the cloaca, where the parasites were housed in frogs of the genus *Boana*. In both the above cases, the anurans in question exhibited nocturnal activity, as was the case with the species reported in this study. Therefore, the active period of the frogs does not coincide with that of the flies, which makes them more vulnerable.

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REFERENCES

- Bolek, M.G. & Coggins, J.R. (2002). Observations on myiasis by the calliphorid, *Bufoleucilia silvarum*, in the eastern American toad (*Bufo americanus americanus*) from southeastern Wisconsin. *Journal of Wildlife Diseases* 38: 598–603.
- Bolek, M.G. & Janovy, J. (2004). Observations on myiasis by the calliphorids, *Bufoleucilia silvarum* and *Bufoleucilia elongata*, in the wood frogs, *Rana sylvatica* from Southeastern Wisconsin. *Journal of Parasitology* 90: 1169–1171.
- Haddad, C.F.B., Toledo, L.F., Prado, C.P.A., Loebmann, D., Gasparini, J.L. & Sazima, I. (2013). *Guia dos Anfíbios da Mata Atlântica: Diversidade e Biologia*. São Paulo: Anolis Books. 544 pp.
- Hall, M.J.R. & Walls, R. (1995). Myiasis of humans and domestic animals. *Advances in Parasitology* 35: 257–334.
- Marcondes, C. B. (ed.). (2009). *Doenças transmitidas e causadas por artrópodes*. Editora Atheneu, Sao Paulo, Brazil. 557 pp.
- Müller, G.A., Lehn, C.R., Bemvenuti, A. & Marcondes, C.B. (2015). First report of myiasis (Diptera: Sarcophagidae) in anuran of Leptodactylidae (Amphibia). *Revista Colombiana de Ciencia Animal* 7: 217–220.
- Oliveira, R.M., Mira-Mendes, C.V., Ruas, D.S., Solé, M., Pinho, L.C. & Rebouças, R. (2012). Myiasis on *Hypsiboas atlanticus* (Caramaschi & Velosa, 1996) (Anura: Hylidae) from Southern Bahia, Brazil. *Herpetology Notes* 5: 493–494.
- Reichenbach-Klinke, H.H. & Elkan, E. (1965) *Principal Diseases of Lower Vertebrates*. Academic Press, London. 612 pp.
- YouTube (2019) Lemos et al Myiasis in three species of the genus. <https://www.youtube.com/watch?v=-lZhKyx2lJE&feature=youtu.be>. Uploaded December 2019.
- Zumpt, F. (1965). *Myiasis in Man and Animals in the Old World*. A textbook for physicians, veterinarians and zoologists. London, Butterworth. 267 pp.

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Autohaemorrhaging in a Bahamian pygmy boa, *Tropidophis curtus barbouri*

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Snakes use a wide variety of antipredatory behaviours, including gaping, exuding musk, defecating, convolving, feigning death, and autohaemorrhaging (Greene, 1994).

Autohaemorrhaging, or the deliberate ejection of blood, in snakes occurs via either the cloaca or the orifices of the head (i.e. nares, mouth and orbits). In reptiles, autohaemorrhaging is already known from a few species of snakes in the genera *Heterodon*, *Nerodia*, *Rhinocheilus*, *Natrix*, *Zamenis*, and *Tropidophis* as well as in the lizard genus *Phrynosoma* (Smith et al., 1993; Greene, 1994; Sherbrooke & Middendorf, 2001; Gregory et al., 2007; lifetime & lftime, 2014). In the genus *Tropidophis* specifically, which is also unusual in that some species are known to be able to change colour, cephalic autohaemorrhaging has so far been recorded in 13 out of 32 species (Smith et al., 1993; Greene, 1994; Torrest et al., 2013; Iturriaga, 2014). In a brief report, Hecht et al. (1955) were the first to describe autohaemorrhaging in *Tropidophis curtus curtus* (formerly known as *Tropidophis pardalis*). Here we offer greater detail of cephalic autohaemorrhaging in a different sub-species, the Bahamian pygmy boa (*Tropidophis curtus barbouri*), including access to the first-ever published video footage of this behaviour in any snake species (YouTube, 2019).

Knowing that *T. c. barbouri* is found on several islands across the Bahamian archipelago where it inhabits mesic, wooded areas (Henderson & Powell 2009; Powell & Henderson, 2012), we conducted an expedition and found an adult individual under a small rock in the Leon Levy Plant Preserve on Eleuthera, The Bahamas, on 20 October 2019 at 16:25 h. The snout vent length of the snake was 352 mm and tail length 42 mm, dorsally it was coloured beige-brown and ventrally the tail was yellow (Fig. 1).

Immediately after capture, it defecated and discharged musk. When gentle pressure was applied to the head, the eyes almost immediately filled with blood and a drop was exuded from the mouth (Fig. 2). After 2.4 sec of pressure both eyes began to fill with blood (starting from the postocular side) and were fully flooded within 0.7 sec. Blood started to exude from the mouth 0.8 sec after the eyes were fully filled and only two drops were expelled. After 4.4 sec the blood was re-absorbed from the postocular side and the eyes were completely clear within 1.6 sec. Overall, it took 6.7 sec from the start of the cephalic autohaemorrhaging to the full clearing of the eyes. After autohaemorrhaging, the snake appeared to be in full health and was returned to the location in which it was found.



Figure 1. Bahamian pygmy boa (*T. curtus barbouri*) found at the Leon Levy Preserve showing the tail with yellow colouring ventrally

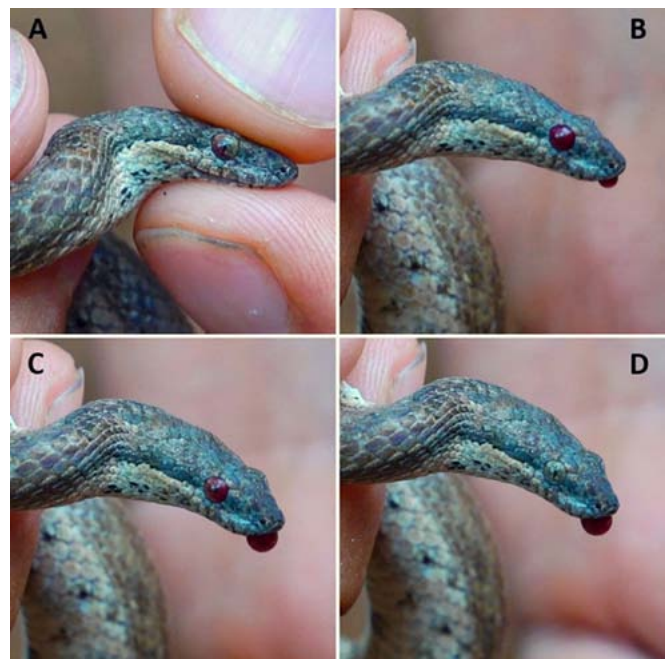


Figure 2. Four different stages of a cephalic autohaemorrhaging display in a Bahamian Pygmy Boa (*T. curtus barbouri*). **A)** Start of the autohaemorrhaging, after 2.4 sec of pressure both eyes began to fill with blood starting from the postocular side; **B)** Fully flooded eyes within 0.7 sec and expelled drop of blood from the mouth 0.8 sec after blood-filled eyes; **C)** Start of the clearing of the eyes from the postocular side 4.4 sec after full flooding; **D)** Fully cleared eyes with a drop of blood still hanging from the mouth within 1.6 sec. Overall, it took 6.7 sec from the initial start of the autohaemorrhaging to the full clearing of the eyes.

The physiological processes involved in cephalic autohaemorrhaging in *Tropidophis* spp. and the effect of expelled blood on predators are still unknown and thus complicate the attribution of this behaviour to a specific function. Hecht et al. (1955) and Greene (1994) suggested that autohaemorrhaging is an antipredatory response. Our observations support this as we witnessed cephalic autohaemorrhaging in *T. c. barbouri* alongside other defensive behaviours including defecating, exuding musk and convolving (forming a tight ball, see cover photo). While defecation and exudation of musk are often thought to render a snake unpalatable to a predator, this could be exacerbated by ejected blood. Toxic or foul smelling compounds are often added to the expelled blood in many species of insects (Blum & Sannasi, 1974; Stocks, 2008; Bateman & Fleming, 2009) to deter predators. In the case of *T. curtus curtus* (Hecht et al., 1955), this was investigated by injecting the snake's autohaemorrhaged blood into a prey animal, which presented no evidence of toxic properties.

The exact function of cephalic autohaemorrhaging is yet to be understood and predatory experiments as well as toxicological analyses of the expelled blood could shed light into how this behaviour could aid *Tropidophis* spp. deter predators. We also suggest that knowledge of the physiological processes involved in the very rapid onset and prompt stop of haemorrhaging in members of *Tropidophis* could have potential applications in medical fields.

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REFERENCES

Bateman, P.W. & Fleming, P.A. (2009). There will be blood: autohaemorrhage behaviour as part of the defence repertoire of an insect. *Journal of Zoology* 278: 342-348.

Blum, M.S. & Sannasi, A. (1974). Reflex bleeding in the lampyrid *Photinus pyralis*: defensive function. *Journal of Insect Physiology* 20: 451-460.

Greene, H.W. (1994): Antipredator Mechanisms in Reptiles. In *Biology of the Reptilia*, Vol. 16, p. 1-53. Gans, C., Huey R. B. (Eds.). Washington, Branta Books.

Gregory, P.T., Isaac, L.A. & Griffiths, R.A. (2007). Death feigning by grass snakes (*Natrix natrix*) in response to handling by human "predators". *Journal of Comparative Psychology* 121: 123.

Hecht, M.K., Walters, V. & Ramm, G. (1955). Observations on the natural history of the Bahaman pigmy boa, *Tropidophis pardalis*, with notes on autohemorrhage. *Copeia* 1955 3: 249-251.

Henderson, R.W. & Powell, R. (2009). *Natural History of West Indian Reptiles and Amphibians*. University Press of Florida, Gainesville, Florida.

Iftime, A. & Iftime, O. (2014). Thanatosis and autohaemorrhaging in the Aesculapian Snake *Zamenis longissimus* (Laurenti, 1768). *Herpetozoa* 26: 173-174.

Iturriaga, M. (2014). Autohemorrhaging behavior in the Cuban Dwarf Boa *Tropidophis melanurus* Schlegel, 1837 (Serpentes: Tropidophiidae). *Herpetology Notes* 7: 339-341.

Powell, R. & Henderson R.W. (2012). Island lists of West Indian amphibians and reptiles. *Florida Museum of Natural History Bulletin*, University of Florida. 51: 85-166.

Sherbrooke, W.C. & Middendorf III, G.A. (2001). Blood-squirting variability in horned lizards (*Phrynosoma*). *Copeia* 2001 4: 1114-1122.

Smith, D.D., Pflanz, D.J. & Powell, R. (1993). Observations of autohemorrhaging in *Tropidophis haetianus*, *Rhinocheilus lecontei*, *Heterodon platyrhinus*, and *Nerodia erythrogaster*. *Herpetological Review* 24: 130-131.

Stocks, I. (2008). Reflex bleeding (Autohemorrhage). In J. L. Capinera (Ed.), *Encyclopedia of Entomology*, p. 3132-3139. Dordrecht: Springer Netherlands.

Torres, J., Torres, O.J. & Marrero, R. (2013). Autohemorrhage in *Tropidophis xanthogaster* (Serpentes: Tropidophiidae) from Guanahacabibes, Cuba. *Herpetology Notes* 6: 579-581.

YouTube (2019). Autohaemorrhaging in a Bahamian pygmy boa. https://www.youtube.com/watch?v=F4GS_VdP_ow (uploaded Nov 2019)

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The Rise of Reptiles: 320 Million Years of Evolution

Hans-Dieter Sues

Johns Hopkins University Press, ISBN 9781421428673, pp. 385, hardback



THE RISE OF REPTILES



320 MILLION YEARS OF EVOLUTION | HANS-DIETER SUES



Some would say that the reptiles don't exist, so why, one might ask, is there a book about their evolution? This denial of existence is a bit of word-play though. The critics mean that there is no formal clade Reptilia, because birds and mammals evolved from reptilian ancestors, and so the group is paraphyletic because they are excluded from its definition. But we all know what reptiles are today,

and their past has been much richer and significant than the current crop of lizards, snakes, crocodiles and turtles might suggest.

Hans-Dieter Sues has produced a book in the style of his mentor, the great Bob Carroll, whose 1988 vertebrate paleontology textbook was a substantial update of the 1966 text by Al Romer (1894–1973), so the present book has a long and distinguished pedigree. Characteristic of these works is a focus on diversity and anatomy, with crisp text matched by excellent illustrations, often carefully crafted drawings, of the fossils and reconstructions of skulls and skeletons. Here we have all of that, but with the addition of colour photographs of fossils and living animals, as well as a modern phylogenetic setting throughout.

Introductory chapters explain the basics of fossils, geological time, anatomical terminology, and the importance of amniotes and reptiles. As in the precursor Romer and Carroll volumes, the remainder is a thorough coverage, group by group, from parareptiles to dinosaurs, with turtles, marine reptiles, lepidosauromorphs, and archosauromorphs in between. For each group, there is a brief history and then a review of key diagnostic characters, taxa, adaptations, and functions. The illustrations include the best available specimen drawings and colour photographs, obtained from dozens of researchers, so many have never featured in a book before, and they are very well reproduced. Sues has redrafted some older figures to add colour and explanation. A further good feature is that each clade is introduced with a standardized cladogram of major taxa, showing the current best estimate of relationships.

There are different ways to write such a book, and the

chapter on Avemetatarsalia (bird-line archosaurs) can illustrate this. Here, we are introduced to the various close relatives of dinosaurs, including pterosaurs, and much of the work is recent, with papers from 2017 and 2018 cited for the Aphanosauria, Silesauridae, and some new pterosaurs. The text is clear in defining the various clades, and providing a rational sequence of taxa, and highlighting key characteristics of each. The figures are a mix of colour photographs of specimens, drawings and some sharp whole-body images of these slick little pre-dinosaurs by Scott Hartman, Carol Abraczinskas and others. This gives an excellent and up-to-date overview of who was who and why, when they lived, and where they sit in the evolutionary tree.

There are some hints at paleobiology ("features suggest omnivorous or herbivorous habits"), but not much, and there are no life restorations such as colour paintings of animals in their habitats. Nor is there anything about macroevolution or macroecology. For example, why were these avemetatarsalian groups diversifying in the Triassic (perhaps part of the recovery fauna from the devastating end-Permian mass extinction; perhaps part of an arms race between synapsids and archosauromorphs as they adopted erect posture and some form of warm-bloodedness)? Debates, discussions and data about triggers for the origin and diversification of the dinosaurs are not included. Computational modelling of jaw and limb mechanics are excluded. However, this sits perfectly in the long tradition of the fundamentals of vertebrate paleontology with the focus on getting the framework right, based on careful exploration of anatomy, and the search for phylogenetically informative characters. Clarity about anatomy and phylogeny are essential before exploring broader themes in function and macroevolution, and such topics are well covered elsewhere.

The writing style is clear and easy, the illustrations are excellent, and the whole design and print quality highly attractive. There is no other book like it, and this will stand as a useful reference for many years. It is also the same size and shape as Romer's and Carroll's books, so I will squeeze them up side-by-side on my book shelf. The hardback volume retails for around £57, Kindle edition slightly less.

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