The Herpetological Bulletin

Issue 164, Summer 2023

Published by the British Herpetological Society


BHS

THE HERPETOLOGICAL BULLETIN

The Herpetological Bulletin is a full colour, 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.

All *British Herpetological Society* (BHS) members have access to issues of *The Bulletin* once they have been uploaded to the Society's website. Certain articles are **open access** from the time of release, these include articles containing a hyperlink to an illustrative video and those **written by *BHS* members** (encouraging authors to become *BHS* members). Other articles remain 'subscription-only' for one year. A printed version of *The Bulletin* is also distributed to those *BHS* members who subscribe to hardcopy.

The Editors are keen to ensure that *The Bulletin* is open to as wide a range of contributors as possible. If a potential author has concerns about compliance with submission guidelines (see below) or the suitability of a manuscript, then please contact the Scientific Editor for discussion.

Abbreviated Submission Guidelines for Contributing Authors

Authors should read and adhere to *The British Herpetological Society's* Ethical Policy and Guidelines, a full version of which can be found at https://www.thebhs.org/images/stories/BHS-ethicspolicy.pdf. All submissions are assessed for ethical considerations and publication may be refused for non-compliance. Contributors may therefore need to justify killing or the use of other animal procedures if these have been involved in the execution of the work. Likewise, full justification is required for studies involving the collection of endangered species or disturbance to their habitat(s).

1. See the BHS website for full 'Instructions for Authors' and a free download of a copy of *The Bulletin* showing house style.
2. Text contributions should be plain formatted with no additional spaces or tabs. Footnotes should not be used.
3. The References section must be formatted following The Bulletin house style (refer to this issue or a recent copy on the BHS website as a guide to style and format). Attention should be given to the format of citations within the text.
4. Images (photographs, graphs, illustrations) may be embedded within the text file of a submitted article but must also be submitted separately as PDF (preferred), TIFF or JPEG files. Images should be entirely relevant to the text and numbered sequentially with Arabic numbers (i.e. Figure 1. etc.). Images should be at a maximum 300 dpi resolution although higher resolution files may be requested in the case of images selected for the front cover or for other promotional purposes.
5. By submitting a manuscript, authors agree that the copyright for their article (including images) is shared with the publisher if and when the article is accepted for publication. This arrangement covers the rights of the BHS to reproduce and distribute the article, including reprints and photographic reproductions. However, authors also retain the right to use and distribute their article and its images as they see fit.
6. Where appropriate, authors will be requested to deposit their data sets, e.g. audio files, genetic analyses etc., in an archive with public access.
7. Authors are encouraged to submit relevant video footage that can be linked to their published articles. To do this, submit a title for the video and a short introductory text along with your manuscript. The video file itself, which is potentially very large, should be sent to the managing editor, Julie Tee, (managingeditor@thebhs.org) using Wetransfer (wetransfer.com) which is available free of charge. When an article is published, the video will be uploaded to the BHS video channel and a hyperlink inserted into the article to connect it to the video and vice versa. Articles with hyperlinks to videos will be open access from the time that they are uploaded to the BHS website (i.e. they will not be embargoed for a year).
8. Authors will be informed promptly of receipt of their manuscript but this does not infer acceptance for publication. All contributions are liable to assessment for suitability and ethical issues and all articles are subject to peer-review.
9. The Editor reserves the right to shorten or amend a manuscript, although substantial alterations will not be made without permission of the primary author. Occasionally, photographs and text from selected articles will be used for publicity purposes on the social media of the British Herpetological Society, where all relevant acknowledgements will be made.
10. Authors will be supplied with a portable document file (pdf) of their published article and a complimentary copy of the full printed issue.
11. All manuscript submissions and correspondence arising from *The Bulletin* should be sent to the Editor, herpbulletineditor@gmail.com
12. Articles reporting the results of experimental research, descriptions of new taxa, or taxonomic revisions should be submitted to *The Bulletin's* sister publication *The Herpetological Journal* (see inside back cover for Editor's address).

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: The northern spectacled salamander *Salamandrina perspicilliata* photographed in southern Italy by Massimo Capula, see article on p.18.

Printed by: Bruce Clark (Printers), Units 7-8, Marybank Lane, Dundee, DD2 3DY, UK. ISSN 2634-1387.

THE HERPETOLOGICAL BULLETIN

Contents: Issue 164, Summer 2023

RESEARCH ARTICLES:

SHORT NOTES:

SHORT COMMUNICATIONS:

NATURAL HISTORY NOTES:

BOOK REVIEW:

Registered Charity No. 205666

The Herpetological Bulletin 164, 2023: 1–6
RESEARCH ARTICLE

https://doi.org/10.33256/hb164.16

Bioacoustics, breeding ecology and range of the Kerala warty frog *Minervarya* cf. *keralensis* from north of the Palghat gap, central Western Ghats

AMIT HEGDE & GIRISH KADADEVARU*

Breeding Behaviour and Bioacoustics Lab, Department of Zoology, Karnatak University, Dharwad 580003, India

*Corresponding author e-mail: kadadevarug@gmail.com

ABSTRACT – *Minervarya* cf. *keralensis* has been detected for the first time north of the Palghat gap in the central Western Ghats, a range extension of about 85 km. This species calls and breeds in the post-monsoon period (Jan–March) and exhibits axillary amplexus. Male call characteristics, natural history observations, and distributional complexities are described. Currently, the *Minervarya nilagirica* group that includes *M. keralensis*, show a complex distribution pattern in peninsular India that is worthy of further phylogeographic study.

INTRODUCTION

The genus *Minervarya* includes 31 species that are distributed in South Asia where the majority of species are known to occur in the Western Ghats (Frost, 2022); most of the species are widespread while a few are range restricted (Garg & Biju, 2021). They occupy a wide diversity of habitats including those close to human habitation, agricultural landscapes, open lands, and mud puddles. For several decades this genus was known to be an "unresolved long standing taxonomic enigma" due to its deeply conserved morphological characters and crypticity (Garg & Biju, 2021; Sanchez et al., 2018; Dinesh et al., 2015).

Frogs of the genus *Minervarya* have received more attention from taxonomists than other anurans (Garg & Biju, 2021; Dinesh et al., 2015). Several attempts have been made to classify minevaryans into different groups based on morphological traits, size and phylogenetic analyses (Garg & Biju, 2017; Phuge et al., 2019; Hegde et al., 2020). In peninsular India, the genus *Minervarya* is currently classified into eight groups (Garg & Biju, 2021). The *Minervarya nilagirica* group is endemic to peninsular India and currently contains three species - *M. nilgarica*, *M. kalinga* and *M. keralensis* which all occur in the Western Ghats. This group is known to show complex distributional patterns (Hegde et al., 2020; Garg & Biju, 2021) where *M. keralensis* has been reported from the southern portion of the Western Ghats with a northern limit at Poomala (Biju & Garg 2021).

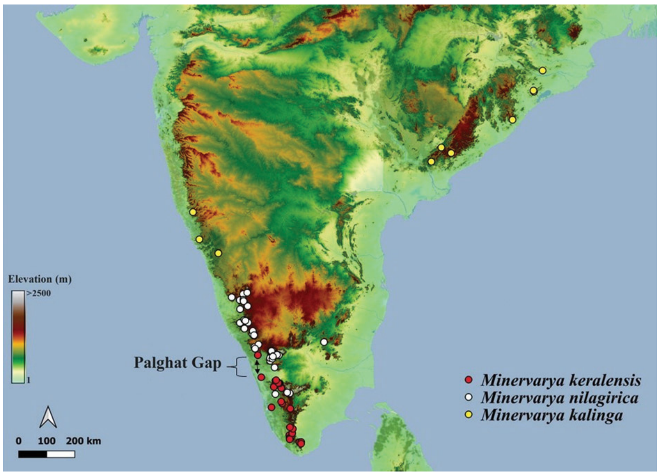


Figure 1. Distribution of the *Minervarya nilagirica* group from peninsular India - *Minervarya kalinga*, *Minervarya nilagirica*, *Minervarya keralensis* (range extension to the north of the Palghat gap)

A previous study (Kuramoto & Joshy, 2001) claimed to have described the advertisement call characteristics of *M.* cf. *keralensis* (*Limnonectes* cf. *keralensis*) from two sites in Karnataka (Talagini and Kudremukh) in the central Western Ghats, but subsequently the subject of their study was revealed to be *M. kudremukhensis* (*Fejervarya kudremukhensis*) (Kuramoto et al., 2007) which is currently synonymised as *M. mysorensis* (Garg & Biju, 2021). Consequently, there are no studies on the breeding behaviour, bioacoustics or natural history of *M. keralensis*. Hence the present study reports the vocal repertoire, breeding ecology and a range extension of *M. keralensis* from central Western Ghats. However, although the morphology of our study species is consistent with *M. keralensis*, we have not undertaken the molecular sequencing that would be needed to substantiate this identification, consequently we refer to *M.* cf. *keralensis* throughout the study.

MATERIALS & METHODS

During this field study, *M.* cf. *keralensis* was observed between January and March at the southern tip of central Western Ghats in Kakandanmpoyii, Nilambur, Kerala 11° 20'36.24" N, 76° 6'16.56" E at an altitude of 700–800 m a.s.l. (Fig. 1). To assess the anuran diversity in different regions of the central Western Ghats, we have undertaken regular visual encounter and acoustics surveys (Rödel & Ernst, 2004) throughout the year. Frogs were identified to species level by observing morphological characters based on the available taxonomic descriptions and literature (Garg & Biju, 2021; Hegde et al., 2020; Raj et al., 2018). The breeding ecology and the natural history of the species encountered were observed and noted. No specimens were collected during the study.

Call recording and analysis

Calling was observed from January to March, 2019 and calls were recorded on 14 February 2019 in the winter season (post-monsoon) when the air temperature ranged 22.0–22.2 °C and the humidity ranged 60–61 %.

Calls were recorded with a Zoom H1 portable digital audio recorder connected to a unidirectional Rode microphone attached to a Rycote Lyre shock mount (sampling rate of 44100 Hz/16 bit). After locating a calling adult male, the unidirectional microphone was directed at its vocal sac, at a distance of about 30–50 cm. Gain settings of the audio recorder were kept constant throughout the call recording. Calls were recorded choosing randomly selected males without disturbing the frogs. Temperature was measured using 310 CIE (Chung Instrument Electronics) digital thermometer and relative humidity was measured using a Barigo hygrometer (Germany) near the calling individual. Calls with high background noise and overlapping calls were excluded from the analysis. Recorded calls were visualised and randomly analysed in Raven Pro 1.6.2 (K. Lisa Yang, 2022). Temporal measurements were quantified using the waveform/oscillogram window and included - call per minute, call type, call series duration, call duration, call series interval, call interval and the number of calls in a call series. Using the spectrogram, we measured the peak frequency and average entropy. We used a call centred approach and call series parameters were based on previous studies (Köhler et al. 2017; K. Lisa Yang Center for Conservation Bioacoustics, 2022). Calls of six adult males and 20 call series (159 calls) were recorded. These can be played back in BHS video (2023).

Statistical analysis

Call measurements were graphically represented using the R studio (ver. 0.7, R Core Team, 2022) and boxplots were obtained using “ggplot2” (Wickham, 2016).

RESULTS

Morphological diagnosis and range extension

The snout vent length (SVL) of large adult males ranged from 32–44 mm (n=9), the body was robust, head length greater than the head width, the first finger was longer than the second finger, a third subarticular tubercle was present on the fourth toe, with long cylindrical inner metatarsal tubercles, prominent discontinuous folds on the dorsal skin and prominent glandular warts along a dorsal chevron, and prominent reticulations on groin and thigh. The upper lip had horizontal bands, minervaryan lines and rictal glands were visible. The digits on the forelimbs were shorter than those on the hind limbs, fingers lacked interdigital webbing, and toes were moderately webbed. Based on the above morphological characteristics, individuals in the population were identified as *M.* cf. *keralensis* (Garg & Biju, 2021; Hegde et al., 2020)(Fig. 2).

Figure 2. Adult male *Minervarya* cf. *keralensis* - **A.** Dorsolateral view, **B.** On calling ground, **C.** Dorsal view

The current study is the first report of *M.* cf. *keralensis* from north of the Palghat gap. This species was commonly observed close to the Nilgiris range of the Western Ghats, near the Chaliyar river basin, which is situated on the south-western side of the central Western Ghats (Fig. 1). In this region, human modified landscapes are common within a mosaic of other land-use types including teak plantations and agriculture landscapes (tea, cocoa beans and other crops).

Breeding biology

Breeding season: Calling and breeding activity was observed from January to March; this frog species is a post-monsoon breeder.

Breeding ground/spawning ground: The breeding areas were typical wetlands, paddy fields and marshes, moist microhabitat beside slow-flowing streams and temporary streams which were about to dry out. The adult males called from the banks of the ditches and wetlands, whilst perched on stones, grass patches, gravel and wet soil surfaces that usually had an open or partially open canopy. Spawning grounds were close to the calling areas (Fig. 2).

Secondary sexual characters: Adult males possessed a single subgular vocal sac that was highly distensible, the skin near the vocal sac was lightly pigmented with black, and slightly transparent. While calling, occasional irregular inflation of the vocal sac was observed. Calling adult males had an oval shaped nuptial pad on the first finger. Calling males exhibited yellow colouration near and below the tympanum and below the eye. Adult females were larger than adult males.

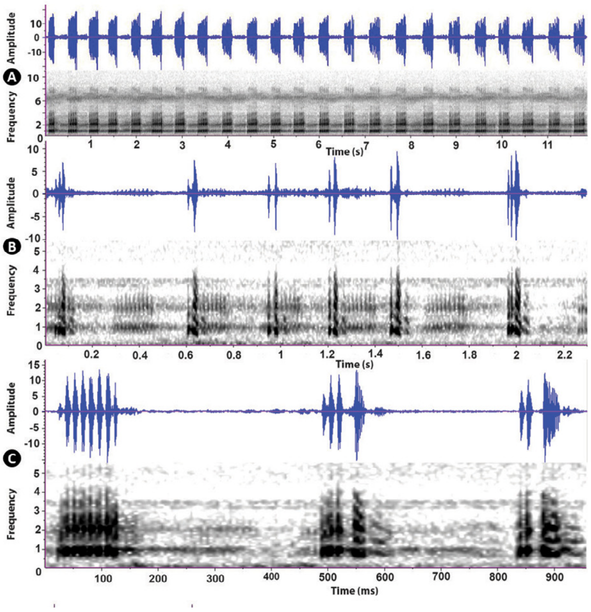


Figure 3. The three advertisement call series of male *Minervarya* cf. *keralensis*, oscillogram showing amplitude (kU) and spectrogram showing frequency (kHz) - **A.** Call series A, **B.** Call series B, and **C.** Call series C (composite call series)

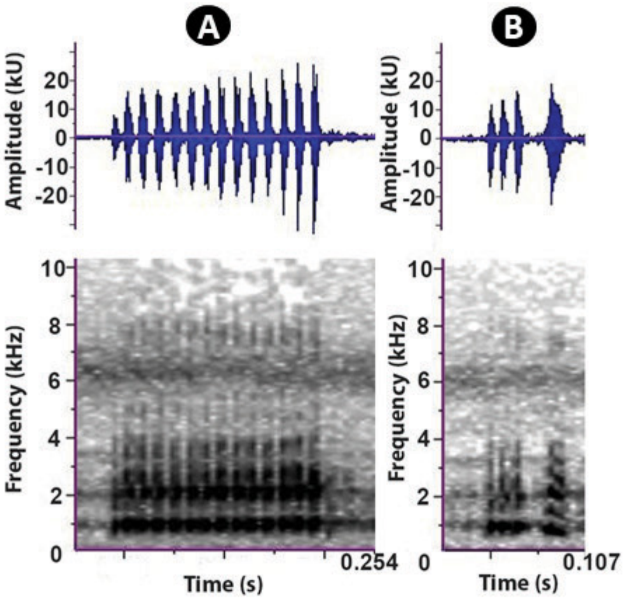


Figure 4. The two types of male advertisement calls (Type A & Type B) of *Minervarya* cf. *keralensis*, oscillogram (blue trace) and spectrogram (grey trace) - **A.** Call type A and **B.** Call type B

Amplexus and egg characteristics: Axillary amplexus was observed (n=8). Multiple small egg clutches were laid in a single spawning. Amplexed pairs moved slowly within the spawning ground and the female laid 3–8 eggs at a time in a clutch (n=8 clutches) and the clutches were laid in more than one place at different distances apart. The eggs were brown in colour and surrounded by a transparent jelly layer. After the eggs were laid, this jelly layer slowly absorbed water from the spawning habitat and the jelly layer swelled gradually.

Bioacoustics

The calls of males were heard from 19:00 h and peak calling time was between 20:00 h and 00:30 h. The majority of amplectant pairs were seen during and after midnight (n=4) and later. After 02:00 h calls slowly subsided.

Call series characters. Calls series contained acoustically multi-call groups that were different and each call comprised several pulsatile groups (Fig. 3). Call series duration/call series length was 3.34 ± 0.32 s and ranged 2.62–3.80 s; Call series interval was 10.71 ± 26.22 s and it ranged from 0.81–117.73 s. The number of calls per call series (n=20) was 8.6 ± 5.91, ranging from 2–25; the average number of call series per minute (n=20) was 16 (range 11–21). The call series contained structurally distinct and different calls, hence we have established three call series categories - A, B, and C.

Call series A: TarrahTarrahTarrah - this was the most frequent call type (n=16), and was most commonly emitted with repetitive and multiple calls, and was relatively long when compared to the call series B and C (Fig. 3A, B & C, Table 1).

Call series B: Kruhrkruhrkruhrrkruhrr - this was emitted before call type A and was more commonly heard in the early hours of the night usually from 18:00 h to 19:30 h and from 04:00 h to 06:00 h on the following day (Fig. 3B; Table 1).

Table 1. The parameters of call series A, B, and C of male *Minervarya* cf. *keralensis*

Call series	**Call parameters**	**Mean ± SD**	**Range**
Series A (n=16)	Call series duration (s)	3.45±3.57	0.51–12.82
	Call series interval (s)	12.88±3.45	0.81–117.73
	No. of calls in a call series	9±6.59	2–25
Series B (n=2)	Call series duration (s)	2.83±1.16	2.01–3.66
	Call series interval (s)	1.39±0.11	1.31–1.74
	No. of calls in a call series	9±3.53	6–11
Series C (composite call) (n=2)	Call series duration (s)	2.52±2.28	0.91–4.14
	Call series interval (s)	4.81±2.33	3.16–6.46
	No. of calls in a call series	6±4.243	3–9

Call series C: TarrahTarrahTarrahkruhr or kruhrkruhrTarrahTarrah - this was a composite series that consists of both type A and type B calls. These were not emitted as frequently as series A calls. They were commonly emitted after the series B calls or at the end of series A calls.

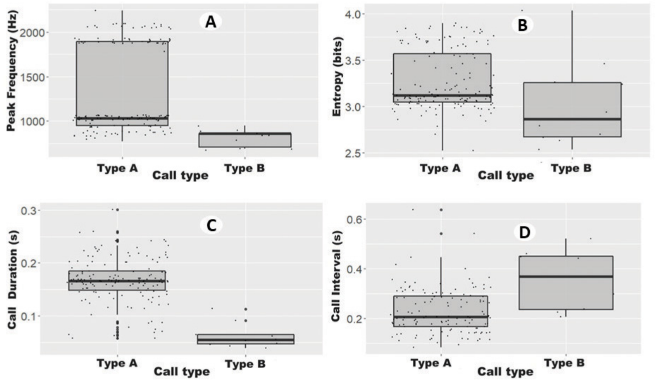


Figure 5. Characteristics of the two types of male advertisement calls of *Minervarya* cf. *keralensis* - **A.** Peak frequency, **B.** Entropy in bits, **C.** Call duration, and **D.** Call intervals

Table 2. Parameters of call types A and B of male *Minervarya* cf. *keralensis*

Call series	Call parameters	Mean ± SD	Range
Type A (n=149)	Peak frequency (Hz)	1334.19±484.62	775.20–2239.45
	Call duration (s)	0.16±0.04	0.05–0.31
	Call interval (s)	6.73±0.08	0.08–0.63
	Average entropy (bits)	3.27±0.31	2.52–3.89
Type B (n=10)	Peak frequency (Hz)	809.64±92.59	689.06–947.46
	Call duration (s)	0.06±0.02	0.03–0.11
	Call interval (s)	0.35±0.12	0.21–0.52
	Average entropy (bits)	3.025±0.47	2.53–4.03

They were frequently not repetitive. In this series, type B calls were emitted at the beginning or at the end of the call series. (Fig. 3C, Table 1).

Call description: Based on structural and audible complexity we have classified calls into two types:-

1) Type A call

In this type, there were more pulsatile groups that were more fused relative to call type B. As there were more pulsatile groups, call duration was greater than in call type B (Figs. 4 & 5, Table 2) and these pulsatile groups were generally more uniformly distanced relative to type B. As the length of call increased, a greater number of pulsatile groups were emitted and pulsatile groups within the call also increased. This call type was the most frequently emitted (n=149). However, type A calls also showed within call type diversity where the number of pulsatile groups were fewer at the beginning of call and there were more pulsatile groups at the end of the call (Fig. 4, Table 2). These contribute to the series A call and were also components of series C.

2) Type B call

These constitute non uniformly placed pulsatile groups which were fused, and with a shorter call duration and with shorter intercall intervals. Type B calls (n=10) were not as frequently emitted as type A (n=149) (Figs. 4 & 5, Table 2). Type B calls were emitted in series B calls and were also components of series C calls.

According to the classification of anuran call guilds (Emmrich et al., 2020), both Call type A and B belong to Call Guild H, which are frequency-modulated, complex calls with multi-unit and multi note or pulsatile groups.

DISCUSSION

During the present study, calling and breeding activities were observed between January and March which is during the dry season. *Minervarya* cf. *kerlaensis* is a post-monsoon breeder, much like *M. kalinga* from the *M. nilagirica* group (Raj et al., 2019; Hegde et al., 2020) (Fig. 6). Among the minervaryans, the *M. nilagirica* group consists of large bodied frogs and large body size may enable them to be active and reproduce after the monsoon (dry periods) in peninsular India, as they are likely to be less prone to desiccation than the smaller minervaryan frogs (Raj et al., 2018; Hegde et al., 2020; unpublished data). Most minevaryan frogs breed in the pre-monsoon period or during the monsoon (Fig. 6) but as many of them are sympatric (Garg & Biju, 2017; 2021; Phuge et al., 2019; 2020; Kadadevaru et al., 2002; Dubois et al., 2001) there may be micro separation in breeding habitat, calling pattern, and spawning pattern (Garg & Biju, 2017).

The secondary sexual characters observed in adult male *M.* cf. *keralensis* include nuptial pad on the first finger of the forelimbs and black colouration or glandular or transparent skin in the vocal sac region and are typical of minervaryans (Garg & Biju, 2017; 2021; Kadadevaru et al., 2002; Phuge et al., 2019; 2020). In addition to these secondary sexual characteristics, adult males also show different colouration near the tympanum, close to mouth commissural region and in forelimbs which have also been described in *M. mysorensis* (Kuramoto & Joshy, 2001). All the minervaryan species of peninsular India employ axillary amplexus (Garg & Biju, 2017; 2021; Kadadevaru et al., 2002; Phuge et al., 2019; Dubois et al., 2001) and lay their eggs in a variety of sites including aquatic vegetation, wet soil, puddles, and other moist places that are used as temporary spawning grounds (Dubois et al., 2001; present study; unpublished data), which may reduce the risk of predation and desiccation of both the eggs and tadpoles.

The frog *M.* cf. *keralensis* exhibits call complexity. Call type A is more frequent and may be related to male-female interaction. Call type B was heard in the beginning or in between multiple Call A types and these were typically short (Fig. 4, Table 1). Playback experiment studies are needed to ascertain the functionality of all the calls. There is a further complication in our understanding of the calls

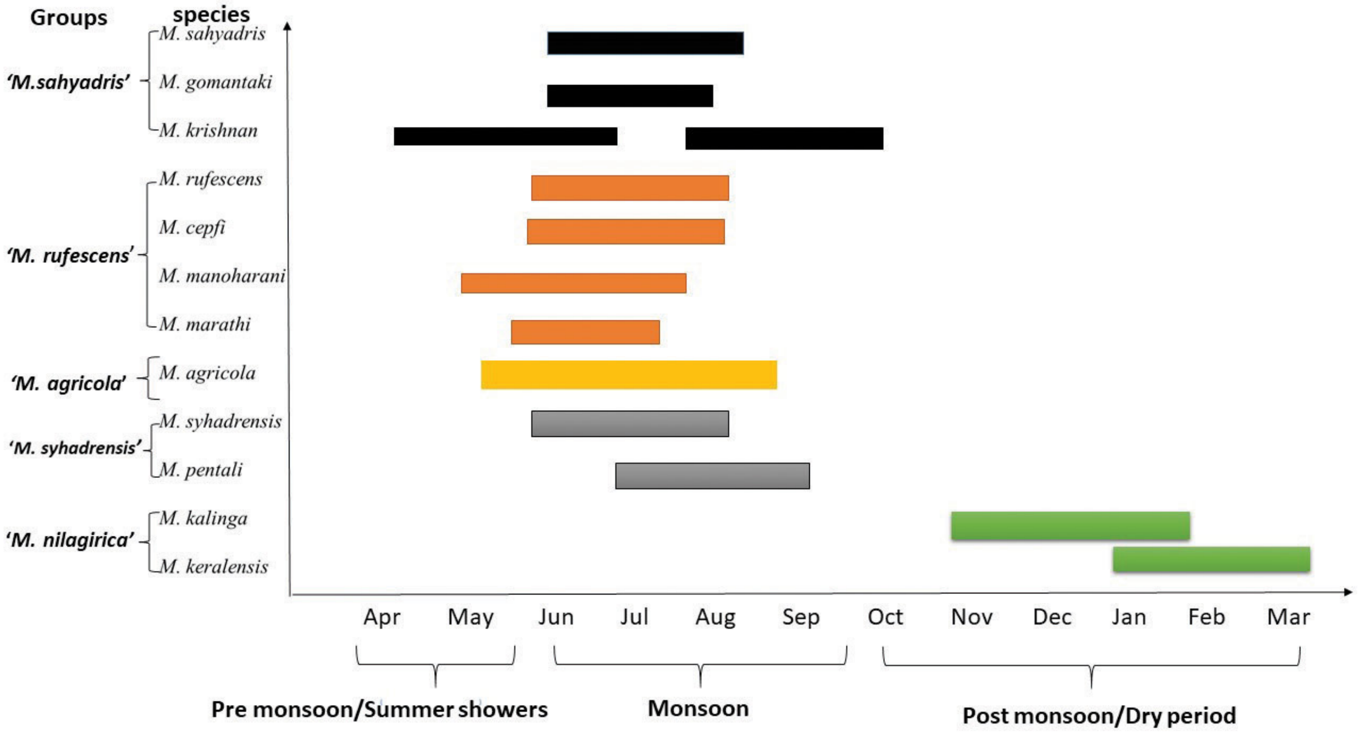


Figure 6. Call phenology of Minervaryan frogs from Western Ghats (after Garg & Biju, 2017; 2021; Phuge et al., 2019; 2020; Kadadevaru et al., 2002; Dubois et al., 2001; present study). Different coloured bars indicate different species groups.

of *M.* cf. *keralensis* as in this study occasionally irregular symmetry was observed when the vocal sac was enlarged during calling. This might have functional significance in producing call variation or different calls, further detailed study of this is needed.

The *M. nilagarica* group of frogs presents an interesting distribution within peninsular India (Fig. 1). *Minervarya nilagirica* is predominantly from the central Western Ghats but is also reported from the southern Western Ghats and the southern part of the Eastern Ghats (Biju & Garg, 2021). *M. kalinga* is known from northern part of the Eastern Ghats (Raj et al., 2018), central Western Ghats (Hegde et al., 2020) and northern Western Ghats (Biju & Garg, 2021) while *M. keralensis* is predominantly from the southern Western Ghats (Biju & Garg, 2021) but this study reports it from north of Palghat gap, in the central Western Ghats with a range extension of about 85 kms. Within the Peninsular India this group is distributed in highly elevated forest areas. Further phylogeographic studies are needed to explain the distribution patterns of the *M. nilagirica* group.

ACKNOWLEDGEMENTS

Both authors are grateful to K.P. Dinesh from the Zoological Survey of India for all his critical comments and suggestions. AH would like to thank Karnatak University for the award of URS (University Research Studentship) funding support. Sunita Rao, Land and Lens India, Batt Anderson, Tom, Amila-Lupé Ellena Aguilar for the gadgets support and grateful to Danita Daniel, Sachet, Deepak P for the help and special thanks to Sarojkumar, Sunil Kumar, Sattva resort for allowing us to work in their field. The travel grant was funded by SAP-II grants from UGC New Delhi.

REFERENCES

BHS video (2023). Advertisement calls of *Minervarya* cf. *keralensis*. https://youtu.be/xsC3264lVr8.

Dinesh, K.P., Vijayakumar, S.P., Channakeshavamurthy, B.H., Torsekar, V.R., Kulkarni, N.U. & Shanker, K. (2015). Systematic status of *Fejervarya* (Amphibia, Anura, Dicroglossidae) from South and SE Asia with the description of a new species from the Western Ghats of Peninsular India. *Zootaxa* 3999(1): 79–94.

Dubois, A., Ohler, A. & Biju, S.D. (2001). A new genus and species of Ranidae (Amphibia, Anura) from south-western India. *Alyte* 19(2–4): 53–79.

Emmrich, M., Vences, M., Ernst, R., Köhler, J., Barej, M.F., Glaw, F., Jansen, M. & Rödel, M.O. (2020). A guild classification system proposed for anuran advertisement calls. *Zoosystematics and Evolution* 96(2): 515–525. Doi: 10.3897/zse.96.38770.

Frost, D.R. (2022). *Amphibian Species of the World: an Online Reference*. Version 6.0 Electronic Database. American Museum of Natural History, New York. https://amphibiansoftheworld.amnh.org/. Accessed on 30 September 2022.

Garg, S. & Biju, S.D. (2017). Description of four new species of burrowing frogs in the *Fejervarya rufescens* complex (Dicroglossidae) with notes on morphological affinities of *Fejervarya* species in the Western Ghats. *Zootaxa* 4277(4): 451–490. Doi: 10.11646/zootaxa.4277.4.1.

Garg, S. & Biju, S.D. (2021). DNA barcoding and systematic review of minervaryan frogs (Dicroglossidae: *Minervarya*) of Peninsular India: Resolution of a taxonomic conundrum with description of a new species. *Asian Herpetological Research* 12(4): 345–378. Doi: 10.16373/j.cnki.ahr.210023.

Hegde, A., Dinesh, K.P. & Kadadevaru, G. (2020). Phenotypic divergence in large sized cricket frog species that crossed the geographical barriers within peninsular India. *Zootaxa* 4838(2): 210–220. Doi: 10.11646/zootaxa.4838.2.3.

K. Lisa Yang Center for Conservation Bioacoustics (2022). Raven Pro: Interactive Sound Analysis Software (Version 1.6.2) [Computer software]. Ithaca, NY. The Cornell Lab of Ornithology. https://ravensoundsoftware.com/.

Kadadevaru, G.G., Kanamadi, R.D. & Schneider, H. (2002). Advertisement call, courtship and mating behaviour of the frog, *Limnonectes syhadrensis* from Western Ghats, India. *Current Science* 82(5): 503–505.

Köhler, J., Jansen, M., Rodríguez, A., Kok, P.J.R., Toledo, L.F., Emmrich, M., Glaw, F., Haddad, C.F.B., Rödel, M.O. & Vences, M. (2017). The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251(1): 1–124. Doi: 10.11646/zootaxa.4251.1.1.

Kuramoto, M. & Joshy, S.H. (2001). Advertisement call structures of frogs from southwestern India, with some ecological and taxonomic notes. *Current Herpetology* 20(2): 85–95.

Kuramoto, M., Joshy, S.H., Kurabayashi, A. & Sumida, M. (2007). The Genus *Fejervarya* (Anura: Ranidae) in central Western Ghats, India, with descriptions of four new cryptic species. *Current Herpetology* 26(2): 81–105.

Phuge, S., Dinesh, K.P., Andhale, R., Bhakare, K. & Pandit, R. (2019). A new species of *Fejervarya* Bolkay, 1915 (Anura: Dicroglossidae) from the northern Western Ghats parts of Maharashtra, India. *Zootaxa* 4544(2): 251–268. Doi: 10.11646/zootaxa.4544.2.6.

Phuge, S., Patil, A.B., Pandit, R., Kulkarni, N.U., Chennakeshavamurthy, B.H., Deepak, P. & Dinesh, K.P. (2020). Importance of genetic data in resolving cryptic species: A century old problem of understanding the distribution of *Minervarya syhadrensis* Annandale, 1919 (Anura: Dicroglossidae). *Zootaxa* 4869(4): 451–492. Doi: 10.11646/zootaxa.4869.4.1.

R Core Team (2022). R: A Language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raj, P., Dinesh, K.P., Das, A., Dutta, S.K., Kar, N.B. & Mohapatra, P.P. (2018). Two new species of cricket frogs of the genus *Fejervarya* Bolkay, 1915 (Anura: Dicroglossidae) from the Peninsular India. *Records of the Zoological Survey of India* 118(1): 1–21.

Rödel, M.O. & Ernst, R. (2004). Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. *Ecotropica* 10(1): 1–14.

Sanchez, E., Kurabayashi, A., Biju, S.D., Islam, M.M. & Hasan, M. (2018). Phylogeny and classification of fejervaryan frogs (Anura: Dicroglossidae). *Salamandra*, 54(20): 109–116.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York, NY. Springer-Verlag. https://ggplot2.tidyverse.org.

Accepted: 1 December 2022

The Herpetological Bulletin 164, 2023: 7–12
RESEARCH ARTICLE

https://doi.org/10.33256/hb164.712

A preliminary assessment of chelonian diversity in the montane forests of two areas in northern Vietnam

PHAM VAN THONG[1,2], OLIVIER LE DUC[1,10], BENJAMIN LEPRINCE[1], CEDRIC BORDES[1,10], CHARLOTTE DUCOTTERD[1,4], LUU QUANG VINH[3], SENG RAVOR[5], TRAN QUANG BAO[6] & LUCA LUISELLI[7,8,9,*]

[1]Turtle Sanctuary Conservation Center, 19 rue Béranger 75003 Paris, France
[2]Save Vietnam's Wildlife, Cuc Phuong commune, Nho Quan district, 430000 Ninh Binh province, Vietnam
[3]Vietnam National University of Forestry, str.21, Xuan Mai town, Chuong My district, 100000 Hanoi, Vietnam
[4]Centre Emys, Protection et Récupération des Tortues, Chavornay, Switzerland
[5]Royal University of Agriculture, Dongkor district, 2696 Phnom Penh, Cambodia
[6]Vietnam Administration of Forestry, no 2 Ngoc Ha street, Ba Dinh district, 100000 Hanoi, Vietnam
[7]Institute for Development, Ecology, Conservation and Cooperation, via G. Tomasi di Lampedusa 33 - 00144 Rome, Italy
[8]Department of Applied and Environmental Biology, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria
[9]Département de Zoologie et Biologie Animale, Faculté des Sciences, Université de Lomé, B.P. 1515, Lomé, Togo
[10]Associated Wildlife and Environmental Conservation Community (AWECC), Paris, France

[*]Corresponding author e-mails: l.luiselli@ideccngo.org; lucamaria.luiselli@uniroma3.it

ABSTRACT - Very little is known of the ecology and distribution of Vietnamese tortoises and freshwater turtles, even though Vietnam is one of the world's hotspots of chelonian diversity and, according to the International Union for the Conservation of Nature (IUCN), almost all its species are threatened. We report the diversity of chelonians for two distinct areas of northern Vietnam (in three provinces) characterised by forested hills and mountains, with ponds and streams. We observed ten species in Lang Son/Bac Giang provinces and 11 species in Lao Cai province. Reportedly, the two most frequently recorded species were *Pelodiscus sinensis* and *Cuora mouhotii*. Most species were Endangered or Critically Endangered according to IUCN, thus making the protection of the forest habitats of these provinces of crucial relevance for the survival of these species. Reliable records for the almost extinct *Rafetus swinhoei* were collected, but all of them refer to the end of the 1970s.

INTRODUCTION

There are currently about 90 species of Asian turtles, which belong to just four families Geomydidae, Testudinidae, Trionychidae, and Platysternididae. This chelonian fauna is among the most diverse and the most threatened in the world (Rhodin et al., 2018; Stanford et al., 2018), with many species listed as Critically Endangered by the World Conservation Union (IUCN, 2022). Since most of the Critically Endangered (CR) and Endangered (EN) species are found in south-eastern China, south-east Asia, Indonesia, northern India and Vietnam (Rhodin et al., 2018), these regions need to be particularly well studied in order to evaluate more precisely the distribution and the ecology of the various species. However, the information available is still scarce, and relatively wide territories, especially in mountainous and comparatively difficult-to-access areas, have to date remained practically unexplored.

In order to cover, at least partially, these mountain territories on which accurate research has never been published, we document in this article the specific diversity of tortoises and freshwater turtles in three provinces of northern Vietnam. The method of investigation (interviews with local hunters and specimens observed in villages) and the short timespan of the study (two missions of a few weeks) prevent us from providing indications on the status of the populations. However, the data presented here significantly increase our knowledge of chelonian communities in the areas in question, and may be useful as a baseline for future surveys and more detailed field studies.

MATERIALS & METHODS

Study areas

There were two study areas; Lang Son province (21° 50.357' N, 106° 44.579' E), including a small part of Bac Giang province, and Lao Cai province (22° 20.285' N, 104° 8.922' E). These are mountainous areas of northern Vietnam near the Chinese border (Fig. 1). Lang Son and Bac Giang province are characterised by hilly forests and limestone forests with altitudes of 252–1541 m a.s.l. (BKHDT, 2022). The temperature range is about 17–22 °C, mean humidity about 80 % and with annual rainfall in the range of 1200–1600 mm. The forest cover is about 300,000 ha with the Huu Lien Nature Reserve being the most important area for wildlife (Bui et al., 2019; Lunde et al., 2007). In Lao Cai, the altitude range is 100–3143 m a.s.l., with the Hoang Lien National Park being the most important protected area of the region. Temperature range 15–29 °C, mean humidity is about 80 %, and the annual rainfall is in the range of 1400–2000 mm (Lao Cai, 2022). This province also contains several big river systems such as the Red and Chay rivers.

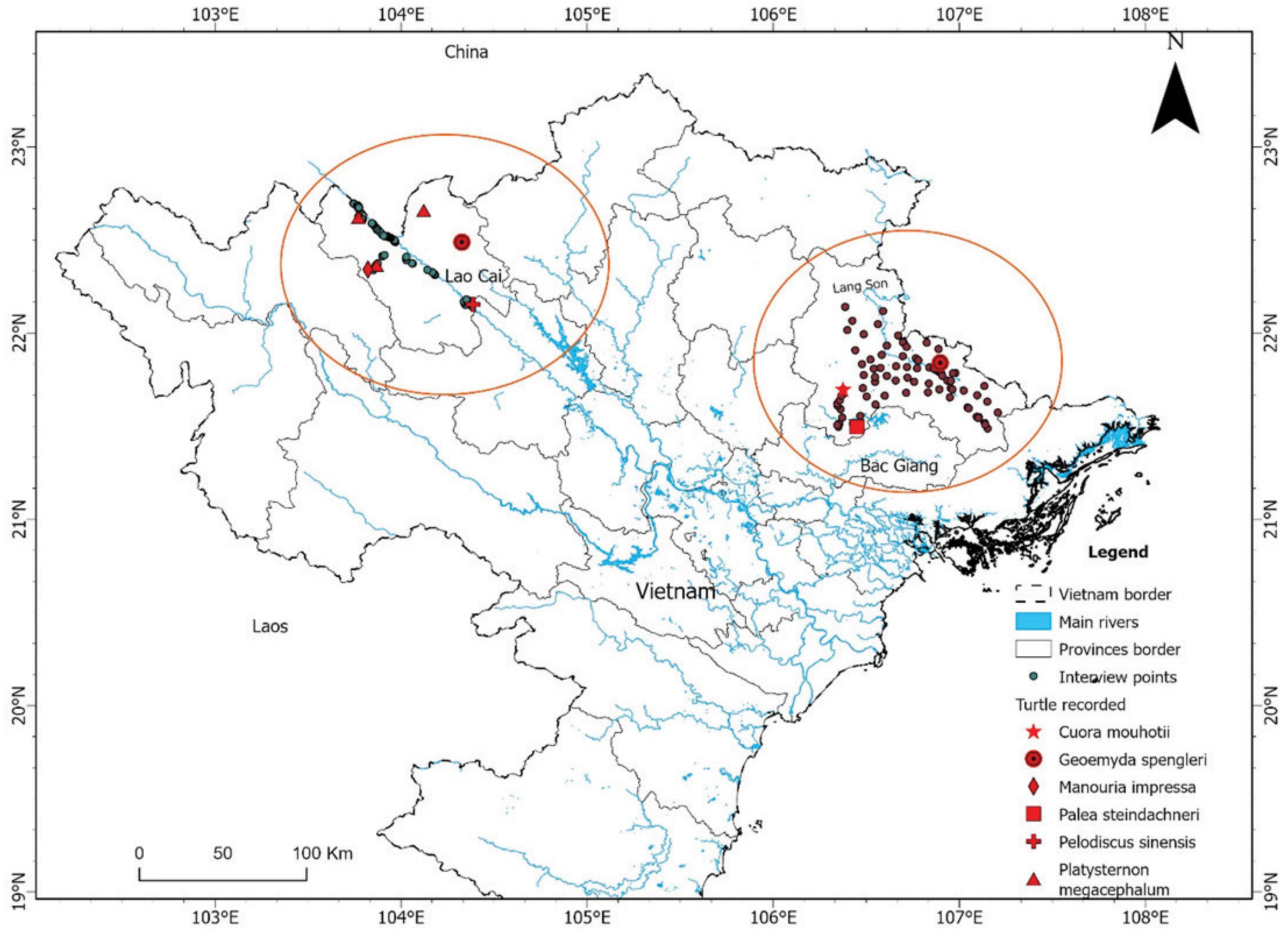


Figure 1. Map of northern Vietnam showing the interview localities and the sites where chelonians were observed directly (as reported in Table 2)

Protocol

This study was based on questionnaire surveys within villages in the study areas. The first survey was conducted in Lang Son and Bac Giang provinces from 8–22 June 2018, and the second survey (Lao Cai) was from 12–28 August 2018. The field team consisted of two persons who conducted semi-structured interviews, in Vietnamese language. The initial interviewees were used to help recruit other knowledgeable interviewees (snowball procedure) who were then interviewed separately. The interviewees were all males, all over 30 years old and were previously hunters or even providers of turtles to traders. The interviewees were informed of the scope of the study in advance and their identities were kept anonymous. Interviews lasted on average 40 minutes, but sometimes up to two hours if the person appeared particularly well aware of the status of turtles in his area. All interviews followed ethical rules (British Sociological Association, 2017).

The interview was based on a semi-structured questionnaire and asked the following questions: (i) Do you know any turtles from your area? (ii) If yes, what are their local names? (iii) What do they look like (carapace colour, hard or softshell, size of the animal, aquatic or terrestrial)? (iv) Which morphological characteristics do you use to determine the different species e.g. hinged (for *Cuora* spp.), serration, size of the head, length of the tail (for *Platysternon megacephalum*)? (v) In what habitats are the various species of turtle found?

After completing the interview, we showed interviewees ID photos of chelonian species to cross-check their description and to ensure uniformity in the descriptions used in their answers. In order to gather further evidence of the various chelonian species found in the area, we also examined any specimens they had in their houses.

In order to evaluate whether the sample size of interviewees was sufficient to obtain an accurate assessment of the total number of species of the two study areas, we applied a saturation curve methodology, calculating the 95 % confidence intervals by 9,999 random bootstraps (Hammer, 2012). Bootstrapping is a re-sampling statistical methodology that uses random sampling with replacement (e.g. mimicking the sampling process) to assign measures of accuracy such as bias, variance, confidence intervals, prediction error, to sample estimates (e.g. Varian, 2005).

RESULTS

Overall, we obtained reliable data on turtle occurrences from 98 interviewees in Lang Son and 138 interviewees in Lao Cai. In Lang Son, a total of ten turtle species were clearly described by interviewees, with the greater number of positive

Table 1. A list of all the chelonian species that have been accurately described during interview surveys in northern Vietnam with the number (and percentage) of interviewees describing each given species, and the districts where they were described

Turtle species	IUCN (2022) red list status	No. and (%) of interviews in which the species was mentioned	Locations (Districts)
Lang Son & Bac Giang province (98 interviews)			
Cuora mouhotii	EN	40 (40.8)	Dinh Lap, Loc Binh, Chi Lang, Huu Lung, Bac Son, Binh Gia, Van lang, Cao Loc
Cuora trifasciata	CR	6 (6.1)	Huu Lung
Cuora galbinifrons	CR	14 (14.3)	Loc Binh, Dinh Lap, Binh Gia, Bac Son, Chi Lang
Mauremys mutica	CR	12 (12.3)	Dinh Lap, Son Dong (Bac Giang province), Luc Ngan (Bac Giang province), Huu Lung, Bac Son, Binh Gia
Geoemyda spengleri	EN	12 (12.3)	Mau Son, Cao Loc
Pelodiscus sinensis	VU	44 (44.9)	Dinh Lap, Loc Binh, Chi Lang, Bac Son, Binh Gia, Lang Son city, Van Quan, Van Lang
Palea steindachneri	CR	12 (12.3)	Dinh lap, Loc Binh, Chi Lang, Bac Son
Cyclemys oldhamii	EN	3 (3.1)	Chi Lang, Bac Son, Binh Gia
Sacalia quadriocellata	CR	13 (13.3)	Dinh lap, Loc Binh, Chi Lang, Bac Son
Indotestudo elongata	CR	8 (8.2)	Dinh Lap, Loc Binh
Lao Cai province (138 interviews)			
Cuora mouhotii	EN	55 (39.9)	Bat Xat, Van Ban, Bac Ha, Bao Yen, Bao Thang, Sa Pa
Cuora galbinifrons	CR	20 (14.5)	Bat Xat, Van Ban, Bac Ha
Geoemyda spengleri	EN	10 (7.3)	Bac Ha
Cyclemys oldhamii	EN	5 (3.6)	Bat Xat, Bac Ha, Bao Yen
Sacalia quadriocellata	CR	12 (8.7)	Van Ban, Bat Xat, Bac Ha, Sa Pa
Pelochelys cantorii	CR	4 (2.9)	Bao Yen, Bao Thang
Rafetus swinhoei	CR	4 (2.9)	Bat Xat
Pelodiscus sinensis	VU	73 (52.9)	Bat Xat, Lao Cai city, Bao Thang, Bao Yen, Bac Ha, Sapa, Van Ban
Palea steindachneri	CR	23 (16.7)	Bat Xat, Van ban, Sapa, Bac Ha
Manouria impressa	EN	20 (14.5)	Sapa, Van Ban, Bac Ha
Platysternon megacephalum	CR	32 (23.2)	Sapa, Muong Khuong, Van Ban, Bat Xat

descriptions for *Cuora mouhotii* and *Pelodiscus sinensis* and the fewest accurate descriptions for *Cyclemys oldhamii* and *Cuora trifasciata* (Table 1). In Lao Cai, 11 turtle species were clearly described by interviewees, with *C. mouhotii* and *P. sinensis* being the most frequently mentioned and three, *C. oldhamii*, *Pelochelys cantorii* and *Rafetus swinhoei*, being the least frequently mentioned (Table 1). All the turtle species found in the two study areas are threatened according to IUCN (2022).

Many interviewees from both study areas concurred in saying that ponds within the mountain forests were the most likely habitats to encounter turtles. A typical example of this type of habitat is presented in Figure 2.

Interestingly, in Lao Cai province, different interviewees mentioned that very large softshell turtles (locally called "Giải mép vàng" that means "yellow mouth corner" clearly referring to *Rafetus swinhoei*) were caught and consumed by local people in Bat Xat district. These turtles were 60–70 kg, and were caught in a muddy swamp right next to the Red river in the late 1970s. Local traders in Bao Yen and Bao Thang districts claimed to have bought two individuals of "Đấm Đấm" (meaning "punching softshell turtle", thus

Figure 2. Typical pond habitat where turtles are captured by local hunters in the study areas

Table 2. Synopsis of the chelonians that were observed directly during interview surveys in northern Vietnam, including the geographic coordinates of the various observations

Species name	No. observed/ total no.	Observation types	District	Lat.	Long.
Lao Cai province					
Platysternon megacephalum	2/30	The owner claimed to keep 30 individuals in his pond. He caught these individuals in forest streams near his village	Bat Sat	22.62581	103.7708
Platysternon megacephalum	5	Observed in Hoang Lien rescue center	Sapa	22.34088	103.8221
Platysternon megacephalum	1	Observed in market where it was offered for sale	Muong Khuong	22.66334	104.1213
Manouria impressa	2	Observed in Hoang Lien rescue center	Sapa	22.34088	103.8221
Geoemyda spengleri	1	Observed in Bac Ha town and kept as a pet	Bac Ha	22.48781	104.327
Pelodiscus sinensis	12/100	Observed with a local hunter, and kept in captivity. He claimed to have about 100 individuals in his pond, which were all caught by himself in water bodies around his village	Bao Ha	22.15401	104.3859
Lang Song province					
Geoemyda spengleri	5	Offered for sale	Mau Son	21.84003	106.8978
Cuora mouhotii	3	Observed with a local trader near Huu Lien Nature reserve	Huu Lung	21.69848	106.3759
Palea steindachneri	1/100	Observed in a Palea farm. The farmer claimed to have about 100 individuals in his farm, but they did not necessarily come from the surrounding area	Huu Lung	21.49686	106.4501

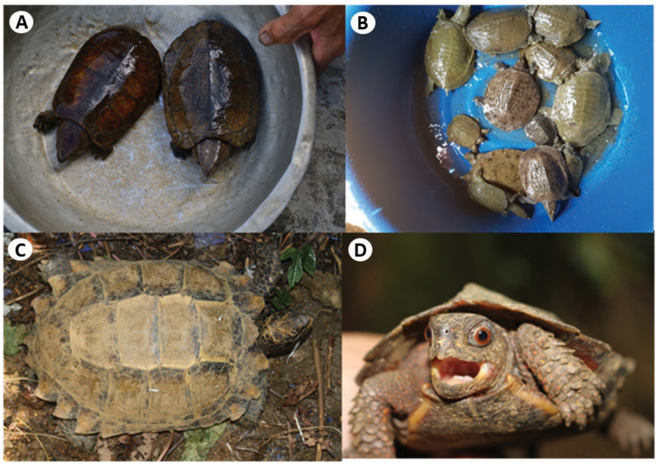


Figure 3. Some of the turtles actually observed in the study areas - **A.** *Platysternon megacephalum* in Bat Xat district, **B.** *Pelodiscus sinensis* in Bao Ha district, **C.** *Manouria impressa* in Sapa district, and **D.** *Geoemyda spengleri* in Bac Ha district

referring to the typical defensive behavior of *P. cantorii*) weighing 29 kg and 40 kg in 2009 and 2012 respectively. These two individuals were captured in the Chay river, and then sold to China across the border in Lao Cai city.

Direct observations were made of 32 turtles, these belonged to six different species (Table 2). *Pelodiscus sinensis*, *Platysternon megacephalum* and *Geoemyda spengleri* were being kept by several interviewees (Fig. 3). All these species had local names fitting exactly with those obtained in the interviews, thus confirming that our interviewees had a good and reliable knowledge of the turtle fauna of their areas. The saturation curves for the number of interviewees (= sample size) against the number of detected species revealed that the sample size was sufficient for obtaining a reliable estimate of the number of turtle species in the two study areas (Fig. 4).

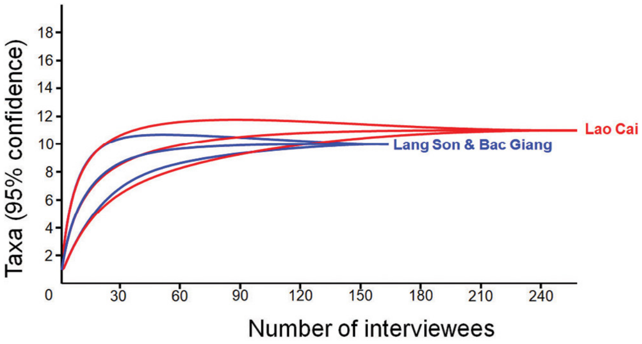


Figure 4. Saturation curves for the two study areas Lang Son (blue) and Lao Cai (red) showing that the number of interviewees was sufficient to adequately capture the turtle diversity. Curves are provided with 95 % confidence intervals, generated from 9,999 random bootstraps.

DISCUSSION

Our study detected a remarkable species richness of turtles in both areas (Lang Son/Bac Giang and Lao Cai), with respectively ten and 11 species reliably described by our interviewees. However, some of these species may now be extirpated. For example, *R. swinhoei* has been accurately described to occur in the area until the end of the 1970s, but there is no recent report showing its survival to the present day. *Rafetus swinhoei* is a gigantic species occurring in large river systems and associated ponds/lakes/marshlands (Le Duc et al., 2020; Van Pham et al., 2022). Given its large size it is much more likely to be known by hunters than the other smaller species and therefore the absence of any recent reports is likely to be a more accurate assessment. In addition, the other smaller sized species are linked to mountainous forest habitat and associated water bodies and streams (e.g. Ihlow et al., 2012; Wanchai et al., 2012; Ly et al., 2013; Pham et al., 2018; Seateun et al., 2019), and these habitats are often difficult to access and relatively remote, thus making an even greater difference in the reliability of absence reports.

The number of species detected in this study was slightly lower than that previously observed in Bac Giang province (n=12, see Pham Van et al., 2018). Results from this survey showed a higher number of species than in Thanh Hoa province (n=9) (Pham Van et al., 2020) but less than in Binh Dinh province in central Vietnam (n=15) (Nguyen et al., 2014). By comparison, in the ecologically similar Hainan province (China), a total of eight native species were recorded so far in villages and local markets (Shiping et al., 2006).

With regard to the habitat types in our study areas, of particular interest are the ponds that are interspersed within the forest patches (such as that shown in Fig. 2). According to the interviewees, these support a remarkable variety of species. Considering that all the species of the two study areas are listed as Threatened by IUCN (2022), careful management of these forest ponds is required by the competent authorities if further declines of these species are to be prevented. Although officially protected by law, these turtles are still frequently offered for sale even on social media (Pham Van et al., 2019), thus some effective protection from hunting/collection should be assured. We would urge the competent authorities to enhance the monitoring of the forested territory by rangers and wildlife soldiers.

ACKNOWLEDGEMENTS

We would like to thank the Turtle Sanctuary Conservation Center (Paris) and the Turtle Conservation Fund for their financial support (grant no. TCF 0643). We are also indebted to the Vietnam National University of Forestry that provided permissions for the survey team during the interview survey in both provinces. We would like to thank Mr. Tran Quang Thai (Lao Cai city) in supporting the logistic for the survey in Lao Cai province.

REFERENCES

BKHDT [Bộ kế hoạch đầu tư]. (2022). Cổng thông tin điện tử Bộ Kế hoạch và Đầu tư. https://www.mpi.gov.vn/Pages/tinhthanhchitiet.aspx?idTinhThanh=56. Accessed on 13 November 2022.

British Sociological Association (2017). Guidelines on Ethical Research. bsa_statement_of_ethical_practice.pdf. https://britsoc.co.uk/. Accessed on 11 Nov 2022.

Bui, D.T., Hoang, N.-D., Nguyen, H. & Tran, X.-L. (2019). Spatial prediction of shallow landslide using Bat algorithm optimized machine learning approach: A case study in Lang Son Province, Vietnam. *Advanced Engineering Informatics* 42: 100978.

Hammer, O. (2012). PAST - Paleontological Statistics, version 2.17. Reference Manual 1999-2012, PAST, Oslo.

Ihlow, F., Geissler, P., Sovath, S., Handschuh, M. & Böhme, W. (2012). Observations on the feeding ecology of *Indotestudo elongata* (Blyth, 1853) in the wild in Cambodia and Vietnam. *Herpetological Notes* 5: 5–7.

IUCN (2022). IUCN Red List of Threatened Species. www.iucnredlist.org. Accessed on 11 November 2022.

Le Duc, O., Van, T.P., Leprince, B., Bordes, C., Tuan, A.N., Benansio, J.S., Pacini, N., Luu, V.Q. & Luiselli, L. (2020). Fishers, dams, and the potential survival of the world's rarest turtle, *Rafetus swinhoei*, in two river basins in northern Vietnam. *Aquatic Conservation: Marine and Freshwater Ecosystems* 30: 1074–1087.

Lao Cai (2022). Geographical location, climate characteristics of Lao Cai province. https://www.laocai.gov.vn/dieu-kien-tu-nhien. Accessed on 11 November 2022.

Lunde, D.P., Son, N.T. & Musser, G.G. (2007). A survey of small mammals from Huu Lien Nature Reserve, Lang Son Province, Vietnam. *Mammal Study* 32: 155–168.

Ly, T., Hoang, H.D. & Stuart, B.L. (2013). Occurrence of the endangered Keeled Box Turtle, *Cuora mouhotii*, in southern Vietnam. *Chelonian Conservation and Biology* 12: 184–187.

Nguyen, T.Q., Duong, L.D. & Ngo, C.D. (2014). New records of turtles from Binh Dinh Province, Vietnam. *Herpetology Notes* 7: 737–744.

Pham Van, T., Le Duc, O., Leprince, B., Bodres, C., Zuklin, T., Ducotterd, C., Quang Lu, V., Lo Van, O., Nguyen Tam, A., Fa, J.E. & Luiselli, L. (2020). Unexpected high forest turtle diversity in hill forests in northern Vietnam. *Biodiversity and Conservation* 29: 4019–4033.

Pham Van, T., Luu, V.Q., Tien, T.V., Leprince, B., Khanh, L.T.T., & Luiselli, L. (2019). Longitudinal monitoring of turtle trade through Facebook in Vietnam. *The Herpetological Journal* 29: 48–56.

Pham Van, T., Vu, T., Dawson, J.E., Bui, T. & Leprince, B. (2018). Natural history observations on the Endangered turtle *Geoemyda spengleri* in Tay Yen Tu Nature Reserve (Vietnam), with notes on other sympatric species. *The Herpetological Bulletin* 146: 1–7.

Rhodin, A.G., Stanford, C.B., Van Dijk, P.P., Eisemberg, C., Luiselli, L., Mittermeier, R.A., Hudson, R., Horne, B.D., Goode, E.V. & Kuchling, G. (2018). Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation and Biology* 17: 135–161.

Seateun, S., Karraker, N.E., Stuart, B.L. & Aowphol, A. (2019). Population demography of Oldham's leaf turtle (*Cyclemys oldhamii*) in protected and disturbed habitats in Thailand. *PeerJ* 7: e7196.

Shiping, G., Jichao, W., Haitao, S., Riheng, S. & Rumei, X. (2006). Illegal trade and conservation requirements of freshwater turtles in Nanmao, Hainan Province, China. *Oryx* 40: 331–336.

Stanford, C.B., Rhodin, A.G.J., van Dijk, P.P., Horne, B.D., Blanck, T., Goode, E., Hudson, R., Mittermeier, R.A. et al. (2018). *Turtles in trouble - the world's 25+ most* *endangered tortoise and freshwater turtle*. Turtle Conservation Coalition, Lunenburg, USA. 79 pp.

Van Pham, T., Le Duc, O., Bordes, C., Leprince, B., Ducotterd, C., Zuklin, T., Quang, V.L., Dinh, D.H. & Luiselli, L. (2022). Female wanted for the world's rarest turtle: Prioritizing areas where *Rafetus swinhoei* may persist in the wild. *Oryx* 56: 396–403.

Varian, H.(2005). Bootstrap Tutorial. *Mathematica Journal*, 9: 768–775.

Wanchai, P., Stanford, C.B., Thirakhupt, K. & Thanhikorn, S. (2012). Home range of the impressed tortoise, *Manouria impressa* (Günther, 1882) at Phu Luang Wildlife Sanctuary, Loei Province, Thailand. *Tropical Natural History* 12: 165–174.

Accepted: 2 December 2022

The Herpetological Bulletin 164, 2023: 13–17
RESEARCH ARTICLE

https://doi.org/10.33256/hb164.1317

A study of individual differences in spatial use of captive sidewinder rattlesnakes *Crotalus cerastes*

JUANITA PARDO-SANCHEZ[1], ASHLEY TAYLOR[2], EMILY G. WEIGEL[3] & JOSEPH R. MENDELSON III[2,3*]

[1]Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, 48109, USA
[2]Zoo Atlanta, 800 Cherokee Ave SE, Atlanta, Georgia, 30315, USA
[3]School of Biological Sciences, Georgia Institute of Technology, 950 Atlantic Dr NW, Atlanta, Georgia, 30332, USA

[*]Corresponding author e-mail: jmendelson@zooatlanta.org

ABSTRACT – Many species of vipers are known to use ambush sites repeatedly, a phenomenon presumably informed by chemical cues from prey. Experimentally, we limited chemical cue availability and recorded site selection in five captive-born sidewinders *Crotalus cerastes* during their first active season. Snakes showed individual differences among the study subjects in spatial movement across the season and repeatedly reused specific sites in their enclosures, suggesting spatial memory and individual differences in choices of resting sites. Individual variability in spatial- and object-use and associated memories suggests attention to such should be considered in developing husbandry routines for these captive individuals.

INTRODUCTION

Multiple studies using different methodologies have demonstrated spatial cognition abilities in a variety of species of lizards, turtles and crocodiles, yet there are few studies on snakes (Wilkinson & Huber, 2012; Burghardt, 2013). Studies of spatial cognition are often conducted using active maze-based protocols (Healy & Jozet-Alves, 2010), but such scenarios are likely to be unrealistic and arguably irrelevant for stationary ambush-predators, such as viperid snakes. Nevertheless, spatial memories have been found to be somewhat similar between ambush-predator and actively foraging lizard species (Day et al., 1999). Viperid snakes are classic ambush predators, and multiple viper species often re-visit specific ambush sites (Greene, 1986; Secor, 1994; Sasa et al., 2009; Reinert et al., 2011). Chemical cues from prey appear to play an important role in selection of ambush sites by snake ambush predators (Clark, 2004), but it is unclear how snakes remember and re-locate previously used sites across complex landscapes and sometimes over relatively great distances. As chemical cues are strongly relied upon in other aspects of feeding in vipers, such as post-strike prey tracking behaviour (Chiszar et al., 1983; Clark, 2006; Teshera & Clark, 2021), chemical cues may be used to identify both novel and familiar ambush sites. Positive reinforcement from prior feeding success may result in spatial memory of the sites of previous success (Clark, 2006), but we are unaware of any explicit tests of such a hypothesis in snakes.

In this study, we tracked the positions of captive neonate sidewinder rattlesnakes *Crotalus cerastes* daily for their first active season, prior to onset of brumation, to describe and compare their individual movements and use of space. Our design purposely excluded chemical cues from prey in the environment in order to examine spatial use in the situation where cues from prey would not influence site selection. In general, our study was designed as a complement to the work of Clark et al. (2016) who emphasised the challenges of studying spatial patterns in wild juvenile snakes. We also focused on intraspecific variation in the form of individual differences in behaviours, which in non-avian reptiles has mostly been in the form of anecdotal observations and observations of anti-predator behaviours (Waters et al., 2017).

MATERIALS & METHODS

Five snakes were born to one female on 24 May 2017 and left with the attendant dam (sensu Greene et al., 2002) until their first ecdysis. By 7 June 2017 all snakes had experienced their first ecdysis and on this date all were placed in identical enclosures in a single-rack unit in an off-exhibit area at Zoo Atlanta. Enclosures were 60 x 30 cm in size with opaque walls, except for clear acrylic doors at one end (Fig. 1). Daytime temperatures were held between approximately 25–32 °C. Lights (compact fluorescent light bulbs within Zoo Med Naturalistic Terrarium Hoods) in the enclosure were on a timer to mimic natural light cycles and there was a window in the room for natural light. Temperatures were maintained by controlling ambient room temperatures, intentionally equalising any possible heat gradients across the enclosure; discrete sources of heat (e.g. heat lamp, heat tape) were not used. Clark et al. (2016) reported typical daily patterns of movement in *C. cerastes* involving refuge in burrows during the heat of the day and emergence to adopt an ambush posture during much of the evening. In the absence of both temperature gradients and potentially lethal extremes, our study is intended to represent a more simplified and controlled environment than is possible when studying wild snakes. Enclosures were fitted out identically with a 5 x 10 grid pattern printed on paper for a substrate, with two

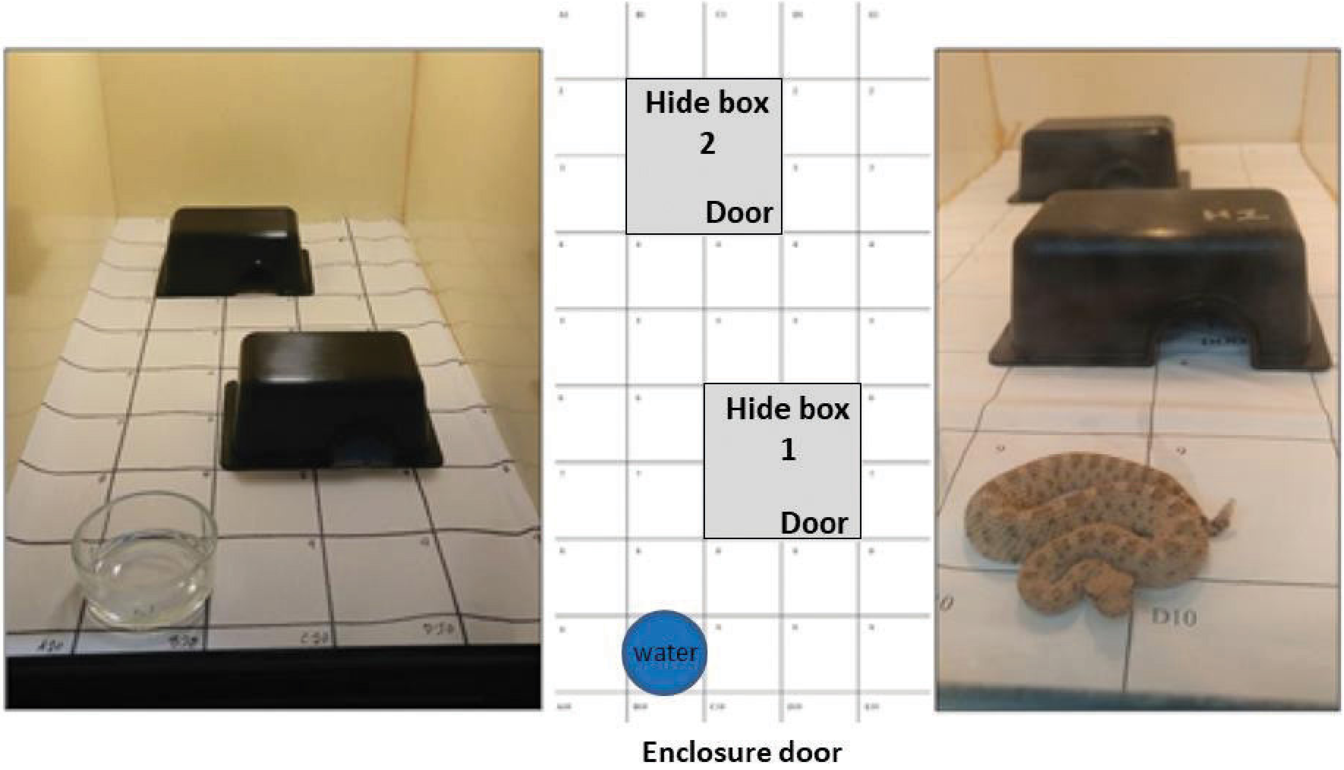


Figure 1. View of enclosure from the doorway, a schematic showing the grid-chart substrate and location of hide boxes and water bowl, and sidewinder in enclosure. Based on location of the snout, this snake is situated in quadrant C10.

identical hide boxes and a water bowl placed consistently in fixed positions through the study (Fig. 1). Each grid measured 6 cm x 6 cm.

Data collection began on 13 June 2017 (when snakes were first fed) and ran until 22 October 2017 (when cooling conditions were applied to the room for brumation). The position of each snake in its enclosure was recorded once per day at approximately (within 1 hour, typically 30 min of) 11:00 h. To standardise our data collection, we chose to determine each grid location by using the position of the tip of the snout, regardless of the direction in which it was oriented. Unless actively moving (a rare event; recorded as "no data"), snakes typically were in a tightly coiled position. The matching hide boxes covered four grids each but had a single entrance and were treated as single units of space (e.g. in Hide box 2). Snakes that were not visible (e.g. deep inside a hide box but could not ascertain which box, or under the grid paper) were scored as "not visible." Instances of "no data" or "not visible" were not included in analyses (3–6 % of observations). Percentage of time in each grid were calculated for each individual and utilised to create heatmaps in R using the lattice package (R Core Team, 2019; Sarkar, 2008).

Feedings generally occurred once every nine days; prey consisted of thawed young mice of appropriate size. Prey were offered to all snakes at the same times and snakes were offered food regardless of their location in the enclosure. Nevertheless, not all feedings were at the same date across snakes in the study because, for example, on a given day an individual may refuse food because of their stage in a shedding cycle. Offering food directly to the snake's location regardless of location reduced the possibility of inadvertently training the snakes to prefer certain sites because food may function as positive reinforcement (response learning, sensu Ruprecht, 2018). Each feeding was subsequently given a number during data analysis. During ingestion, prey items inevitably contacted the paper substrate, so data was first taken on snake location, snakes were then fed, and substrates were changed (which included both replacement of the paper substrate and bleaching of the bottom of the enclosure) immediately after each feeding to remove prey cues; the same was done following defecation. After each substrate change, clean hide boxes and water bowls were replaced in their original positions relative to one another in the enclosure.

To quantify movement, we measured the displacement between each daily observation. Thus, we converted grid locations to numerical co-ordinates to obtain positions for any two consecutive days, denoting the previous position of the snake the day before (Point x_1, y_1) and its current location (Point x_2, y_2). We then calculated the displacement from the previous position during that day span using the formula:

$$\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

We also quantified how much each snake used its enclosure by calculating Plowman's modified spread-of-participation index for each snake (Plowman, 2003). This index provides a number from zero to one where numbers closer to one indicate that an organism is using specific areas of an enclosure preferentially.

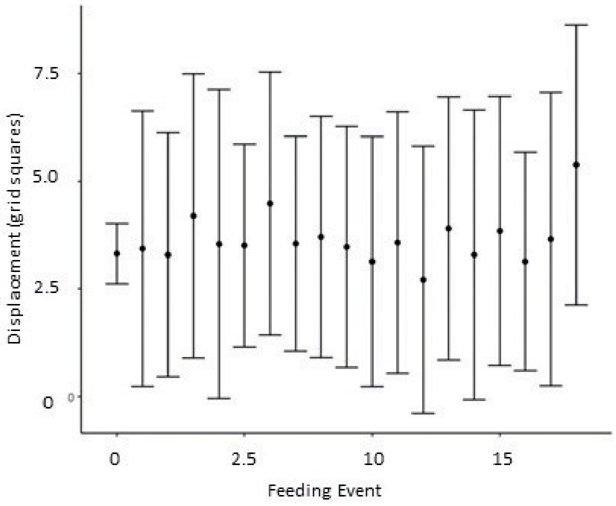


Figure 2. Mean displacement ± standard deviation by all neonate sidewinders after each feeding event. Feeding events began as soon as a snake had eaten and ended on the observation before the next feeding.

All statistical analyses were conducted using R software (R Core Team, 2019). Our base model used displacement between observations as our dependent variable and feeding number and age in days as fixed effects. We included snake ID as a random effect to control for individuals having multiple data points. We checked the appropriateness of our model using variance inflation factors of including age in days and feeding number in the same model (examined with the car package; Fox & Weisberg, 2019), Levene's tests of variance, normality (examined visually and though Kolmogorov-Smirnov analyses), and by graphing the residuals using the R package ggResidpanel (Goode & Rey, 2019). These analyses suggested a generalised linear mixed-effects model to be appropriate to deal with overdispersion due to zeros, which we constructed using the package glmmTMB (Brooks et al., 2017). The final model included days in age and feeding events as predictors because both independently describe age and experience.

RESULTS

Displacements (in cm) per snake varied markedly between each snake (mean ± standard deviation; snake 1: 24.0 ± 20.5; snake 2: 18.7 ± 16.3; snake 3: 21.5 ± 20.0; snake 4: 20.5 ± 14.8; snake 5: 23.2 ± 15.7). Time and feeding number were not predictive across snakes for displacement (GLMM; z = -1.079, p = 0.281 and 1.088 and p = 0.276, respectively). Snake movements generally were highly variable per snake over time. Most notable was a remarkable increase in variability in displacements after the first feeding event (Fig. 2). We also ran two additional models with the variables time and feeding number as one fixed effect in each, in order to confirm that neither fixed effect masked the other. Both model results support the findings of our main model. Nevertheless, there is considerable variation, or "noise" in our data.

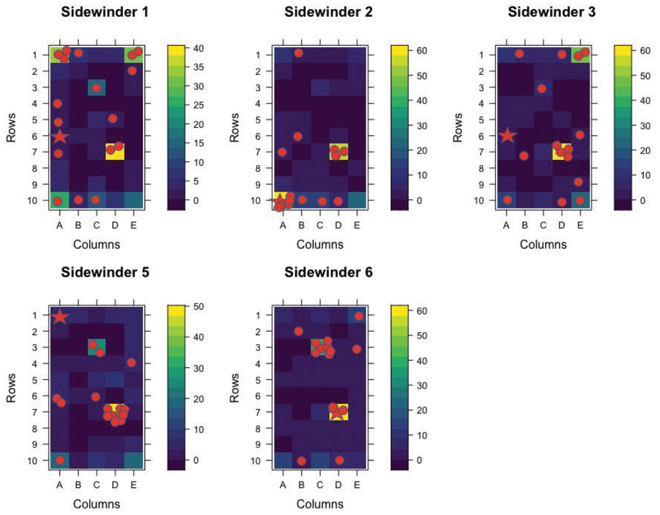


Figure 3. Heatmaps for all sidewinders representing where they spent most of their time. The entrances for the hide boxes were located at quads (3C) and (7D). Yellow represents grid where each snake was most frequently observed, followed by deepening shades of green, then deepening shades of blue.

Table 1. Modified spread-of-participation indices calculated for each snake

Snake	Modified spread-of-participation index
1	0.667
2	0.734
3	0.701
4	0.553
5	0.622

Heatmaps represent where each snake spent the most time throughout the experiment (Fig. 3). Modified spread-of-participation indices calculated for each snake indicate that snakes were using their enclosure spaces preferentially (Table 1). Most often, snakes were found in association with the hide boxes and corners of the enclosure, but much individual variation in specific locations was evident between snakes. For example, snakes 2 and 3 never used one of the hide boxes available to them, while all of the other snakes used both boxes at varying frequencies. Snakes generally spent approximately 20 % of their time in association with at least one hide box and roughly equivalent amounts of time at specific (but variable between snakes) corners of the enclosure.

DISCUSSION

After an initial period of consistent, moderate movement by all snakes at first feeding (Fig. 2), snakes were later highly variable in their displacements. We attribute this, at least in part, to a novelty effect - i.e. exploratory behaviours of individuals in new environments (Reinert & Rupert, 1999; Wolfe et al., 2018), as translocated snakes in the wild often move greater distances than do snakes in familiar surroundings (Roe et al., 2010), which here may have required a short acclimation period, and perhaps feeding, to become manifest. Regardless, the variability of movement across snakes suggests this may be reflective of individual variation rather than a more general ontogenetic, or perhaps seasonal, trend of spatial use. Bonnet et al. (1999) found that mortality was highest among post-hatchling snakes as they dispersed, and mortality decreased as older snakes became more sedentary. Howze et al. (2021) found that wild neonatal *C. horridus* increased their dispersal differences during the three weeks after leaving their birth site, but they had no information regarding their feeding. However, both Secor (1994) and Clark et al. (2016) found that juvenile and adult sidewinders had generally similar patterns of movements, suggesting that our observations may be better explained by the novel conditions upon introduction to the enclosure rather than to ontogenetic trends.

The spatial-use heatmaps (Fig. 3) and modified spread-of-participation indices (Table 1) indicate that snakes showed individual differences in spatial use and did selectively re-use specific sites in their enclosure. However, we noted no general pattern across all snakes (e.g. all snakes preferring one of the two hide boxes, or the furthest corner from the enclosure door, etc.); such observations would have indicated a potential systematic influence bearing upon all snakes in the room. Site selection for hunting or retreat is likely to depend on a variety of factors potentially including thermoregulation, hunger, memory of past feeding events, and perhaps substrate. Our focus here was on spatial use in a purposely simplified experimental environment. Decisions concerning site use by snakes are surely multi-modal including, for example, information such as chemical cues from potential prey items (Clark, 2004). However, our design indicates that snakes do form individual spatial preferences and repeated behaviours even in the absence of such cues.

The modified spread-of-participation indices and heatmaps suggest that the snakes preferred to spend time in specific hide boxes and corners; use of such spaces generally is consistent with the thigmophilic tendencies of vipers (JRM pers. obs.). The lack of similar patterns among the five snakes is minimally an important demonstration of variability, or individual differences, that may be indicative of personality (sensu de Vere, 2017) in these snakes. Compared to other groups of vertebrates (e.g. felid mammals; Gartner & Weiss, 2013), personality has been poorly investigated in the non-avian reptiles; as is the case for most aspects of cognition (De Meester & Baeckens, 2021). Repeated individual differences in behaviours indicate awareness of objects and stimuli in the environment and making cognitive decisions with respect to them; this qualifies as the sentience discussed by Learmonth (2019). Waters et al. (2017) reviewed literature related to personality, much of which was anecdotal or specific to predatory and anti-predatory behaviours. However, they noted considerable evidence for individual differences in a diversity or other behaviours, such as boldness or exploratory tendencies, in lizards. As such, our results contribute to their encouragement of more studies in other non-avian reptiles.

Our evidence of individual differences and choice in these snakes suggests that their individual welfare may be influenced by factors such as object and spatial recognition. It may be appropriate to incorporate simple protocols into husbandry routines, such as returning enclosure items to their original positions after a cleaning event, for example.

Our small study suggests that future researchers should take into account the growth of individual snakes during the study period when designing their enclosures and grid patterns. By the end of our study, the snakes occupied multiple grids when at rest; however, this should not affect our data as they were scored based only on position of the tip of the snout. Nevertheless, future studies should implement larger grids as the small size of our grids created certain levels of non-independence among grids; a minor repositioning of the snake would place the tip of the snout in a different grid.

ACKNOWLEDGEMENTS

We thank the staff of the Zoo Atlanta Herpetology for assistance in collecting raw data; Jay Diffendorfer and Carl Anthony provided early advice regarding study design. Marieke Gartner and Chris Michaels provided useful input on the draft manuscript. Hannah Dallas participated in the original study design and early data collections, supported by funding from a USDA/NIFA/ELI grant (#11903419, to J. Leavey, Georgia Tech): Bee-INSPIRED: INtegrating Service Projects Into REsearch and Design. Chris Michaels and anonymous reviewers provided helpful advice on the final manuscript. This project was approved by the Scientific Review Committee of Zoo Atlanta.

REFERENCES

Bonnet, X, Naulleau, G. & Shine, R. (1999). The dangers of leaving home: dispersal and mortality in snakes. *Biological Conservation* 89: 39–50.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M. & Bolker, B.M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9: 378–400.

Burghardt, G.M. (2013). Environmental enrichment and cognitive complexity in reptiles and amphibians: concepts, review, and implications for captive populations. *Applied Animal Behavioral Science* 147: 286–298.

Chiszar, D., Radcliffe C.W., Scudder K.M. & Duvall, D. (1983). Strike-induced chemosensory searching by rattlesnakes: the role of envenomation-related chemical cues in the post-strike environment. In *Chemical Signals in Vertebrates* 3, 1–24 pp. Müller-Schwartze, D. & Silverstein, D.R.M. (Eds.). New York: Springer Publishing.

Clark, R.W. (2004). Timber rattlesnakes (*Crotalus horridus*) use chemical cues to select ambush sites. *Journal of Chemical Ecology* 30: 607–617.

Clark, R.W. (2006). Post-strike behavior of timber rattlesnakes (*Crotalus horridus*) during natural predation events. *Ethology* 112: 1089–1094.

Clark, R.W., Dorr, S.W., Whitford, M.D., Freymiller, G.A. & Putnam, B.J. (2016). Activity cycles and foraging behaviors of free-ranging sidewinder rattlesnakes (*Crotalus cerastes*): the ontogeny of hunting in a precocial vertebrate. *Zoology* 119: 196–206.

Day, L.B., Crews, D. & Wilczynski, W. (1999). Spatial and reversal learning in congeneric lizards with different foraging strategies. *Animal Behavior* 57: 393–407.

De Meester, G. & Baeckens, S. (2021). Reinstating reptiles: from clueless creatures to esteemed models of cognitive biology. *Behaviour* 158: 1057–1076.

De Vere, A.J. (2017). Personality in animals. In *Encyclopedia of Animal Cognition and Behavior*. Vonk, J. & Shackelford, T. (Eds.). New York: Springer Publishing. https://doi.org/10.1007/978-3-319-47829-6_984-1.

Fox, J. & Weisberg, S. (2019). *An {R} Companion to Applied Regression* (Third). Los Angeles: Sage Publishers.

Gartner, M.C. & Weiss, A. (2013). Personality in felids: a review. *Applied Animal Behavioral Science* 144: 1–13.

Goode, K. & Rey, K. (2019). ggResidpanel: Panels and Interactive Versions of Diagnostic Plots using ‘ggplot2’. R package version 0.3.0. https://CRAN.R-project.org/package=ggResidpanel.

Greene, H.W. (1986). Natural history and evolutionary biology. In *Predator–Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates,* 99–108 pp. Feder, M.E. & Lauder, G.V. (Eds.). Chicago: University of Chicago Press.

Greene, H.W., May P.G, Hardy Sr., D.L., Sciturro, J.M. & Farrell, T.M. (2002). Parental behavior by vipers. *Biology of the Vipers,* 179–205 pp. Schuett, G.W., Höggren, M., Douglas, M.E. & Greene, H.W. (Eds.). Eagle Mountain: Eagle Mountain Publishing.

Healy, S.D. & Jozet-Alves, C. (2010). Spatial memory. In *Encylopedia of Animal Behavior*, Vol. 2, 304–2307 pp. Breed, D.M. & Moore, J. (Eds.). Amsterdam: Elsevier Science.

Howze, J.M., Stohlgren, K.M., Schlimm, E.M. & Smith, L.L. (2012). Dispersal of neonate timber rattlesnakes (*Crotalus horridus*) in the southeastern coastal plain. *Journal of Herpetology* 46: 417–422.

Learmonth, M.J. (2019). The matter of non-avian reptile sentience, and why it “matters” to them: A conceptual, ethical and scientific review. *Animals* 9: 821.

Plowman, A.B. (2003). A note on a modification of the spread of participation index allowing for unequal zones. *Applied Animal Behavior Science* 83: 331–336.

R Core Team (2019). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Reinert, H.K., MacGregor, G.A., Esch, M., Bushar, L.M. & Zappalorti, R.T. (2011). Foraging ecology of timber rattlesnakes, *Crotalus horridus*. *Copeia* 2011: 430–442.

Reinert, H.K. & Rupert Jr, R.R. (1999). Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. *Journal of Herpetology* 33: 45–61.

Roe, J.H., Frank, M.R., Gibson, S.E., Attum, O. & Kingsbury, B.A. (2010). No place like home: an experimental comparison of reintroduction strategies using snakes. *Journal of Applied Ecology* 47: 1253–1261.

Ruprecht, C. (2018). Place versus response learning. In *Encyclopedia of Animal Cognition and Behavior*. Vonk, J. & Shackelford, T. (Eds.). New York, USA. Springer Publishing. doi.org/10.1007/978-3-319-47829-6_743-1.

Sarkar, D. (2008). Lattice: Multivariate Data Visualization with R. Springer. New York: Springer Science & Business Media.

Sasa, M., Wasko, D.K. & Lamar, W.W. (2009). Natural history of the terciopelo *Bothrops asper* (Serpentes: Viperidae) in Costa Rica. *Toxicon* 54: 904–922.

Secor, S.M. (1994). Ecological significance of movements and activity range for the sidewinder, *Crotalus cerastes*. *Copeia* 1994: 631–645.

Teshera, M.S. & Clark, R.W. (2021). Strike-induced chemosensory searching in reptiles: a review. *Herpetological Monographs* 35: 28–52.

Waters, R.M., Bowers, B.B. & Burghardt, G.M. (2017). Personality and individuality in reptile behavior. In *Personality in nonhuman animals.* Vonk, J., Weiss, A. & Kuczaj, S.A. (Eds.). New York: Springer Publishing.

Wilkinson, A. & Huber, L. (2012). Cold-blooded cognition: reptilian cognitive abilities. In *The Oxford Handbook of Comparative Evolutionary Psychology*, 129–143 pp. Shackelford, T.K. & Vonk, J. (Eds.). Oxford: Oxford University Press.

Wolfe, A.K., Fleming, P.A. & Bateman, P.W. (2018). Impacts of translocation on a large urban-adapted venomous snake. *Wildlife Research* 45: 316–324.

Accepted: 15 December 2022

The Herpetological Bulletin 164, 2023: 18–23
SHORT NOTE

https://doi.org/10.33256/hb164.1823

The distribution of amphibians in the Matese Massif of southern Italy

LINO CIRUCCI[1], LORENZO DE LISIO[2], MARCO CARAFA[3], ANGELA PELOSI[4], GIOVANNI CAPOBIANCO[5], PASQUALE BUONPANE[6] & MASSIMO CAPULA[7*]

[1]WWF OA Molise, Via Eugenio Cirese (Terzo Spazio), 86100, Campobasso, Italy
[2]Via F. Pietrunto 9, 86100, Campobasso, Italy
[3]Societas Herpetologica Italica, sez. Abruzzo-Molise, Via Valle Yuva 21/2, 67039, Introdacqua (Aquila), Italy
[4]Viale di Valle Aurelia 93, 00167, Roma, Italy
[5]ARDEA, Via Ventilabro 6, 80126, Napoli, Italy
[6]Via Largo Tiratoie 10, 81016, Piedimonte Matese (Caserta), Italy
[7]Museo Civico di Zoologia, Via U. Adrovandi 18, 00197, Roma, Italy

[*]Corresponding author e-mail: massimo_capula@yahoo.it

The Matese Massif is a wide mountainous area that is located between the Campania and Molise regions of southern Italy. The only study of the local herpetological fauna, although relating specifically to the Campania side of the massif, is that of Guarino et al. (2002) in the protected area of the Matese Regional Park (Province of Caserta), where a total of six amphibian species were found. The present work presents the results of original investigations undertaken from 2008 to 2019 on the presence and distribution of the amphibian species in the Matese Massif, which we have reported alongside data taken from previous studies. The purpose of the research was to acquire better information on the presence and distribution of amphibians in support of the conservation of local populations.

MATERIALS & METHODS

Study area

The study area corresponds entirely to the Matese Massif (Fig. 1), including both the Campania side and the Molise side. The massif spreads over an area of about 50,000 hectares and extends, with an east/west orientation, between the Volturno River and the upper valley of the Tammaro River, and culminates with the Monte Miletto (2050 m a.s.l.). The massif is characterised by a predominance of limestone soils and karst phenomena. There are some lake basins, among which Lake Matese stands out, lying in a karst depression about 8 km long and about 2 km wide, at the foot of the Monte Miletto. Some of the main protected natural reserves of the Molise and Campania regions fall within the Matese area. On the Molise side there are the WWF Guardiaregia-Campochiaro Regional Nature Reserve, and the Callora Torrent Nature Reserve, both falling within a special conservation zone (ZSC IT 7222287) called ‘La Gallinola-Monte Miletto-Monti del Matese’. On the Campania side there is the Matese Regional Park, which extends for 33,327 ha. The Campania side is administratively assigned to the provinces of Caserta and Benevento, while the Molise side is assigned to the provinces of Isernia and Campobasso. On both sides of the Matese Massif there is a mosaic of habitats: plant formations typical of the Mediterranean scrub, semi-desert stony ground on the south-western side, wood formations consisting of beech forests, calcicolous and rocky grassy formations of the sunny slopes, up to the mountain and alpine calcareous scree.

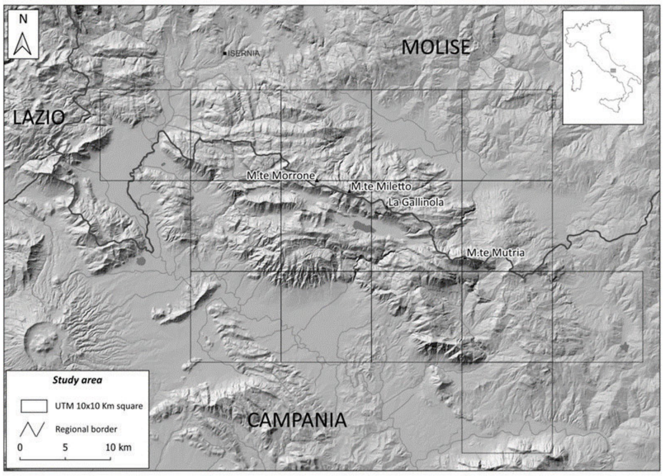


Figure 1. The survey grid of 15 UTM 10 x 10 km squares superimposed on the Matese Massif. At the top right there is a map of the Italian peninsula showing the position of the study area.

On both sides of the massif the guide species for the phytoclimatic type are: *Fagus sylvatica*, *Taxus baccata*, *Ilex aequilifolium*, *Acer lobelii*, *Ostrya carpinifolia*, *Sorbus aria*, *Arum lucanum*, *Linum capitatum* and *Geranium cinereum*.

Survey methodology

The current study is based mostly on records from field surveys performed over a period of 12 years (2008–2019) although also included were records from previous study obtained by screening the scientific literature and records from the herpetological database “Progetto Atlante degli Anfibi e dei Rettili del Molise” (Capula et al., 2008; 2010; 2018).

Data on amphibians in the field were collected by visual observation, and no individual was caught during the

Table 1. Amphibian species occurring in the study area, number of UTM 10 x 10 km squares in which the species were found, their altitudinal range, and number of records. Records marked p.s. = previous study, c.s. = current study, all other records come from both sources.

Species	No. of UTM squares	% study area occupied by the species	Altitudinal range (min–max m a.s.l.)	No. of records
Salamandra salamandra (Linnaeus, 1758)	4	27	864–1259	41
Salamandrina perspicillata (Savi, 1821)	2	13	456–1128	76
Salamandrina terdigitata (Bonnaterre, 1789) (p.s.)	2	13	403–832	5
Salamandrina sp. (c.s.)	6	40	427–959	18
Lissotriton italicus (Peracca, 1898)	5	33	340-–1400	50
Lissotriton vulgaris (Linnaeus, 1758)	4	27	105–1400	16
Triturus carnifex (Laurenti, 1768)	8	53	790–1330	27
Bombina pachypus (Bonaparte, 1838)	5	33	552–1269	49
Bufo bufo (Linnaeus, 1758)	10	67	140–1459	47
Bufotes balearicus (Boettger, 1880)	1	7	172	1
Hyla intermedia Boulenger, 1882	4	27	187–1076	11
Pelophylax bergeri (Günther, in Engelmann, Fritzsche, Günther & Obst, 1986) / *P.* kl. *hispanicus* (Bonaparte, 1839)	9	60	94–1330	44
Rana dalmatina Fitzinger, in Bonaparte, 1838	3	20	450–1105	20
Rana italica Dubois, 1987	10	67	235–1341	105

research. Surveys were mainly undertaken during spring and summer months (March–October), according to the methodologies reported by Maio et al. (2000). These surveys each lasted one day (for a total of 103 research days) and were undertaken mainly during daylight hours by travelling along predetermined transects that were based on cartographic and vegetational characteristics. During field investigations, data relating to distribution, frequency and type of activity (reproduction, feeding) of the observed species were collected, and the macro-environmental characteristics of the observation sites were recorded. Particular attention was paid to the collection of data relating to the species included in the Habitat Directive 92/43/EEC, and to the threatened species included in the Italian and European Community red lists (Rondinini et al., 2013; IUCN, 2022). During the 12 years of monitoring, we covered 93.3 % of the study area, i.e. 14 out of the 15 10 x 10 km grid cells of the Matese Massif (Fig. 1).

The records were collected from 26 municipalities that are listed in Table S1 (see Supplementary Material). Both the original records from the field and those from previous study were screened, validated and entered as records in a database prepared using Microsoft EXCEL software. Each record was geo-referenced using WGS 84/UTM33N. The database was projected to the same co-ordinate system (WGS84) and transformed into a 10 × 10 km grid. We aggregated the occurrence records to the Universal Transverse Mercator (UTM) grid system at a spatial resolution of 100 km^2 (UTM 10 × 10 km). We mapped the species occurrence by assigning each species to the corresponding UTM 10 × 10 km square following Sillero et al. (2005).

Species were identified according to their morphology and coloration following Lanza et al. (2007). The nomenclature adopted for the species in this paper is that proposed by Di Nicola et al. (2019).

RESULTS & DISCUSSION

Overall, a total of 510 records were collected, 170 from field investigations and 340 from an analysis of the literature (Table 1). This gave a total of 14 autochthonous species of amphibians (Caudata n=6, Anura n=8) that are listed in Table 1. The 14 species listed correspond to the total number of amphibian species occurring in much wider regions of southern Italy, i.e. Campania (13.670 km^2; Guarino et al., 2012) and Molise (4.438 km^2; Capula et al., 2018). To date, the Matese Massif has the greatest number of recorded amphibian species of any mountainous area of central or southern Italy (Table 2). The large number of species probably reflects the mosaic of natural habitats, the optimal biological and chemical conditions of local waterbodies, and the absence of threats such as water pollution.

As to the species richness, the analysis shows that in five out of 15 UTM 10 x 10 km squares, that is one third of the area investigated, the number of recorded species ranges from 7 to 11, and in two UTM squares (13.3 % of the investigated area) this number ranges from 4 to 6 (Fig. 2). In one UTM 10 x 10 km square no amphibian species were recorded. Two localities (Guardiaregia and Campitello di Sepino) can be considered as very important hotspots for amphibians within the study area as well as in southern Italy. In Guardiaregia, nine species have been recorded - *Salamandra salamandra*, *Salamandrina perspicillata*, *Lissotriton italicus*, *Bombina pachypus*, *Bufo bufo*, *Hyla intermedia*, *Rana italica* and *Pelophylax bergeri* / *P.* kl. *hispanicus*. In Campitello di Sepino, six species were found - *Salamandra salamandra*, *Salamandrina perspicillata*, *L. italicus*, *Lissotriton vulgaris*, *Triturus carnifex*, and *R. italica*.

The distribution maps of the species recorded in the study area can be found in the Supplementary Material (Figs. S1

Table 2. Number of amphibian species recorded in some mountainous areas of central and southern Italy

Mountainous area	No. of species	Reference
Sibillini Mounts (Sibillini National Park, Central Italy)	13	Fiacchini, 2013
Majella Massif (Majella National Park, Central Italy)	13	Scalera et al., 2006
Simbruini Mountains (Central Italy)	9	Crucitti et al., 2010
Prenestini Mountains (Central Italy)	10	Bologna et al., 2001
Lepini Mountains (Central Italy)	11	Corsetti & Capula, 1992
Lucretili Mountains (Central Italy)	9	Carpaneto, 2000
Aurunci Mounts (Central Italy)	9	Romano et al., 2007
Ausoni Mountains (Central Italy)	8	Corsetti & Romano, 2007
Vesuvio (Vesuvius National Park, Southern Italy)	8	Maio et al., 2000
Alburni Massif (Southern Italy)	11	Caputo et al., 1985
Matese Massif (Southern Italy)	14	Current study

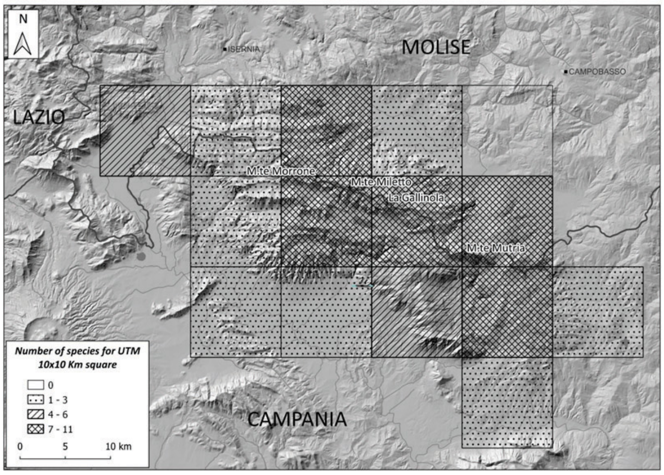


Figure 2. Number of amphibian species in the Matese Massif recorded for each of the UTM 10 x 10 km squares investigated

Figure 3. Adult *Salamandrina perspicillata* from the Molise side of the Matese Massif

– Fig. S14). The most widespread species in the 15 UTM 10 x 10 km squares of the study were *R. italica* (10 squares), *B. bufo* (10 squares), *P. bergeri* / *P.* kl. *hispanicus* (9 squares), and *T. carnifex* (8 squares) which were found in more than 67 % of the monitored localities (Table 1). Species with relatively narrow ranges were *R. dalmatina* (3 squares), *S. perspicillata* (2 squares), *S. terdigitata* (2 squares), and *Bufotes balearicus* (1 square). *Bombina pachypus*, though recorded in the past in a number of sites (> 40 literature records), now appears to be very rare and extremely localised. The current rarity of the latter species seems to be real and not due to lack of field investigations, since its range is currently shrinking fast throughout the Italian Peninsula (Bulgarini et al., 1998; Stagni et al., 2004; Rondini et al., 2013; Di Nicola et al., 2019) and it is considered as Endangered in the Red List of the Italian threatened species (Rondini et al., 2013). The altitudinal range for Caudata (6 species) was 105–1400 m a.s.l., and the Anura (8 species) was very similar at 94–1459 m a.s.l. (Table 1); *L. vulgaris* and *B. bufo* were respectively Caudata and Anura with the widest altitudinal ranges.The Matese Massif is extremely interesting from the herpetological perspective as it is the only Italian geographic area in which the two species of the genus *Salamandrina* (*S. perspicillata* and *S. terdigitata*) - which is strictly endemic to the Italian Peninsula - are sympatric. *Salamandrina perspicillata* is present on both sides of the Matese Massif, and would be the only *Salamandrina* species occurring in the Molise region according to Romano et al. (2009). *Salamandrina terdigitata* has been reported with certainty in some localities sited on the Campania side (province of Benevento, Romano et al., 2009)(Fig. S3). Extremely interesting are the populations of *S. perspicillata* occurring in some localities of the Molise side (see Romano et al., 2009 for the list of localities), as they are characterised by a genome in which alleles of *S. terdigitata* are present (Fig. 3). This could be the evidence of past hybridisation and introgression between the two species (Hauswaldt et al., 2011; Mattoccia et al., 2011). However, it should be noted that the taxonomic distinction between *S. perspicillata* and *S. terdigitata* is based on mitochondrial (Mattoccia et al., 2005; Nascetti et al., 2005) and nuclear analyses (Nascetti et al., 2005), while no morphological traits allow a clear and easy distinction between the two species in the field (Angelini et al., 2007). Although some small and hardly noticeable differences in size and coloration could allow a tentative distinction between adults of the two species (Romano et al., 2009), neither young individuals nor larvae can be identified exclusively on the basis of morphological characters. Since our observations were based on field investigations it did not allow the unambiguous taxonomic attribution to one or the other species. In the present contribution the original data

Table 3. Phenology of 11 amphibian species in the Matese Massif, * = adults; E = eggs; L= larvae. The months indicated are those in which there were field investigations.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Salamandra salamandra		*	*	*L	L			*
Salamandrina perspicillata	*	*	*E	*L	L			*
Lissotriton italicus	*	*E	*E	*L	L	L	L	
Lissotriton vulgaris	*	*	*E	*L	L	L		
Triturus carnifex	*	*	*E	*L	*L	L		
Bombina pachypus			*	*E	*L	*L	*L	
Bufo bufo	*E	*EL	*L	*L	L	L	*	*
Hyla intermedia	*	*E	*EL	*L	*L		*	
Pelophylax bergeri / P. kl. *hispanicus*	*	*E	*E	*L	*L	*L	*	*
Rana italica	*	*EL	*EL	*L	*L	*L	*	*

relating to specimens (adults, young, larvae) observed in the wild on the Campania side, where both species are known to occur, were treated as *Salamandrina* sp. (Table 1; Fig. S4).

As in other regions of central and southern Italy, green frogs are represented in the Matese Massif by a sinklepton, that is, by two closely related taxa from the genetic point of view, one of which is the parent species, *Pelophylax bergeri*, and the other is the hybridogenetic hybrid or klepton hybrid, *Pelophylax* kl. *hispanicus* (Dubois & Ohler, 1994; Günther & Plötner, 1994; Capula et al., 2007; Dubey & Dufresnes, 2017). The genetic makeup of the Italian hybridogenetic hybrids has recently been analysed and a new endemic lineage of eastern-Mediterranean origin as one parental ancestor of *P.* kl. *hispanicus* identified. Apparently, this ancestor "is nowadays extinct in the wild but its germline subsists through its hybridogenetic descendant (*P.* kl. *hispanicus*), which can thus be considered as a semi living fossil" (Dubey & Dufresnes, 2017). *Pelophylax bergeri* and *P.* kl. *hispanicus* are morphologically and chromatically very similar (Capula et al., 2007) and in Molise and Campania, as in the rest of central and southern Italy, they usually coexist in the same habitat (Capula et al., 2007; Di Nicola et al., 2019). As the two taxa can only be distinguished with certainty by genetic and/or molecular analyses, all records relating to the presence of green frogs in the study area were processed in the database as *P. bergeri* / *P.* kl. *hispanicus* (Table 1, Fig. S14).

The most commonly observed reproductive habitats by the three newt species (*L. italicus*, *L. vulgaris*, *T. carnifex*) and *B. pachypus* were drinking troughs and small ponds located in small valleys or pastures along the border of dry grassland and woods (Fig. 4). For reproduction *Salamandra salamandra*, *Salamandrina perspicillata*, *S. terdigitata* and *R. italica* mainly frequented streams and water springs located inside or at the edge of mixed oak woods and beech woods. *Rana dalmatina* was only found in localities characterised by riparian hygrophilous woods close to ponds and swamps. *B. bufo*, *H. intermedia* and the taxa of the genus *Pelophylax* were eurytopic species and reproduced in several wet habitats, i.e. swamps, ponds, streams, small lakes and drinking troughs, either in woods or pastures. According to the available records, the phenology of 11 amphibian species is summarised in Table 3.

Figure 4. Drinking trough in which adults and larvae of *Lissotriton italicus*, *Bufo bufo*, *Hyla intermedia* and *Pelophylax bergeri* / *P.* kl. *hispanicus* were observed

The observation of uncommon levels of species richness, with 7 to 11 species in certain UTM 10 x 10 km squares, suggests that the Matese Massif area is important for the conservation of amphibian species as well as their natural wet and freshwater habitats, and thus it should be continuously monitored and strictly protected by local authorities. Within this area intensive agriculture and pastoral activities and the increase in the extension of land used for agricultural purposes should be avoided as much as possible. It has been mentioned that drinking troughs are important for the reproductive biology of the three species of newts as well for *B. pachypus* and, occasionally, for other amphibian species (*B. bufo*, *H. intermedia*, *P. bergeri* / *P.* kl. *hispanicus*). These small water bodies are widespread, but they are under threat due to 1) periodic clearing and emptying by farmers and shepherds, and to 2) introduction of fish by humans, causing the rapid disappearance of eggs, larvae and adults of amphibians as well as the aquatic invertebrate fauna (Scoccianti, 2001; Peria et al., 2016). Streams and fresh water springs are extremely important for conservation of local populations of some species that tolerate only a narrow range of environmental conditions (*Salamandra salamandra*, *Salamandrina perspicillata*, *S. terdigitata* and *R. italica*) and they should be strictly protected from water pollution, drying up of springs, introduction of fish and alien species of vertebrates and invertebrates (Scoccianti, 2001; Capula & Contini, 2009; Peria et al., 2016). It should be noted that the species considered in the present study are monitored and strictly protected inside the WWF Guardiaregia-Campochiaro

Regional Nature Reserve, and the Callora Torrent Nature Reserve (Province of Campobasso, Molise side), and within the Matese Regional Park (Province of Caserta, Campania side). Moreover, all species of amphibians are formally protected by the regional law of Molise (Regional Law 26/1996), and some species (*S. perspicillata*, *S. terdigitata*, *L. italicus*, *T. carnifex*, *B. pachypus*, *R. dalmatina* and *R. italica*) are also included in the Appendices of the European Council Directive 92/43/EEC (Habitat Directive on the conservation of natural habitats and wild fauna and flora) as deserving high conservation priority.

ACKNOWLEDGEMENTS

We thank Arianna Ceccarelli, Roberto Colella, Andrea Corso, Egidio Di Zinno, Anna Loy, Michele Marinelli, Valeria Ramacciato and Antonio Romano for their valuable help in collecting data and helpful field co-operation. The "WWF Molise", the Riserva Naturale Regionale WWF Guardiaregia-Campochiaro, and the Parco Regionale del Matese are acknowledged for supporting our field research.

REFERENCES

Angelini, C., Vanni, S. & Vignoli, L. (2007). *Salamandrina terdigitata* (Bonnaterre, 1789), *Salamandrina perspicillata* (Savi, 1821). In *Fauna d'italia*. Vol XLII. Amphibia, 228–236 pp. Lanza, B., Andreone, F., Bologna, M.A., Corti, C. & Razzetti, E. (Eds.). Edizioni Calderini, Il Sole 24 ORE, Editoria specializzata S.r.l., Bologna.

Bologna, M.A., Capula, M. & Carpaneto, G.M. (2001). Gli anfibi e i rettili dei Monti Prenestini. In *Aspetti naturalistici dei Monti Prenestini*, 135–149 pp. Angelici, F.M. (Ed.). Associazione Naturalistica "Orchidea" - Genazzano (Roma), Regione Lazio, Assessorato Ambiente, Tipografia Nuova Stampa, Cave (Roma).

Bulgarini, F., Calvario, E., Fraticelli, F., Petretti F. & Sarrocco S. (Eds.) (1998). Libro Rosso degli Animali d'Italia – Vertebrati. WWF Italia, Roma, 210 pp.

Capula, M., Carafa, M., De Lisio, L. & Loy A. (2010). Il progetto atlante degli Anfibi e Rettili del Molise. In Atti VIII Congresso Nazionale Societas Herpetologica Italica, Chieti 22-26 Settembre 2010, 37–49 pp. Di Tizio, L., Di Cerbo, A.R., Di Francesco, N. & Cameli, A. (Eds). Ianieri Edizioni, Pescara.

Capula, M. & Contini, F.(2009). Le specie aliene di Rettili in Italia Le specie aliene di Anfibi in Italia. In *L'impatto delle specie aliene sugli ecosistemi: proposte di gestione*. Verso la strategia nazionale per labiodiversità. I contributi della conservazione ecoregionale. - Progetto Ministero Ambiente e della Tutela del Territorio e del Mare, 9–11 pp.,15–16 pp. WWF Italia Onlus. Ministero Ambiente e della Tutela del Territorio, Roma.

Capula, M., De Lisio, L., Cianfrani, C., Di Florio, M., Invasile, D., Ramacciato, V. & Loy, A. (2008). Recenti acquisizioni sull'erpetofauna del Molise In *Atti 1° Congresso Societas Herpetologica Italica Sezione Abruzzo "Antonio Bellini"* (Caramanico Terme, PE, 25–27 maggio 2007), 13–21 pp. Carafa, M., Di Francesco, N., Di Tizio, L. & Pellegrini, Mr. (Eds.). Talea Edizioni, Atessa, CH.

Capula, M., Di Tizio, L., De Lisio, L., Carafa, M. & Brugnola, L. (2018). *Anfibi e Rettili del Molise*. Atlante e guida. Ianieri Edizioni Srls, Pescara, 278 pp.

Capula, M., Sacchi, R. & Razzetti, E. (2007). *Pelophylax bergeri*, *Pelophylax hispanicus*. In *Fauna d'italia*. Vol XLII. Amphibia, 381–386 pp. Lanza, B., Andreone, F., Bologna, M.A., Corti, C. & Razzetti, E. (Eds.). Edizioni Calderini, Il Sole 24 ORE, Editoria specializzata S.r.l., Bologna,

Caputo, V., Kalby, M. & De Filippo, G. (1985). Gli Anfibi e i Rettili del Massiccio degli Alburni (Appennino Campano-Lucano). *Natura*, Milano 76: 94–104.

Carpaneto, G.M. (2000). Anfibi e rettili dei Monti Lucretili. In: *Monti Lucretili. Parco Regionale Naturale. Invito alla lettura del territorio*, 353–364 pp. De Angelis G. (Ed.). Parco Regionale Monti Lucretili, Consorizio di gestione – Comitato promotore. Grafica Salaria s.n.c., Monterotondo.

Corsetti, L. & Capula, M. (1992). The Amphibians and Reptiles of the Lepini Mountains (Latium, Central Italy): checklist and provisional atlas. *The Herpetological Bulletin* 39: 8–16.

Corsetti, L. & Romano, A. (2007). Amphibians of the Ausoni Mountains (Latium, Central Italy). *Acta Herpetologica* 2: 129–137.

Crucitti, P., Brocchieri, D., Emiliani, F., Malori, M., Pernice, S., Tringali, L. & Welby, C. (2010). Amphibians of the Simbruini Mountains (Latium, Central Italy). *Acta Herpetologica* 5: 91–101.

Di Ncola, M., Cavigioli, L., Luiselli, L. & Andreone, F. (2019). *Anfibi & Rettili d'Italia* Edizioni Belvedere, Latina, 568 pp.

Dubey, S. & Dufresnes, C. (2017). An extinct vertebrate preserved by its living hybridogenetic descendant. *Scientific Reports* 7(1): 12768. Doi: 10.1038/s41598-017-12942-y.

Dubois, A. & Ohler, A. (1994). Frogs of the subgenus *Pelophylax* (Amphibia, Anura, genus Rana): a catalogue of available and valid scientific names with comments on name-bearing types, complete synonymies, proposed common names, and maps showing all type localities. *Zoologica Poloniae* 39: 139–204.

Fiacchini, D. (2013). *Atlante degli Anfibi e dei Rettili del Parco Nazionale dei Monti Sibillini*. Parco Nazionale dei Monti Sibillini, Quaderni scientifico-divulgativi n. 16, Editrice GESP.

Guarino, F.M., Aprea, G., Caputo Barucchi, E., Maio, N., Odierna, G. & Picariello, O. (2012). *Atlante degli Anfibi e dei Rettili della Campania*. Massa Editore, Napoli, 344 pp.

Guarino, F.M., Odierna, G. & Maio, N. (2002). Gli Anfibi e i Rettili del Parco Regionale del Matese. In *IVertebrati ectotermi del Parco Regionale del Matese*, 9–23 pp. Odierna, G. & Guarino, F.M. (Eds.). Università Federico II Ed., Napoli.

Günther, R. & Plötner, J. (1994). Morphometric, enzymological and bioacoustic studies in Italian water frogs (Amphibia, Ranidae). *Zoologica Poloniae* 39: 387–415.

Hauswaldt, J.S., Angelini, C., Pollok, A. & Steinfartz, S. (2011). Hybridization of two ancient salamander lineages:

molecular evidence for endemic spectacled salamanders on the Apennine peninsula. *Journal of Zoology* 284: 248–256.

IUCN (2022). The IUCN Red List of Threatened Species. Version 2022-1. Accessed on 10 September 2022.

Lanza, B., Andreone, F., Bologna, M.A., Corti, C. & Razzetti, E. (2007). *Fauna d'italia*. Vol XLII. Amphibia. Edizioni Calderini, Il Sole 24 ORE, Editoria specializzata S.r.l., Bologna.

Maio, N., Guarino, F.M., D'Amora, G. & Picariello, O. (2000). L'erpetofauna del Parco Nazionale del Vesuvio. In *Elementi di biodiversità del Parco nazionale del Vesuvio* (Biodiversity in the Vesuvius national park), 139–169 pp. Picariello, O., Di Fusco, N. & Fraissinet, M. (Eds.). Ente Parco Nazionale del Vesuvio Ed., Napoli.

Mattoccia, M., Marta, S., Romano, A. & Sbordoni, V. (2011). Phylogeography of an Italian endemic salamander (genus *Salamandrina*): glacial refugia, postglacial expansions and secondary contact. *Biological Journal of the Linnean Society* 104: 903–922.

Mattoccia, M., Romano, A. & Sbordoni, V. (2005). Mitochondrial DNA sequence analysis of the spectacled salamander, *Salamandrina terdigitata* (Urodela: Salamandridae), supports the existence of two distinct species. *Zootaxa* 995: 1–19.

Nascetti, G., Zangari, F. & Canestrelli, D. (2005). The spectacled salamanders, *Salamandrina terdigitata* (Lacépède, 1788) and *S. perspicillata* (Savi, 1821): genetic differentiation and evolutionary history. *Rendiconti Lincei, Scienze Fisiche e Naturali Accademia dei Lincei* 16: 159–169.

Peria, E., Dell'Anna, L., Fattori, C., Mancinella, D. & Peroni, E. (2016). Programma Territorio e Biodiversità. Buone pratiche per la conservazione degli anfibi nell'agroecosistema. Edizioni ARP, Roma, 63 pp.

Romano, A., Mattoccia, M., Marta, S., Bogaerts, S., Pasmans, F. & Sbordoni, V. (2009). Distribution and morphological characterization of the endemic Italian salamanders *Salamandrina perspicillata* (Savi, 1821) and *S. terdigitata* (Bonnaterre, 1789) (Caudata: Salamandridae). *Italian Journal of Zoology* 76: 1–11.

Romano, A., Montinaro, G., Mattoccia, M. & Sbordoni, V. (2007). Amphibians of the Aurunci Mountains (Latium, Central Italy). Checklist and conservation guidelines. *Acta Herpetologica* 2: 17–25.

Rondinini, C., Battistoni, A., Peronace, V. & Teofili, C. (Eds.) (2013). *Lista Rossa IUCN dei Vertebrati Italiani*. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 56 pp.

Scalera, R., Venchi, A., Carafa, M., Pellegrini, M., Capula, M. & Bologna, M.A. (2006). Amphibians and reptiles of the Majella National Park (Central Italy). *Aldrovandia* 2: 31–47.

Scoccianti, C. (2001). *Amphibia: aspetti di ecologia della conservazione*. [Amphibia: Aspects of Conservation Ecology]. WWF Italia, Sezione Toscana, Editore Guido Persichino Grafica, Firenze.

Sillero, N., Celaya, L. & Martín-Alfageme, S. (2005). Using Geographical Information Systems (GIS) to make an atlas: a proposal to collect, store, map and analyse chorological data for herpetofauna. *Revista Españolade Herpetologia* 19: 87–101.

Stagni, G., Dall'Olio, R., Fusini, U., Mazzotti, S., Scoccianti, C. & Serra, A. (2004). Declining populations of Apennines yellow-bellied toad *Bombina pachypus* (Bonaparte, 1838) in northern Appennines, Italy: is *Batrachochytrium dendrobatidis* the main cause? *Italian Journal of Zoology* 71(Suppl. 2): 151–154.

Accepted: 4 December 2022

Please note that the Supplementary Material for this article is available online via the Herpetological Bulletin website: https://thebhs.org/publications/the-herpetological-bulletin/issue-number-164-summer-2023

The Herpetological Bulletin 164, 2023: 24–25
SHORT COMMUNICATION

https://doi.org/10.33256/hb164.2425

High frequency of hindlimb malformation in froglets *Pelophylax* sp. in Ukraine

ANNA FEDOROVA*, ELEONORA PUSTOVALOVA & MYKOLA DROHVALENKO

Department of Zoology and Animal Ecology, V.N. Karazin Kharkiv National University, Kharkiv, 61022, Ukraine

*Corresponding author e-mail: anna.fedorova@karazin.ua

Hindlimb anomalies are the most widespread among all developmental anomalies of tailless amphibians (Meteyer et al., 2000; Ballengee & Sessions, 2009). Severe limb malformation may reduce the viability and reproduction success of an individual. Herein we report noticeable hindlimb malformations in froglets of European water frogs *Pelophylax* Fitzinger, 1843, from one particular locality.

In September 2018, we collected a sample of 21 water frog froglets in the old artificial pond, the Lower Dobrytskyi pond (NP "Homilshanskilisy", Kharkiv region, Ukraine, 49° 37'40" N, 36° 16'58" E). It was previously shown that the Lower Dobrytskyi pond was inhabited by a population system of hybrid edible frogs *Pelophylax esculentus* Linnaeus, 1758, (both diploid and triploid forms) and one of its parental species, the marsh frog *Pelophylax ridibundus*, Pallas, 1771 (Meleshko et al., 2014; Kryvoltsevych et al., 2022). Adult hybrids are usually easily distinguishable from *P. ridibundus* by a complex of morphological traits that include colouration of hips and vocal sacs (for males) and shape of the inner metatarsal tubercle (e.g. Kierzkowski et al., 2013). However, these differences are not reliable for the identification of subadults, consequently we refer to all the froglets only as *Pelophylax*. We assessed the froglets sex and ploidy, by examination of gonadal morphology and chromosomal slides prepared from intestine and/or bone marrow (Macgregor & Varley, 1983). For this purpose, the froglets were euthanised with ethylacetate vapours and dissected; specimens were further stored in 96 % ethanol. Data from the investigation of ploidy is described in detail in Fedorova et al. (2018). Water frogs are not listed as Endangered species either in IUCN Red List or Red Data Book of Ukraine. All manipulations were undertaken according to the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research (ASIH, 2004).

In most literature sources, the terminology concerning hindlimb malformations lacks consistency. Here we follow Nekrasova (2008) by referring to the total absence of any part of the limb as ectromelia and shortened limb as hemimelia. The disturbed body plan of the limb is described as taumelia.

Among the 21 froglets studied, 13 were females, and eight were males. All froglets were diploid. Seven individuals (33 %, five females and two males) had severe malformations of

Figure 1. *Pelophylax* froglets with serious hindlimb malformations- **A.**–**E.** Ectromelia, **F.** Hemimelia, and **G.** Taumelia (arrow) and hemimelia

hindlimbs. Five individuals had ectromelia (Fig. 1A–E), one had hemimelia (Fig. 1F), and one had hemimelia accompanied by taumelia (Fig. 1G). The presence of malformations was not associated with the sex of froglets ($\chi^2 = 0.2074$, $p = 0.6488$).

Our previous research on 190 adult water frogs from the same locality showed only one frog with a missing hindlimb and one with taumelia (Kryvoltsevych et al., 2022). Based on that research and our data on the froglets presented in the current study we applied Fisher's exact test to estimate the difference between the frequency of hindlimb malformations among froglets and among adults. The test results showed a significant difference (two-tailed, $p < 0.0001$), indicating that such types of malformations may affect the viability of frogs.

Revealing the primary reason of each malformation would be a complex task because malformations could result from either natural or anthropogenic factors, including parasitic infections, exposure to UV radiation and chemical pollutants, physical traumas, and genetic defects (Johnson et al., 1999; Ouellet, 2000; Henle et al., 2017). Furthermore, Ballengee & Sessions (2009) showed that the most likely reason for missing limbs and missing limb segments is predation by dragonfly nymphs who can injury or even amputate the limbs of tadpoles. The extent of subsequent limb regeneration, and as a result the extent of limb deformity, depends on the tadpole's stage of development: younger tadpoles have a greater chance of full limb regeneration. The local occurrence of this phenomenon may indicate a link to genetic traits of the quite complex local population system. Such frequent limb malformation has not been reported from other *Pelophylax* populations in this national park, although they have been monitored for almost a decade.

Currently, we are unable to state why there was such a high frequency of missing hindlimbs in these froglets. Additional investigations with larger samples (including both larvae and metamorphosed individuals) are required.

ACKNOWLEDGEMENTS

We are thankful to Oleksii Korshunov for his invaluable help during the fieldtrip. We are also deeply grateful to all the defenders of Ukraine whose efforts gave us a chance to complete this work.

REFERENCES

ASIH (American Society of Ichthyologists and Herpetologists) (2004). Guidelines for use of Live Amphibians and Reptiles in Field and Laboratory Research. Second Edition, Revised by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, Washington, D.C. 43 pp.

Ballengee, B. & Sessions, S.K. (2009): Explanation for Missing Limbs in Deformed Amphibians. *Journal of Experimental Zoology (Molecular and Developmental Evolution)* 312B: 1–10.

Fedorova, A., Drohvalenko, M. & Pustovalova, E. (2018). Unusual ratio of hybrids among juvenile water frogs in Lower Dobrytskyi pond. In *Abstract book of the Conference of young zoologists*, 26 pp. Institute of Zoology, Kyiv, Ukraine.

Henle, K., Dubois, A. & Vershinin, V. (2017). A review of anomalies in natural populations of amphibians and their potential causes. In *Studies on Anomalies in Natural Populations of Amphibians*, 57–164 pp. Henle, K. & Dubois, A. (Eds.). Mertensiella 25. Supplement zu Salamandra. Deutschen Gesellshaft für Herpetologie und Terrarienkunde e.V., Mannheim, Germany.

Johnson, P.T.J., Lunde, K.B., Ritchie, E.G. & Launer, A.E. (1999). The effect of trematode infection on amphibian limb development and survivorship. *Science* 284: 802–804.

Kierzkowski, P., Kosiba, P., Rybacki, M., Socha, M. & Ogielska, M. (2013). Genome dosage effect and colouration features in hybridogenetic water frogs of the *Pelophylax esculentus* complex. *Amphibia-Reptilia* 34(4): 493–504.

Kryvoltsevych, A., Fedorova, A., Shabanov, D. & Pustovalova, E. (2022). Anomalies in marsh frogs (*Pelophylax ridibundus*) and hybrid waterfrogs (*P. esculentus*) (Anura: Ranidae) from two ponds in the Kharkiv Region of Ukraine. *Reptiles & Amphibians* 29(1): 204–209.

Macgregor, H.C. & Varley, J.M. (1983). *Working with animal chromosomes*. Wiley. 250 pp.

Meleshko, O.V., Korshunov, O.V. & Shabanov, D.A. (2014). The study of three hemiclonal population systems *Pelophylax esculentus* complex from the Seversko-Donetskiy center of green frogs diversity. *The Journal of V.N. Karazin Kharkiv National University* 20: 153–158.

Meteyer, C.U. (2000). Field guide to malformations of frogs and toads with radiographic interpretations. Biological Science Report USGS/BRD/BSR–2000–0005. 16 pp.

Nekrasova, O.D. (2008) Classification of anomalies of tailless amphibians. In *Proceedings of the Ukrainian Herpetological Society* 1: 55–58. (In Russian)

Ouellet, M. (2000): Amphibian deformities: current state of knowledge. In *Ecotoxicology of Amphibians and Reptiles*, 617–661 pp. Sparling, D.W., Linder, G., Bishop, C.A. & Krest, S.K. (Eds.). Pensacola, Florida, USA, SETAC Technical Publications Series.

Accepted: 1 December 2022

The Herpetological Bulletin 164, 2023: 26–27
SHORT COMMUNICATION

https://doi.org/10.33256/hb164.2627

First record of hypopigmentary anomaly in the Moorish gecko *Tarentola mauritanica* with an overview of the cases reported for wild geckos

FRANCESCO PAOLO FARAONE[1], FILIPPO FARAONE[2] & MATTEO RICCARDO DI NICOLA[3,4*]

[1]Dipartimento Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, University of Palermo, Via Archirafi, 18, 90123 Palermo, Italy
[2]Viale Regione Siciliana S.E., 532, 90129 Palermo, Italy
[3]San Raffaele Hospital, Unit of Dermatology, Via Olgettina 60, 20132 Milan, Italy
[4]Asociación Herpetológica Española, Apartado de Correos 191, 28911 Leganés, Madrid, Spain

*Corresponding author e-mail: dinicola.matteo@hsr.it

Hypopigmentary anomalies (HAs) are chromatic disorders of various etiologies that result in lighter colourations due to a decrease or lack of pigments. A standardised terminology for chromatic anomalies in reptiles has recently been proposed by Borteiro and colleagues, including definitions for the main HAs such as amelanism, albinism, hypomelanism, leucism, and piebaldism (Borteiro et al., 2021). Among wild reptiles, HAs are mostly known in snakes while they are rarer in other squamates, including geckos.

In the case of wild geckos, HAs have been reported in less than 0.4 % of the known species (Table 1). There have been no previous reports of HA in the Moorish gecko *Tarentola mauritanica* Linnaeus, 1758, which may be a complex of six cryptic species (Rato et al., 2016). The species is chromatically variable with a grey or brown colouration dorsally, sometimes with yellowish tones, and a ladder-shaped dark pattern (Aprea et al., 2011). Moreover, *T. mauritanica* is able to quickly darken or lighten the dorsal ground colour and pattern in relation to the substrate and light conditions (Vroonen et al., 2012).

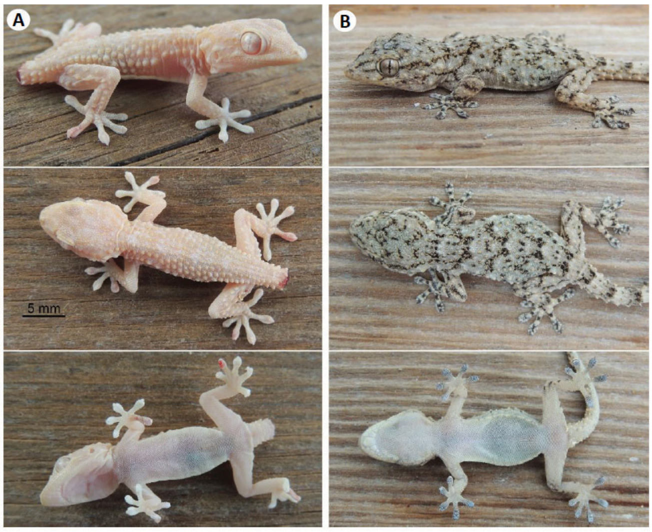


Figure 1. Two young Moorish geckos *Tarentola mauritanica* from Sicily - **A**. Dorsal and ventral views of a specimen affected by an hypopigmentary anomaly, and **B.** An individual of normal colouration and similar size observed at the same location

On 1 September 2022 at 22:40 h, in the seaside locality Triscina di Selinunte (Province of Trapani, Sicily; 37° 34’57’’ N, 12° 48’44’’ E; 10 m a.s.l.) a young Moorish gecko with a freshly shed tail (Fig. 1) was observed on the ground of a private garden. Specific identification was made on a morphological basis (Aprea et al., 2011). The gecko snout-vent length (SVL) was 27 mm, which is compatible with a recently hatched individual (Martinez-Rica, 1974). The dorsal

Table 1. Overview of gecko species for which hypopigmentary abnormalities in the wild have been reported in scientific literature, there is only a single record in each case

Species	**Family**	**Declared anomaly**	**Age class (sex)**	**References**
Woodworthia maculata	Diplodactylidae	Leucism	Adult (female)	Baling, 2015
Cyrtodactylus deccanensis	Gekkonidae	Xanthism	Unspecified	Mayekar et al., 2022
*Lygodactylus capensis**	Gekkonidae	Amelanism	Unspecified	Raw, 2021
Homonota taragui	Phyllodactylidae	Partial albinism	Unspecified	Courtis et al., 2015
Tarentola boettgeri	Phyllodactylidae	Piebaldism	Adult (male)	Rocha & Rebelo, 2010
Tarentola mauritanica	Phyllodactylidae	Amelanism	Young	This study
Euleptes europaea	Sphaerodactylidae	Albinism	Young	Delaugerre, 1981
Gonatodes albogularis	Sphaerodactylidae	Leucism	Adult (female)	Grisalez-Martínez & Arias-Alvarez, 2018

*The individual is likely young from what can be assessed from the photo ground colour was whitish with pinkish hues, marbled with a very faint yellow pattern, while the ventral parts were whitish and slightly translucent. The subdigital lamellae also appeared depigmented (Fig. 1). The iris was pale pink, affected by a faded whitish part around the pupil, the latter appearing distinctly reddish when dilated. The chromatic condition of the gecko would appear to be amelanism sensu Borteiro et al. (2020; i.e. lack of melanin expression -dark colours-, including eyes).

Although *T. mauritanica* is a common, widespread, and adaptive species (Speybroeck et al., 2016), no cases of HAs have been described previously in the scientific literature suggesting a low survival rate of affected individuals in nature.

ACKNOWLEDGEMENTS

The gecko was handled following ministerial and regional permits: MATTM reg. 0039049, 15/04/2021 and Prot. ISPRA 10201, 24/03/2021.

REFERENCES

Aprea, G., Lo Cascio, P., Corti, C. & Zuffi, M.A.L. (2011). *Tarentola mauritanica*. In *Fauna d'Italia, vol. XLV, Reptilia*, 277–285 pp. Corti C., Capula M., Luiselli L., Sindaco R. & Razzetti E. (Eds.). Bologna: Calderini.

Baling, M. (2015). *Woodworthia maculata* (common gecko). Leucism. *Herpetological Review* 46: 97–98.

Borteiro, C., Diesel Abegg, A., Hirouki Oda, F., Cardozo, D.E., Kolenc, F., Etchandy, I., Bisaiz, I, Prigioni, C. & Baldo, J.D. (2021). Aberrant colouration in wild snakes: case study in Neotropical taxa and a review of terminology. *Salamandra* 57: 124–138

Courtis, A., Cajade, R., Piñeiro, J.M., Hernando, A. & Marangoni, F. (2015). First record of albinism in the Taragüi Gecko *Homonota taragui* (Squamata: Phyllodactylidae). *Herpetology* 8: 425–427.

Delaugerre, M. (1981). Un cas d'albinisme chez *Phyllodactylus europaeus* Gené, premier cas signalé dans la famille des Gekkonidés. *Bulletin Mensueldela Societe Linneenne de Lyon* 50: 213–216.

Grisalez-Martínez, F.A. & Arias-Alvarez, L. (2018). Leucism in the yellow-headed gecko *Gonatodes albogularis* (Duméril and Bibron, 1836). *Herpetology Notes* 11: 1003–1005.

Martínez-Rica, J.P. (1974). Contribución al estudio de la biología de los gecónidos ibéricos (Rept., Sauria). *Publicaciones del Centro Pirenaico de Biologia Experimental* 5: 1–291.

Mayekar, P., Ghumare, M., Raul, S., Ghadekar, P. & Yadav, O. (2022). Xanthism in the Deccan Banded Gecko, *Cyrtodactylus deccanensis* (Günther 1864). *Reptiles & Amphibians* 29: 316–317.

Rato, C., Harris, D.J., Carranza, S., Machado, L. & Perera, A. (2016). The taxonomy of the *Tarentola mauritanica* species complex (Gekkota: Phyllodactylidae): Bayesian species delimitation supports six candidate species. *Molecular Phylogenetics and Evolution* 94: 271–278.

Raw, L.R.G. (2021). Observations on Abnormalities in South African Reptiles. *ZooNova* 6: 1–15.

Rocha, R. & Rebelo, R. (2010). First record of a piebald Selvagens Gecko *Tarentola boettgeri bischoffi* (Squamata: Gekkonidae). *Herpetology Notes* 3: 361–362.

Speybroeck, J., Beukema, W., Bok, B. & Van Der Voort, J. (2016). *Field Guide to the Amphibians and Reptiles of Britain and Europe*. London: Bloomsbury. 432 pp.

Virens, J., Davis, R.A. & Doherty, T.S. (2017). Two records of amelanism in the Australian skink *Ctenotus fallens* (Storr, 1974). *Herpetology Notes* 10: 453–455.

Vroonen, J., Vervust, B., Fulgione, D., Maselli, V. & van Damme, R. (2012). Physiological colour change in the Moorish gecko, *Tarentola mauritanica* (Squamata: Gekkonidae): effects of background, light, and temperature. *Biological Journal of the Linnean Society* 107: 182–191.

Accepted: 1 December 2022

The Herpetological Bulletin 164, 2023: 28–29
SHORT COMMUNICATION

https://doi.org/10.33256/hb164.2829

Tail regrowth in wall lizards *Podarcis muralis* from a population introduced into England

RAYMOND WERGAN

Devon Reptile and Amphibian Group

Author e-mail: wergan48@btinternet.com

A population of introduced wall lizards *Podarcis muralis* in a small (15 m x 8 m) undisturbed garden in the village of Newton Ferrers in Devon, England, has been studied intensively for 4 years. The lizards correspond in appearance to the subspecies *nigriventris* Boneparte, 1838, and so probably originated from central Italy. Individuals were identified by the pattern, shape and colouration of the scales around their eyes and cheeks, which, with one exception out of 18, were the same on both cheeks. In 2021, three adult males and six adult females were observed and photographed on 105 days, often three times a day, for periods from one minute to one hour, providing 4800 photographs. In 2022, one male and eight females were photographed on 161 days providing 6400 photographs. In 2021, a male lizard (M1) was seen to have suffered tail loss, as was a gravid female lizard (F2) in 2022. The subsequent progress of tail regeneration in these two cases was documented with photographs and tail measurements.

Measurements of total body length and tail length were made using a ruler which was laid horizontally beneath a paving slab that was often used as a basking place (Fig. 1). Regrowth was measured on the computer screen by enlarging the known part of the lizard's length to its actual size, enabling the new growth to be measured. The error in this measurement system was not determined but measurements over time gave progressive increases in the length of regenerated tails, indicating a reasonable degree of accuracy.

The male lizard (M1) was observed with a recently broken tail on 13 July 2021 and photographs and measurements were taken on five subsequent occasions until 16 August (Figs. 2 & 4). In this 34-day period the tail had regrown 83 mm. This occurred at a minimum rate of 0.7 mm/day and a maximum of 2.6 mm/day, as determined between the intervals that photographs were taken.

The gravid female lizard (F2) was observed with a recently shed tail on the 27 May 2022 (Figs. 3 & 4) and laid eggs 13 to 15 days later (9–11 June). At that time the regenerated tail (blastema) measured only about 1 mm in length, i.e. there was negligible regrowth before egg laying. Between 11 June and 18 July, the tail grew 53 mm, at an average rate of 1.6 mm/day.

The observations on male and female specimens were made in two different years, 2021 and 2022. In the period April to the end of July, these years showed marked differences

Figure 1. A female lizard on the paving slab with the ruler used to measure length

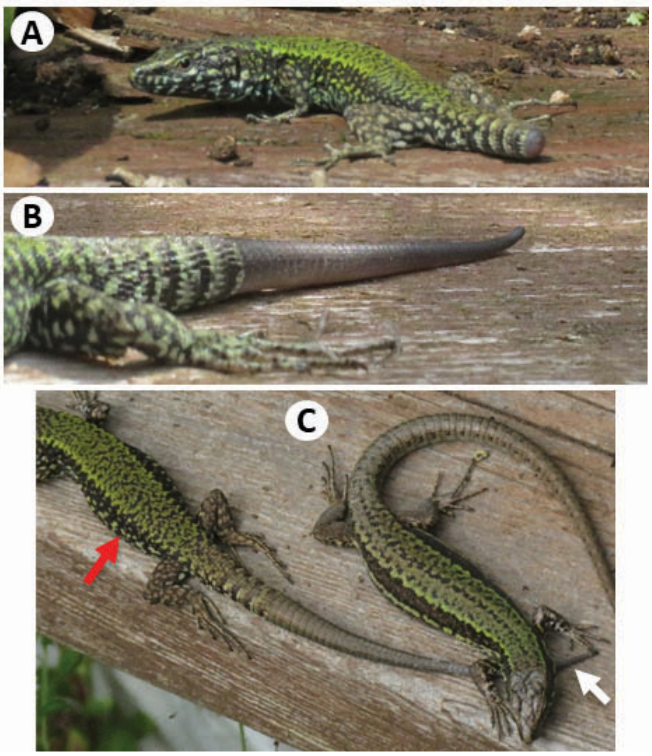


Figure 2. Tail regeneration in an adult male *Podarcis muralis* (M1) in 2021 - **A**. Soon after tail loss (13 July), **B.** After 12 days (25 July) with significant tail regeneration, and **C.** After 34 days (16 August) with further regeneration (the male indicated by red arrow, tail tip by white arrow)

Figure 3. Tail regeneration in an adult female *Podarcis muralis* (F2) in 2022 - **A.** Soon after tail loss on 27 May while still gravid, **B.** After 33 days (30 June) when eggs had been laid and with significant tail regeneration, and **C**. After 52 days (18 July) with further regeneration

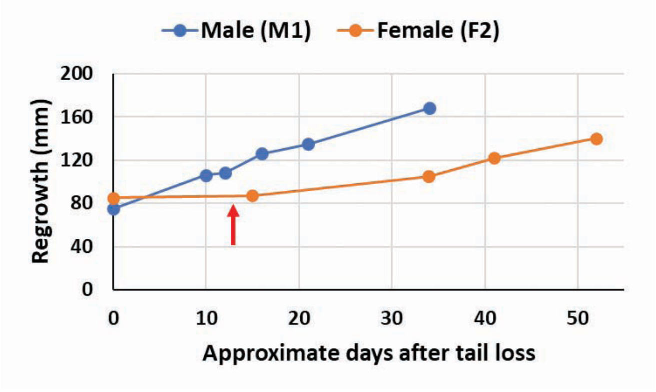


Figure 4. Tail regrowth of a male and a female wall lizard *Podarcis muralis* that were photographed and measured several times after tail loss. The male was assessed from 13 July to 16 August 2021 and the female from 27 May to 18 July 2022 (red arrow indicates approximate time of egg laying)

from long-term average weather conditions; 2021 was relatively cool and moist while 2022 was exceptionally hot and dry. These differences would be expected to have impacted physiological processes in the lizards and also perhaps even food availability. Consequently, no direct comparisons can be made between male and female tail regrowth rates. There appear to be no data in the literature with which the current observations can be compared directly, although under laboratory conditions the tail of *P. muralis* is said to be able to regenerate in 20–30 days (Alibardi, 2010; 2018) which concurs with at least the male in this study. The lack of regeneration during the period that the female was gravid is particularly interesting, especially as it is known that the rate of tail regeneration in *Zootoca vivipara* is reduced in individuals that are heavily infected by blood parasites (Oppliger & Clobert, 1997). The extra physical stress involved in reproduction may account for very slow regrowth rate until after egg laying although this could in part be attributed to the more extreme weather conditions of 2022, which may have been an important stress factor.

ACKNOWLEDGEMENTS

I am very grateful for advice on fieldwork and on the preparation of the manuscript from Oliver Ellis, Geoffrey Sharp, George Cameron and Roger Avery.

REFERENCES

Alibardi, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards: A Model System with Implications for Tissue Regeneration in Mammals.* Springer; Heidelberg, Germany, 112 pp.

Alibardi, L. (2018). Tail regeneration reduction in lizards after repetitive amputation or cauterization reflects an increase in immune cells in blastemas. *Zoological Research* 39: 413–423.

Oppliger, A. & Clobert, J. (1997). Reduced tail regeneration in the Common Lizard *Lacerta vivipara* parasitized by blood parasites. *Functional Ecology* 11: 652–655.

Accepted: 8 December 2022

The Herpetological Bulletin 164, 2023: 30–32
SHORT COMMUNICATION

https://doi.org/10.33256/hb164.3032

Insights into the courtship and copulation of the worm snake *Xerotyphlops vermicularis*

ANGEL DYUGMEDZHIEV[*], KOSTADIN ANDONOV & GEORGI KRASTEV

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

[*]Corresponding author e-mail: angel_diugmedjiev@abv.bg

The worm snake *Xerotyphlops vermicularis* Merrem, 1820, is a small semi-fossorial snake, distributed in the southern parts of the Balkan Peninsula (along the Adriatic coast, parts of North Macedonia, parts of southern Bulgaria, reaching north to the Central Black Sea coast, and most of Greece including many Aegean islands), Turkey, the Middle East, Caucasus and eastwards to Iran and Afghanistan (Speybroeck et al., 2016; Koynova et al., 2021). Due to its secretive life, the species' biology and ecology have been little studied across its range. In Bulgaria between the end of April and the end of June, *X. vermicularis* is found during the day under stones, and rarely on the surface (only at dusk and at night, or after heavy rains); in July and August it is not usually found under stones, but comes to the surface at night; in September it migrates to the deeper soil layers and it is almost impossible to find (Naumov, 2007; Stojanov et al., 2011). Copulation is in May and June, and the eggs (2–8) are laid in July–August in deep layers of soil (Naumov, 2007; Stojanov et al., 2011; Speybroeck et al., 2016). To date, there are apparently no published descriptions of the mating behaviour of *X. vermicularis* or of other blind snakes of the families Typhlopidae, Anomalepididae and Leptotyphlopidae. In this report, we provide some insights into the courting ritual and the copulation behaviour of *X. vermicularis*.

On 16 May 2022, during an ecological study of snakes in Bulgaria, we found two adult *X. vermicularis* under a stone at 23:44 h (local time), near Dositeevo Village (41° 55'44" N, 26° 0'51" E; 149 m a.s.l.) in south-eastern Bulgaria, aided with an artificial light. We noticed that the two snakes were coiled together and appeared to be conducting courting behaviour, prior to copulation. We carefully removed the stone, then started observing and filming their behaviour without disturbing the snakes. Following copulation, we captured the snakes to measure snout to vent length (SVL) and tail length (TL) (precision 0.1 cm), and weigh (W) them (precision 0.01 g) before releasing them at the site of capture.

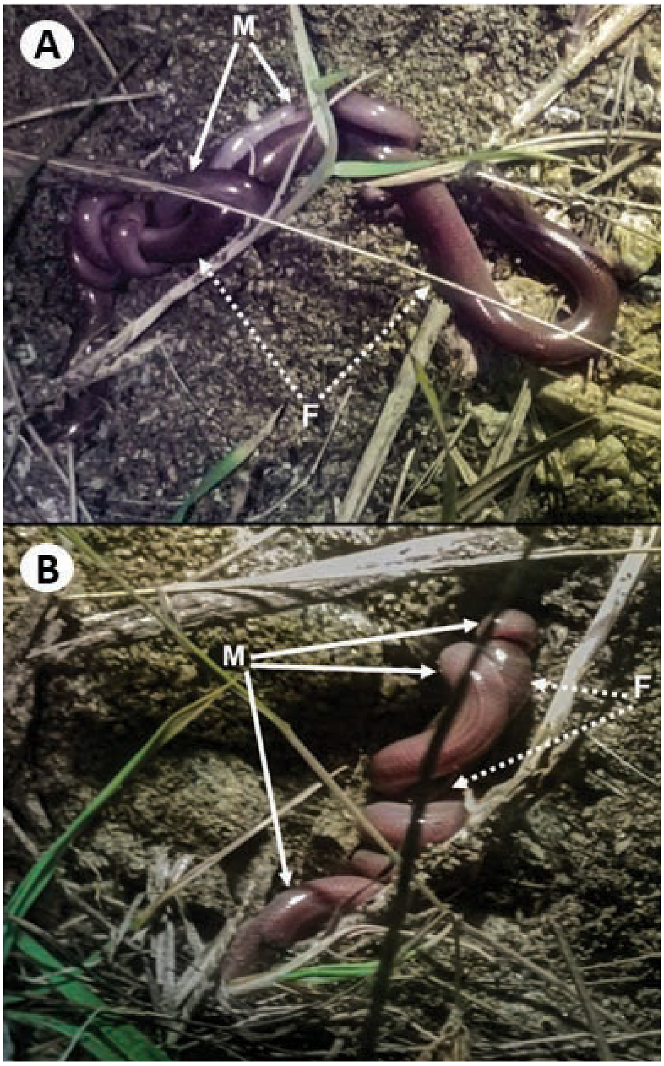


Figure 1. Mating behaviour of *Xerotyplops vermicularis* observed in situ on 16 May 2022 - **A.** Courting behaviour, in which the male coiled its body along that of the female, **B.** Copulating behaviour, during which the two snakes align and press their cloacae together. White dotted arrow indicates the presumed female snake (F); white continuous arrow indicates the presumed male snake (M). For more details see BHS videos 2023a & b.

During the courtship, the larger snake (SVL = 26.8 cm, TL = 0.5 cm, W = 5.32 g), which we presumed was female, was laying relatively still on a sandy-soil substrate, its body positioned in a W-shape form with the head at one end, hiding in the grass, and the tail exposed on the surface at the other (Fig. 1A, BHS video 2023a). The smaller snake (SVL = 20.5 cm, TL = 0.6 cm, W = 2.43 g), which we presumed was the male, was coiled and moving around the body of the female. The head and the interior 1/3 of the male's body was coiled tightly around the mid-part of the female's body while the other 2/3 of the male's body, including its tail, was coiled more loosely around the female's body. The male was making spiral movements along the axis of the middle part of the female's body, without releasing the tight grip, with the head leading the way and gently rubbing the female's body, while the tail was providing a loose hold and was slowly moving along the female's body (Fig. 1A, BHS video 2023a). This courting behaviour of the male continued for about two minutes. However, since it had already begun at the time the snakes were first observed, its full duration remains uncertain.

After these two minutes, the two snakes started to copulate. During the copulation, the female's body remained in its previous position, although the snake was slowly moving forward. The head and the anterior 1/3 of the male's body was positioned away from the female's body. The middle 1/3 of the male's body was positioned on top of the mid-part of the body of the female, while the posterior 1/3 of the male made two tight coils around the corresponding parts of the female body, followed by a slightly loosened coil, and ending with the cloacae of the two snakes being pressed together. During this phase, the male tried to remain in this fixed position, so he adjusted the posterior part of its body after each movement of the female (Fig. 1B, BHS video 2023b). The cloacae of the two snakes remained pressed together for around 1.2 minutes, after which the male removed his cloaca for 3 seconds, and then pressed again against the cloaca of the female for an additional 10 seconds. Thereafter, the male released the hold on the female's body and moved away from her (BHS video 2023b).

As is generally the case in the Typhlopidae, sexual dimorphism in *X. vermicularis* is very weak, which makes determining the sex of an individual difficult and uncertain without dissection. Because of the species' small size and frail body structure, the usual methods for determining the sex of live snakes, such as probing (Schaefer, 1934) or squeezing the base of tail to reveal the hemipenes (Gregory, 1983) cannot be applied. Males and females differ to a small degree in the tail length and the number of subcaudal scales, with males usually having longer tails with more scales (Perry, 1984). However, because of the small size of those scales, a magnifying glass should be used for accurate counting (Perry, 1984). Additionally, at least in another member of Typhlopidae, *Anilios nigrescens* Gray, 1845, females are larger than males (Shine, 1978). Although sex determination in our case could not be confirmed, based on the difference between the body proportions of the two individuals (the larger snake was approximately 6 cm longer than the smaller one but its tail was 0.1 cm shorter), as well as the clear difference between the behaviours of the two individuals, it is reasonable to assume that the smaller and more active individual was the male.

The mating behaviour of snakes is usually rather complex and includes specific mating rituals where males are much more active and involved than females (Shine, 1978; Andrén & Nilson, 1983; Andrén, 1986; Vitt & Caldwell, 2014). This usually includes three phases: tactile-chase, tactile-alignment, and intromission coitus (Vitt & Caldwell, 2014). The tactile-chase phase includes the initial contact between the snakes, when males often use chemosensory sampling to determine the sex of the other snake. During this phase, the male places his body alongside or over the dorsal surface of the female, contracting segments of his body musculature in a wave-like manner, while often also rubbing his chin on the females back, or even biting her. The tactile-alignment phase begins when the first attempt to copulate occurs, which involves tail-searching and alignment of the male's tail with that of the female, by rapid caudal vibrating movements. The tactile behaviours expressed during the first phase may also continue during the tactile-alignment phase. During the intromission coitus phase, the female gapes her cloaca and allows the insertion of one of the hemipenes of the male (Vitt & Caldwell, 2014). It appears that courtship and copulating behaviour of *X. vermicularis* involves the same three phases. However, because of the species' very short tail, the end of each phase and the respective beginning of the next is hard to distinguish. We suggest that such behaviour with coiled body and tight grip on the female along with the strong pressing of cloacae are required considering the general morphology of the species and males' typical non-ornamented hemipenes (Heyder, 1968).

ACKNOWLEDGEMENTS

This work was partially supported by the Bulgarian Ministry of Education and Science under the National Research Program "Young scientists and postdoctoral students - 2" approved by DCM № 206 / 07.04.2022. All fieldwork was carried in accordance to Ministry of Environment and Water of Bulgaria Permit № 861/13.01.2021.

REFERENCES

Andrén, C. & Nilson, G. (1983). Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. *Amphibia-Reptilia* 4(1): 63–79. https://doi.org/10.1163/156853883X00274.

Andrén, C. (1986). Courtship, mating and agonistic behaviour in a free-living population of adders, *Vipera berus* (L.). *Amphibia-Reptilia* 7: 353–383.

BHS video (2023a). Courting behaviour of *Xerotyplops vermicularis* (Merrem, 1820). Recorded by Angel Dyugmedzhiev. https://youtu.be/V6jcQZEd-6c.

BHS video (2023b). Copulating behaviour of *Xerotyplops vermicularis* (Merrem, 1820). Recorded by Angel Dyugmedzhiev. https://youtu.be/0UQF1HAEUhM.

Gregory, P.T. (1983). Identification of sex of small snakes in the field. *Herpetological Review* 14: 43–44.

Heyder, G. (1968). The urogenital system of *Typhlops vermicularis* Merrem (1820). *Gegenbaurs Morphologisches Jahrbuch* 112(4): 594–605.

Koynova, T., Marinova, P., Stanchev, N., Natchev, N. & Jablonski, D. (2021). New records of *Xerotyphlops vermicularis* (Merrem, 1820) indicate the northernmost locality of the species in the Balkan Peninsula. *Check List* 17(6): 1623–1626. https://doi.org/10.15560/17.6.1623.

Naumov, B. (2007). Worm Snake. In *A field guide to amphibians* *and reptiles of Bulgaria*, 109–110 pp. Biserkov, V. (Ed.). Sofia: Green Balkans. (In Bulgarian).

Perry, G. (1985). Sexual dimorphism in *Typhlops vermicularis* (Reptilia: Ophidia). *Israel Journal of Zoology* 33: 11–13.

Schaefer, W.H. (1934). Diagnosis of sex in snakes. *Copeia* 4: 181.

Shine, R. (1978). Sexual size dimorphism and male combat in snakes. *Oecologia* 33: 269–277.

Speybroeck, J., Beukema, W., Bok, B. & Voort, J.V.D. (2016). *Field guide to the Amphibians and Reptiles of Britain and Europe*. London: Bloomsbury Publishing Plc. 432 pp.

Stojanov, A., Tzankov, N. & Naumov, B. (2011). *Die Amphibien und Reptilien Bulgariens*. Frankfurt am Main: Chimaira. 588 pp.

Vitt, L.J. & Caldwell, J.P. (2014). Herpetology. *An introductory biology of Amphibians and Reptiles*. Fourth Edition. Amsterdam: Elsevier. 757 pp.

Accepted: 12 December 2022

The Herpetological Bulletin 164, 2023: 33–36
SHORT COMMUNICATION

https://doi.org/10.33256/hb164.3336

New distribution records for the Aesculapian snake *Zamenis longissimus* in Greek Thrace

APOSTOLOS CHRISTOPOULOS[1*], CHRISTOS KOTSELIS[2], PLATON STEFANOPOULOS[3] & YIANNIS G. ZEVGOLIS[4]

[1]Department of Zoology and Marine Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece
[2]Diagora 24, 11636 Athens, Attica, Greece
[3]Filothei, 15237 Athens, Attica, Greece
[4]Biodiversity Conservation Laboratory, Department of Environment, University of the Aegean, 81100 Mytilene, Greece

[*]Corresponding author e-mail: laniusapo@yahoo.gr

The Aesculapian snake *Zamenis longissimus* (Laurenti, 1768) is widely distributed in Europe from north-eastern Spain through central and southern Europe to the Caspian Sea, northern Asia Minor and Caucasus region, with many isolated populations in Britain, Czech Republic, Germany, Poland, Ukraine, south-western Russia, and Elba Island (Italy), while its presence in Sardinia is disputed (Musilová et al., 2010; Di Nicola, 2020). In Greece, the species has been found in various parts of the mainland including the Peloponnese (Fig. 1), Samothraki island in the Aegean sea, and the islands of Corfu and Paxos in the Ionian Sea (Valakos et al., 2008; Stille & Stille, 2017; Pafilis & Maragou, 2020), while recently it was also confirmed in the north part of Euboea island (Strachinis, 2021). The species prefers woodland areas, especially deciduous forests, however it can be found in many habitat types, provided there is dense vegetation, shrubs, maquis, open forests, and thickets. It tends to frequent humid places, from sea level up to an altitude of 2,000 m (Valakos et al., 2008; Geniez, 2018).

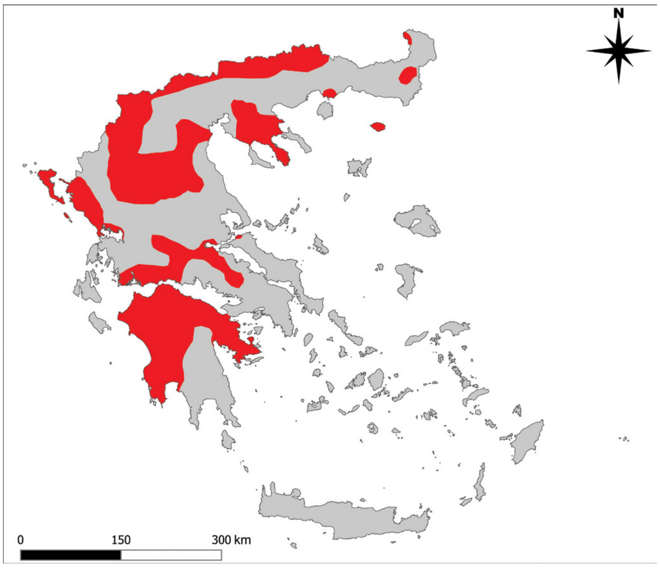


Figure 1. Map of Greece showing confirmed areas of distribution (in red) of the Aesculapian snake *Zamenis longissimus*

The geographical region of Greek Thrace is located in the north-eastern part of Greece; bordering the Marmara Region (Turkey) to the east, and Eastern Macedonia (Greece) to the west, the Aegean sea to the south, and south-central Bulgaria to the north (Fig. 2). The region of Thrace consists of three prefectures (Xanthi, Rhodope and Evros), with a total area of 8,578 km^2 and is mainly characterised by lowland areas (46.2 %) followed by mountainous (28.5 %) and semi-mountainous (25.3 %) areas. Most of its land is covered by agro-pastoral (cultivations and pastures) activities (61 %), forests (32.3 %), and wetlands (2.6 %) (NSSG, 1995). There have been some herpetological surveys in Thrace, in which a total of 15 amphibian and 31 reptile species have been recorded (Valakos et al., 2008; Cattaneo & Cattaneo, 2014; Georgiev & Mollov, 2016; Cattaneo, 2017; Pafilis & Maragou, 2020; Christopoulos & Kotselis, 2021; Christopoulos, 2022). Most of these species occur in all three of the prefectures, while some are limited to one or two of them. The Aesculapian snake has been recorded in the Xanthi and Evros prefectures. According to the literature and our knowledge, in Xanthi the species is known from the Nestos River Delta, and in Evros it has been located in two areas: in the north-western part and in the wider area of the Dadia-Lefkimi-Soufli Forest in the central part of the prefecture. Here, we report the Aesculapian snake for the first time from Rhodope prefecture in two locations, as we have also identified it in new locations of Xanthi and Evros prefectures.

In spring–summer 2021 and 2022, we undertook small-scale herpetological surveys in several areas of the three prefectures of Greek Thrace region. To locate the animals, we used line transects combined with searching for possible shelters and sunny spots that are frequently used by reptiles for basking. Furthermore, snakes were detected both on asphalt and on dirt roads when we were travelling by car. On roads, we recorded both alive and roadkill specimens. In all cases, we noted total length (TL) and snout-vent length (SVL), as well as the weather conditions, a brief habitat description, and geographical co-ordinates. Some specimens that were found dead on roads were collected and preserved in 96 % ethanol.

We found at total of eight specimens (Table 1) from widely ranging locations (Fig. 2), with the very first records for the Rhodope prefecture. Full details, including habitat descriptions and weather conditions, are given in Table 1S

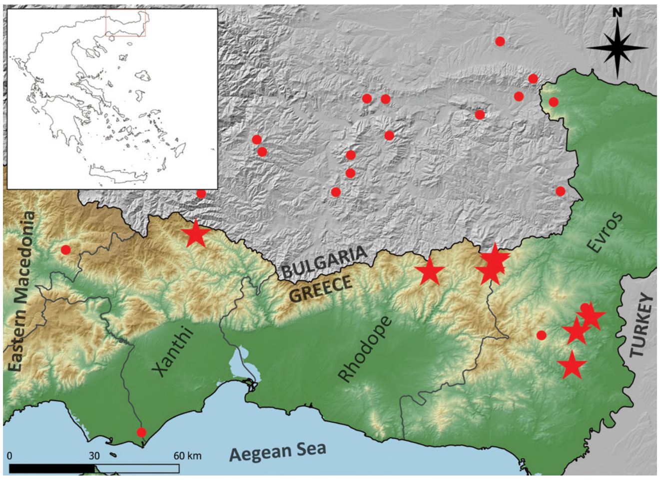


Figure 2. Location of Greek Thrace in Greece (inset) and map of the three prefectures of Greek Thrace and the areas it borders. The red stars show the localities where we found *Zamenis longissimus*, and the red dots show some other published records close by in Greece and Bulgaria. The records in Rhodope are the first for this prefecture.

Figure 4. Detail of the axanthic juvenile *Zamenis longissimus*, next to Hilia Plateau (#5, see Table 1)

(see Supplementary Material). Photographs of seven of the eight specimens recorded are given in Figure 3.

Our observations include new records for the Aesculapian snake in the prefectures of Xanthi and Evros, while the species is reported for the first time from Rhodope prefecture. The presence of the species in our survey area was expected, as the area falls within the species' geographical range limits and its habitat type. To the best of our knowledge, there are a large number of published records in southern Bulgaria (Petrov, 2004; Petrov et al., 2006) and some from north-eastern Greece (Valakos et al., 2008; Pafilis & Maragou, 2020) (Fig. 2).

During our survey we made two unusual observations. Two juveniles (#3 & #7, Table 1) vibrated their tails when captured. Tail vibration was recently reported for the first time in an Aesculapian snake from Bulgaria (Dyugmedzhiev, 2020) and has also been observed in the Italian Aesculapian snake *Zamenis lineatus* Camerano, 1891 (Di Nicola et al., 2022). In addition, we observed (Hilia Plateau - 16 May 2022) a young axanthic individual (Fig. 3F & Fig. 4). The typical colouration of juvenile Aesculapian snakes is brown to dark brown on the head and back, with white or pale-yellow streaks and yellow labial plates and collar (Geniez, 2018). In axanthic juveniles, the yellow colouration is absent and replaced by light grey, while the brown colouration of head and back is replaced by dark brown or black, with numerous small light markings. Axanthic colouration in Aesculapian snakes has rarely been seen in Greece, but has been reported from other countries (Zadravec & Lauš, 2011; Cattaneo, 2015).

New knowledge concerning the distribution and ecology of forest reptile species, such as the Aesculapian snake, can contribute to more appropriate and targeted actions in managing and protecting a sensitive faunal group that live in an ecosystem that is increasingly exploited, leading to deforestation, fragmentation and degradation.

Figure 3. Photographic documentation of the *Zamenis longissimus* individuals found in Greek Thrace (specimen numbers refer to Table 1) - **A.** Young road-killed specimen within the protected area of Lefkimmi forest (#1), **B.** Subadult road-killed snake between the villages of Organi and Smigada, dorsal and **C.** ventral view (#2), **D.** Juvenile individual, within the protected forest of Dadia (#3), **E.** Adult individual between the villages of Dadia and Giannouli (#4), **F.** Juvenile individual (axanthic) within the protected area of Mountainous Evros-Dereio Valley, next to Hilia Plateau (#5), and **G.** A road-killed juvenile snake in the same area (#6). **H.** Juvenile individual within the protected area of Filiouris River Valley, close to the village of Kechros (#7)

Table 1. Aesculapian snake individuals recorded in Greek Thrace, 2021 and 2022

#	Date & time	Dimensions	Life stage	Location	Notes
1	8 May 2021 09:56 h	TL: 536 mm SVL: 445 mm	Young, road-kill	41° 2'25" N, 26° 9'54" E, 228 m a.s.l. Protected area of Dadia-Soufli Forest, Evros. About 3 km north-west of the village of Lefkimmi	Fig. 3A, specimen deposited in the first author's collection
2	10 May 2021 14:20 h	TL: 673 mm SVL: 545 mm	Subadult, fresh road-kill	41° 15'55" N, 25° 42'47" E, 582 m a.s.l. Near north-western Filiouris River valley limit	Fig. 3B & C, specimen deposited in first author's collection. Appears to be first record of the species in the prefecture of Rhodope
3	13 May 2022 19:06 h	TL: 282 mm SVL: 233 mm	Juvenile, basking on road	41° 7'22" N, 26° 10'47" E, 184 m a.s.l. Protected area of Dadia-Soufli Forest - Evros Mountains, about 3.7 km west of the village of Dadia	Fig. 3D, released where captured. Presence of the species in the area already known
4	13 May 2022 dusk	TL: 974 mm SVL: 785 mm	Adult, crossing road	41° 9'26" N, 26° 13'29" E, 95 m a.s.l. Between the villages of Dadia and Giannouli, within the same protected area as record #3	Fig. 3E, released where captured. Presence of the species in the area already known
5	16 May 2022 15:10–17:00 h	TL: 317 mm SVL: 265 mm	Juvenile, on dirt road	41° 17'28" N, 25° 55'12" E, 803 m a.s.l. North-western part of the protected area of Mountainous Evros-Dereio Valley close to the Rhodope Prefecture's borders	Fig. 3F, species not reported previously from this area of Evros
6	Ditto #5	TL: 323 mm SVL: 267 mm	Juvenile, road-killed	41° 17'54" N, 25° 55'14" E, 800 m a.s.l. Ditto #5 but about 430 m further north	Fig. 3G, ditto #5
7	16 May 2022 18:50 h	TL: 342 mm SVL: 285 mm	Juvenile, basking on road	41° 15'54" N, 25° 54'17" E, 752 m a.s.l. North-eastern part of the protected area of Filiouris River Valley, at about 5.3 km north-east of the village of Kechros	Fig. 3H. Specimen released back into its habitat. Second record of the species in the prefecture of Rhodope
8	13 July 2022 17:45 h	TL: 481 mm SVL: 398 mm	Young, road-kill in poor condition	41° 21'18" N, 24° 58'14" E, 515 m a.s.l. Northern part of the prefecture of Xanthi, in the area of Thermes settlements	Presence of the species has not been reported previously reported from this area of Xanthi

ACKNOWLEDGEMENTS

All aspects of this study were conducted in full compliance with Hellenic national law (Presidential Decree 67/81) on the humane use of animals.

REFERENCES

Cattaneo, A. (2015). The *Zamenis longissimus* (Laurenti) axanthic phenotype found on the Castelporziano Presidential Estate: considerations on its morphology, genetic nature and probable extinction (Serpentes: Colubridae). *Rendiconti Lincei* 26(3): 385–389. Doi: 10.1007/s12210-015-0384-7.

Cattaneo, A. (2017). Note sull'erpetofauna dell'Evros sud-occidentale (Grecia NE) e nuovo contributo alla conoscenza di *Montivipera xanthina* (Gray, 1849) della Tracia greca centro-orientale, con descrizione di *Montivipera xanthina occidentalis* subsp. nova (Reptilia Serpentes Viperidae). *Naturalista siciliano* 41(1): 53–74.

Cattaneo, A. & Cattaneo, C. (2014). Sulla presenza di *Montivipera xanthina* (Gray, 1849) nella Tracia greca centrale e note eco-morfologiche sull'erpetofauna locale (Reptilia Serpentes). *Naturalista siciliano* 38(2): 307–330.

Christopoulos, A. (2022). New record of *Telescopus fallax* Fleischmann, 1831 (Squamata: Colubridae) in Rhodope Prefecture, Thrace, Greece. *Herpetology Notes* 15: 873–876.

Christopoulos, A. & Kotselis, C. (2021). A northernmost range shift: the first record of *Eryx jaculus* Linnaeus, 1758 (Squamata: Boidae) in northern Evros, Thrace, Greece. *Herpetology Notes* 14: 1323–1326.

Di Nicola, M.R., Russo, V.G., Senese, A., Colnaghi, S. & Faraone, F.P. (2022). First records of defensive tail vibration in the Italian Aesculapian Snake *Zamenis lineatus* (Camerano, 1891). *Herpetology Notes* 15: 233–236.

Di Nicola, M.R. & Vaccaro, A. (2020). New data on the presence of the Aesculapian snake *Zamenis longissimus* (Laurenti, 1768) (Serpentes Colubridae) on Elba Island (Tuscany, Italy). *Biodiversity Journal* 11(2): 611–614. Doi: 10.31396/Biodiv.Jour.2020.11.2.611.614.

Dyugmedzhiev, A. (2020). Tail vibration – a newly described defensive behaviour of the Aesculapian snake *Zamenis longissimus*. *The Herpetological Bulletin* 154: 31–32. Doi: 10.33256/hb154.3132.

Geniez, P. (2018). *Snakes of Europe, North Africa and the Middle East: a photographic guide*. New Jersey, Oxfordshire: Princeton University Press. 384 pp.

Georgiev, D. & Mollov, I. (2016). Notes on the herpetofauna of Ismaros Mts. (Northern Greece, Rhodopi County). *Ecologica Montenegrina* 5: 41–43. Doi: 10.37828/em.2016.5.7.

Musilová, R., Zavadil, V., Marková, S. & Kotlík, P. (2010). Relics of the Europe's warm past: Phylogeography of the Aesculapian snake. *Molecular Phylogenetics and Evolution* 57(3): 1245–1252. Doi: 10.1016/j.ympev.2010.09.017.

NSSG - National Statistical Service of Greece (1995). Distribution of the country's area by basic categories of land use (Pre-census data of the Agriculture–Livestock census of the year 1991). Athens. http://dlib.statistics.gr/Book/GRESYE_02_0901_00006.pdf. Accessed on 29 October 2022.

Pafilis, P. & Maragou, P. (Eds.). (2020). *Atlas of Amphibians and Reptiles of Greece*. Nicosia, Cyprus: Broken Hill Publishers. 232 pp. [in Greek]. http://herpatlas.gr/wp-content/uploads/2021/02/Atlas_Amphibians_and_Reptiles_of_Greece.pdf. Accessed on 25 October 2022.

Petrov, B. (2004). The herpetofauna (Amphibia and Reptilia) of the Eastern Rhodopes (Bulgaria and Greece). In *Biodiversity of Bulgaria. 2. Biodiversity of Eastern Rhodopes (Bulgaria and Greece)*, 863–879 pp. Beron, P. & Popov, A. (Eds.). Sofia, Bulgaria: Pensoft & National Museum of Natural History.

Petrov, B.P., Tzankov, N., Strijbosch, H., Popgeorgiev, G. & Beshkov, V. (2006). The herpetofauna (Amphibia and Reptilia) of the Western Rhodopes mountain (Bulgaria and Greece). In *Biodiversity of Bulgaria. 3. Biodiversity of Western Rhodopes (Bulgaria and Greece)*, 863–912 pp. Beron, P. (Ed.). Sofia, Bulgaria: Pensoft & National Museum of Natural History.

Stille, B. & Stille, M. (2017). *The Herpetofauna of Corfu and Adjacent Islands*. Frankfurt, Germany: Edition Chimaira. 354 pp.

Strachinis, I. (2021). First evidence on the occurrence of the Greek newt *Lissotriton graecus* and the Aesculapian snake *Zamenis longissimus* on Evia Island, Greece. *Parnassiana Archives* 9: 115–117.

Valakos, E.D., Pafilis, P., Lymberakis, P., Maragou, P., Sotiropoulos, K. & Foufopoulos, J. (2008). *The Amphibians and Reptiles of Greece*. Frankfurt, Germany: Edition Chimaira. 463 pp.

Zadravec, M. & Lauš, B. (2011). Melanism variations in *Natrix natrix* (Linnaeus, 1758) and *Zamenis longissimus* (Laurenti, 1768) in Croatia. *Hyla* 2: 39–42.

Accepted: 25 January 2023

Please note that the Supplementary Material for this article is available online via the Herpetological Bulletin website: https://thebhs.org/publications/the-herpetological-bulletin/issue-number-164-summer-2023

The Herpetological Bulletin 164, 2023: 37–38
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.3738

Missing toes in the graphic spiny lizard *Sceloporus grammicus* from central Mexico

CÉSAR A. DÍAZ-MARÍN*, TONANTZIN CARMONA-ZAMORA & AURELIO RAMÍREZ-BAUTISTA

Laboratorio de Ecología de Poblaciones, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, km. 4.5 carretera Pachuca-Tulancingo, 42184, Mineral de La Reforma, Hidalgo, México

*Corresponding author e-mail: cesaardm@hotmail.com

One of the most reported morphological anomalies in lizards is missing toes. This may occur naturally for various reasons, including attempted predation (Hudson, 1996; Vervust et al., 2009), intraspecific aggression (Gvoždík, 2000; Vervust et al., 2009; Passos et al., 2013), or progressive digital necrosis (Hazell et al., 1985). The incidence of missing toes may vary across habitats (Vervust et al., 2009) and follow different sexual patterns, such as male-bias (Gvoždík, 2000; Vervust et al., 2009), female-bias (Passos et al., 2013), or no sexual bias (Hudson, 1996); longer digits are more likely to be missing (Vervust et al., 2009).

The graphic spiny lizard *Sceloporus grammicus* is widely distributed throughout most of the northern and central states of Mexico (Sites, 1982). During studies on the ecological aspects of this species in four different localities in central Mexico (three in Hidalgo and one in Puebla states), several lizards were captured (by hand, rubber banding, or noosing) that had missing toes (Table 1S - see Supplementary Material). Once captured, we photographed all lizards and measured their snout-vent length and body mass with a digital caliper (± 0.01 mm, Mitutoyo) and a spring balance (± 0.02, Pesola®), respectively. Considering all localities from the years 2018, 2021 and 2022, we collected a total of twelve adult lizards that had some missing toes, which we believe were missing naturally. Two thirds (8) were male and one third (4) were female. According to the definitions of Rothschild et al. (2012), six of these lizards had ectrodactyly (absence of one or more digits, Fig. 1A), two had brachydactyly (shortened digits, Fig. 1B), and four had both anomalies (Fig. 1C-D). All of these lizards were seen perching and performing their usual activities so that initially we failed to notice their missing toes. After inspection, all individuals were returned to their capture site.

Other *Sceloporus* species have been reported to have lost toes naturally (e.g. *Sceloporus merriami* and *Sceloporus occidentalis* [Huey et al., 1990]) but this is the first account of this anomaly in *S. grammicus*. It is interesting to note that the removal of toes as a means of marking lizards is claimed to have no apparent effect on either locomotor performance (Huey et al., 1990, Vervust et al., 2009) or the ability of lizards to climb (Paulissen & Meyer, 2000). Given that all the lizards we observed with missing toes were large adults (54.18–71.5 mm) and were performing their activities as usual, we consider that the naturally missing toes also probably had no effect on performance; as was the case in *S. merriami* and *S. occidentalis* (Huey et al., 1990). It appears from our study that male *S. grammicus* may lose toes more frequently than females; this could result from intraspecific aggression (Gvoždík, 2000; Vervust et al., 2009). However, given the small sample size no definite conclusion can be drawn about this. Further data collection is required.

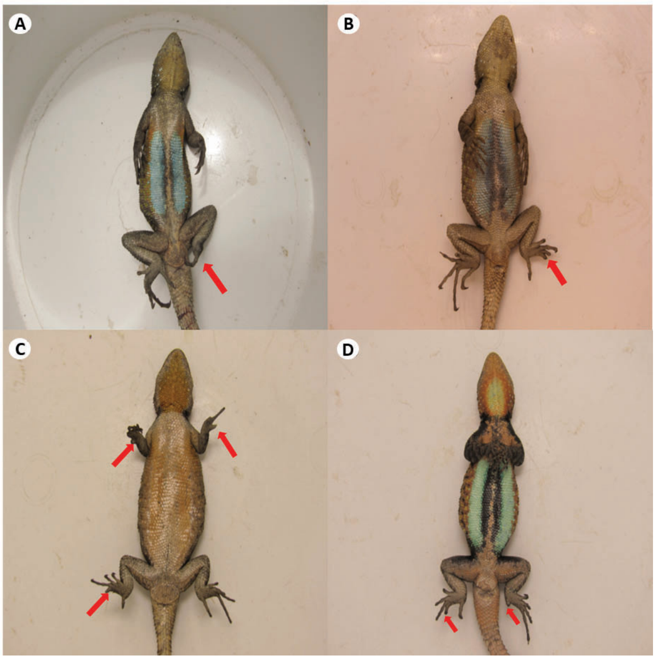


Figure 1. Naturally missing toes in *Sceloporus grammicus* from central Mexico - **A.** Male with ectrodactyly (absence of one or more digits), **B.** Male with brachydactyly (shortened digits), **C.** Female with both ectrodactyly and brachydactyly, **D.** Male with both ectrodactyly and brachydactyly. Red arrows show the missing toes.

ACKNOWLEDGEMENTS

We are grateful to authorities and land owners in the study areas for giving permission to access their land and providing accommodation during our fieldwork. We thank Lizzeth A. Torres-Hernández, S. Aldape, Ismael Reaño-Hernández, Ferdinand Torres-Ángeles, Irany Herrera-Tellez, Elizabeth Martín-Martín, Rubén Zúñiga, Aaron García-Rosales, Alejandra Bautista-Espinosa, and Elizabeth Ortiz-Escobar for their valuable assistance in the field. Fieldwork was approved under collecting permits SGPA/DGVS/06181/17,

SGPA/DGVS/004007/18, SGPA/DGVS/2970/20, and SGPA/DGVS/00959/22. A scholarship was granted to César A. Díaz-Marín (Number 771295) by Consejo Nacional de Ciencia y Tecnología.

REFERENCES

Gvoždík, L. (2000). Intrapopulation variation in injury frequencies in the sand lizard, *Lacerta agilis* (Squamata, Lacertidae). *Biologia Bratislava* 55: 557–561.

Hazell, S.L., Eamens, G.J. & Perry, R.A. (1985). Progressive digital necrosis in the eastern blue-tongued skink *Tiliqua scincoides* (Shaw). *Journal of Wildlife Diseases* 21: 186–188.

Hudson, S. (1996). Natural toe loss in southern Australian skinks: implications for marking lizards by toe-clipping. *Journal of Herpetology* 30: 106–110.

Huey, R.B., Dunham, A.E., Overall, K.L. & Newman, R.A. (1990). Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiological Zoology* 63: 845–872.

Passos, D.C., Galdino, C.A.B., Bezerra, C.H. & Zanchi, D. (2013). Indirect evidence of predation and intraspecific agression in three sympatric lizard species from a semi-arid area in northeastern Brazil. *Zoologia* 30: 467–469.

Paulissen, M.A. & Meyer, H.A. (2000). The effect of toe-clipping on the gecko *Hemidactylus turcicus*. *Journal of Herpetology* 34: 282–285.

Rothschild, B.M., Schultze, H.P. & Pellegrini, R. (2012). *Herpetological osteopathology: annotated bibliography of amphibians and reptiles*. Springer Science & Business Media. 450 pp.

Sites Jr, J.W. (1982). Morphological variation within and among three chromosome races of *Sceloporus grammicus* (Sauria: Iguanidae) in the north-central part of its range. *Copeia* 1982: 920–941.

Vervust, B., Van Dongen, S., Grbac, I. & Van Damme, R. (2009). The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. *Functional Ecology* 23: 996–1003.

Accepted: 3 February 2023

Please note that the Supplementary Material for this article is available online via the Herpetological Bulletin website: https://thebhs.org/publications/the-herpetological-bulletin/issue-number-164-summer-2023

The Herpetological Bulletin 164, 2023: 39–40
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.3940

First account of armadillo burrow use by *Caiman yacare* in Brazil

SEAN KEUROGHLIAN-EATON[1,2*], JOÃO EMÍLIO DE ALMEIDA-JÚNIOR[1], THOMAZ RICARDO FAVRETO SINANI[1], ANA ALICE CABRAL[1], KELVIN YUITI MORI[1], LAUANY LIMA SERAFIM[1] & DIEGO JOSÉ SANTANA[1]

[1]Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79002-970 Campo Grande, Mato Grosso do Sul, Brazil
[2]Programa de Pós-Graduação em Biodiversidade, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", 15054-000, São José do Rio Preto, São Paulo, Brazil

[*]Corresponding author e-mail: s.keuroghlian-eaton@unesp.br

Burrow-creating species are considered ecosystem engineers, and armadillos are one of the most important burrow digging animals in the Neotropics (Rodrigues et al., 2019). Their burrows can be used by other animals as shelter from fire (Friend, 1993), predators (Layne & Jackson, 1994; Waterman & Roth, 2007), as foraging sites (Desbiez & Kluyber, 2013), and can be used as a thermal refuge to escape from extreme environmental conditions (Walde et al., 2009; Pike & Mitchell, 2013). Even in some areas where armadillos are considered invasive species, 33 native species have been reported to use their burrows (Butler, 2020).

Many species of reptiles may exhibit hibernation-like behaviours, when faced with lack of food or drought, remaining inactive for long periods (Fagundes et al., 2016; Campos et al., 2004). This behaviour has already been recorded for many crocodilian species during droughts, including the yacare caiman *Caiman yacare* (Campos, 2020). The yacare caiman is a medium-sized crocodilian that occurs throughout the Pantanal wetlands, in Brazil, Bolivia and Paraguay (Campos et al., 2010). They can bury themselves in mud, hide in leaf litter, and remain in a comatose state for long periods as a way to save energy (Campos et al., 2004; Campos & Magnusson, 2011; Farias et al., 2013).

On 14 February 2022 at about 21:40 h, during fieldwork in the southern Pantanal, we found an individual *C. yacare* (Fig. 1) deep inside an armadillo burrow. This was at Pousada Aguapé, in the municipality of Aquidauana, state of Mato Grosso do Sul, Brazil at (20° 5'7.9296" S, 56° 1'36.8436" W). The armadillo burrow was likely made by either *Dasypus novemcinctus* (nine-banded armadillo) or *Euphractus sexcinticus* (six-banded armadillo). The burrow identification was based on the size of the hole, and that these are the most common armadillo species in the region (Abba & Superina, 2010; Rodrigues, 2002). The burrow was only 84 m away from a vazante (a drainage field) (Bazzo, 2012; Leite, 2021). It is well known that February is not the usual time for *C. yacares* to aestivate (Campos 2020), since it is one of the wetter and rainy months of the year (Faria et al., 2010). Nonetheless, the lack of rainfall in the last three year's rainy season could trigger such behaviour. The Pantanal has been facing one of the worst droughts in its history (Marengo et al., 2021; Naumann et al., 2022), which is consequently affecting the population of Pantanal caimans, which depend on water for their food, reproduction, and development.

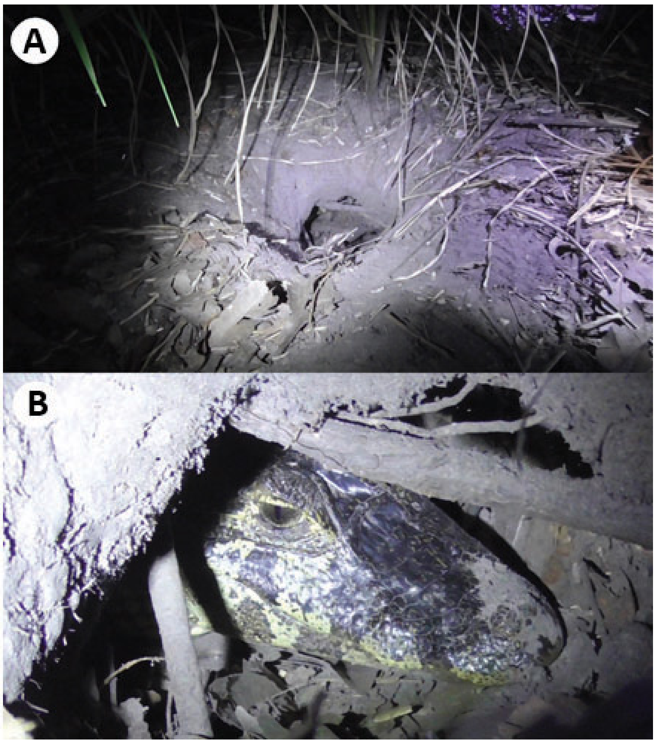


Figure 1. Use of an armadillo burrow by a *Caiman yacare* - **A.** General view of the burrow **B.** Medium sized *C. yacare* taking refuge in the armadillo burrow

Behaviours such as burying in mud or under leaf litter have already been recorded for *C. yacare* (Campos & Mourão, 2020; Campos et al., 2003). However, this is the first record of a *C. yacare* using an armadillo burrow as a refuge. The nearby vazante, which would normally be at least 30 cm deep in water at this time of year, was completely dry (Bazzo et al., 2012; Leite et al., 2021). It was so dry that there was no mud for the caiman to bury itself. We believe that the presence of the caiman in the burrow was an attempt by the animal to protect itself from this irregular dry period, avoiding hot temperatures, and minimising energy expenditure until its environment becomes more favourable. Since both *D. novemcinctus* (nine-banded armadillo) and *E. sexcinticus* (six-banded armadillo) are common in the region, their burrows may represent an important landscape feature for caimans, and even other Pantanal ectothermic species, during this severe drought.

REFERENCES

Abba, A.M. & Superina, M. (2010). The 2009/2010 armadillo red list assessment. *Edentata* 11(2): 135–184.

Bazzo, J.C., de Freitas, D.A.F., Silva, M.L.N., Cardos, E.L. & Santos, S.A. (2012). Aspectos geofísicos e ambientais do pantanal da Nhecolândia. *Revista de Geografia (Recife)* 29(1): 141–161.

Butler, Z.P. (2020). *Assessing the ecological roles of nine-banded armadillos (Dasypus novemcinctus) on Georgia's Barrier Islands*. Master of Science Thesis, Fort Lewis College, Athens Georgia. 96 pp.

Campos, Z., Llobet, A., Piña, C.I. & Magnusson, W.E. (2010). Yacare Caiman *Caiman yacare*. In *Crocodiles. Status Survey and Conservation Action Plan*. Third Edition. 23–28 pp. Manolis S.C. & Stevenson C. (Eds.). Crocodile Specialist Group: Darwin.

Campos, Z., Coutinho, M. & Magnusson, W. (2003). Terrestrial activity of caimans (*Caiman crocodilus yacare*). *Copeia* 3: 628–634.

Campos, Z., Coutinho, M.E. & Magnusson, W. (2004). Estivação de jacarés no Pantanal Sul. *Comunicado Tecnico Embrapa* 39: 1–3.

Campos, Z. & Magnusson, W.E. (2011). Emergence behavior of yacare caimans (*Caiman crocodilus yacare*) in the Brazilian Pantanal. *The Herpetological Journal* 21(1): 91–94.

Campos, Z. & Mourão, G. (2020). Como o jacaré-do-pantanal, *Caiman yacare*, resiste a períodos secos na região central do Pantanal. *Comunicado Técnico Embrapa* 165: 1–17.

Desbiez, A.L.J. & Kluyber, D. (2013). The role of giant armadillos (*Priodontes maximus*) as physical ecosystem engineers. *Biotropica* 45(5): 537–540.

Fagundes, A.C.A., Oliveira, A.C.L., Carmo, A.G.B., Carneiro, J.O.S. & Chagas, R.B. (2016). Mecanismo de sobrevivência dos animais no nordeste do Brasil. *Anais do Congresso Nordestino de Biólogos* 6: 439–442.

Faria, T.G., Cardoso, M.R.D. & Melo, D.C.D.R. (2010). Chuvas no Pantanal brasileiro: análise histórica e tendência futuro. In *Anais 3º Simpósio de Geotecnologias no Pantanal*, Cáceres, MT, 16-20 de outubro, Embrapa Informática Agropecuária/INPE. 170–180 pp.

Farias, I.P., Marioni, B., Verdade, L.M., Basseti, L., Coutinho, M.E., Mendonça, S.H.S.T., Vieira, T.Q., Magnusson, W.E. & Campos, Z. (2013). Avaliação do risco de extinção do jacaré-do-pantanal *Caiman yacare* (Daudin, 1802) no Brasil. *Biodiversidade Brasileira* 3(1): 21–30.

Friend, G.R. (1993). Impact of fire on small vertebrates in mallee woodlands and heathlands of temperate Australia: a review. *Biological conservation* 65(2): 99–114.

Layne, J.N. & Jackson, R.J. (1994). Burrow use by the Florida mouse (*Podomys floridanus*) in south-central Florida. *American Midland Naturalist:* 17–23.

Leite, E.F., de Carvalho, E.M., de Moraes, E.M.V. & Farias, F.R. (2021). Uso e ocupação da terra, aspectos físicos e econômicos do município de Aquidauana-MS. *Revista Pantaneira* 19: 1–19.

Marengo, J.A., Ambrizzi, T., Barreto, N., Cunha, A.P., Ramos, A.M., Skansi, M., Carpio, J.M. & Salinas, R. (2021). The heat wave of October 2020 in central South America. *International Journal of Climatology* 42: 2281–2298.

Naumann, G., Podesta, G., Marengo et al. (2022). *O episódio de seca extrema de 2019-2021 na bacia de La Plata*. Serviço das Publicações da União Europeia, Luxemburgo. 50 pp.

Pike, D.A. & Mitchell, J.C. (2013). Burrow-dwelling ecosystem engineers provide thermal refugia throughout the landscape. *Animal Conservation* 16(6): 694–703.

Rodrigues, F.H.G., Medri, I.M., Tomas, W.M. & Mourão, G.D.M. (2002). Revisão do conhecimento sobre ocorrência e distribuição de mamíferos do Pantanal. *Embrapa Pantanal-Documentos* (INFOTECA-E).

Rodrigues, T.F., Mantellatto, A.M.B., Superina, M. & Chiarello, A.G. (2019). Ecosystem services provided by armadillos. *Biological Reviews* 2019: 1–21

Walde, A.D., Walde, A.M., Delaney, D.K. & Pater, L.L. (2009). Burrows of desert tortoises (*Gopherus agassizii*) as thermal refugia for horned larks (*Eremophila alpestris*) in the Mojave Desert. *The Southwestern Naturalist* 54(4): 375–381.

Waterman, J.M. & Roth, J.D. (2007). Interspecific associations of Cape ground squirrels with two mongoose species: benefit or cost? *Behavioral Ecology and Sociobiology* 61(11): 1675–1683.

Accepted: 28 October 2022

The Herpetological Bulletin 164, 2023: 41–42
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.4142

Unilateral anophthalmia in a recently metamorphosed blue-sided leaf frog *Agalychnis annae* in Costa Rica

ADAM W. BLAND* & ELLIE J. MCLAREN

25 Place Road, Broadheath, Altrincham, WA14 4HH, UK

*Corresponding author e-mail: adamwbland@gmail.com

The blue-sided leaf frog *Agalychnis annae* is currently assessed by the IUCN as Vulnerable (IUCN, 2020). The species was considered endemic to Costa Rica, but a single individual has since been observed in Panama (Hertz et al., 2012). Within Costa Rica the known range of the species has recently been extended (Hildalgo-Mora et al., 2021). Despite these recent extensions the species is still found predominantly in a few, isolated populations within urban areas of the Central Valley of Costa Rica (Hoffmann, 2005; Hildalgo-Mora et al., 2021; Arguedas et al., 2022), and remains extirpated from previously known sites of more natural habitat, such as Monteverde and Tapantí national parks, where the species was once considered common (IUCN, 2020).

On 15 September 2022 at approximately 18:30 h in Heredia province Costa Rica, 1188 m a.s.l., during a visit to a breeding site for *A. annae* within an urban area, the authors observed a juvenile specimen at Gosner stage 45 (Gosner, 1960) with a missing right eye, where instead there was a small fold or scar, and a normal left eye (Fig. 1A & B). The specimen was active, observed on the upper surface of a leaf approximately 1 m above the ground and within 0.5 m from a breeding pond. It appeared otherwise in good health and body condition when compared to numerous individuals observed at the site at a similar developmental stage. Anophthalmia, the absence of one or both eyes, is a deformity that has previously been recorded in both anurans (Ramalho et al., 2017; Castro-Torreblanca & Blancas-Calva, 2021) and urodeles (Ayres et al., 2022). Individuals with this condition exhibiting the loss of a single eye have been known to survive to adulthood whilst appearing in otherwise good health (Ramalho et al., 2017).

This observation is the first recorded account of anophthalmia in a wild individual of this vulnerable species. Due to the fragile nature of amphibian populations, and observed dramatic declines recorded previously in this species (Hoffmann, 2005; IUCN, 2020), it is important to keep a record of observations that may be indicative of the health of their populations.

Figure 1. *Agalychnis annae* from Costa Rica with unilateral anophthalmia - **A.** Missing right eye, **B.** Unaffected left eye

REFERENCES

Arguedas, V., Barquero, M.D. & Mora, J.M. (2022). Population structure and dynamics, breeding activity and phenology of the blue-sided treefrog (*Agalychnis annae*). *Biotropica* 54(2): 455–466.

Ayres, C., Asensi-Cabirta, M. & Dominguez, M. (2022). Anophthalmia in Palmate Newts (*Lissotriton helveticus*) in Galicia (Northwestern Spain). *Reptiles & Amphibians* 29: 113–114.

Castro-Torreblanca, M. & Blancas-Calva, E. (2021). Anophthalmia in a juvenile pine toad, *Incilius occidentalis* (Anura: Bufonidae), from Laguna de Tixtla, Guerrero, Mexico. *Reptiles & Amphibians* 28(1): 22–23.

Gosner, K.L. (1960). A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica* 16: 183–190.

Hertz, A., Lotzkat, S., Carrizo, A., Ponce, M., Köhler, G. & Streit, B. (2012). Field notes on findings of threatened amphibian species in the central mountain range of western Panama. *Amphibian and Reptile Conservation* 6(2): 9–30.

Hidalgo-Mora, E., Valverder-Castillo, A. & Abarca, J.G. (2021). Range Extension of the Blue-Sided Leaf Frog, *Agalychnis annae* (Anura: Hylidae): Using Citizen Science across Suburban Areas of Costa Rica. *Reptiles & Amphibians* 28(2): 264–267.

Hoffmann, H. (2005). Some ecological notes on *Agalychnis annae* (Anura: Hylidae). *Brenesia* 65: 73–77.

IUCN SSC Amphibian Specialist Group & NatureServe. (2020). *Agalychnis annae*. *The IUCN Red List of Threatened Species 2020*: e.T55288A158518518. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T55288A158518518.en. Accessed on 18 October 2022.

Ramalho, W.P., Maffei, F., Guerra, V., Da Silvia, D.P., Matos, L.R.A. & Vieira, L.J.S. (2017). Anophthalmia in adults of two Amazonian treefrogs (Anura:Hylidae). *The Herpetological Bulletin* 139: 43–44.

Accepted: 3 November 2022

The Herpetological Bulletin 164, 2023: 43
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.43

Smooth newts *Lissotriton vulgaris* as more than just occasional items in the diet of the Eurasian kingfisher *Alcedo atthis*

NIGEL DAVIES[1*] & STEVEN J.R. ALLAIN[2]

[1]The Cottage, Cory Manor, West Putford, Devon, EX22 7XB, UK
[2]Cambridgeshire & Peterborough Amphibian and Reptile Group

[*]Corresponding author e-mail: nigeldavies@ymail.com

Smooth newts *Lissotriton vulgaris* are a widespread and common newt species found throughout Europe, growing to approximately 11 cm in length (Speybroeck et al., 2016). The species is widespread in Great Britain and is well adapted to urban environments, often being observed in garden ponds (Beebee & Griffiths, 2000) and may also inhabit natural ponds, lakes, and slow-flowing streams (Speybroeck et al., 2016). In February or March, adults enter water to breed and by July most have left water for terrestrial habitats, although some remain in water until September or October and then leave to locate hibernation sites in the terrestrial environment. Metamorphs remain on land until they are sexually mature.

The Eurasian kingfisher *Alcedo atthis* is an unmistakable blue bird found throughout Eurasia, that may grow to 16 cm in length (Fry & Fry, 2010). The species inhabits clear, slow-flowing streams and rivers, ponds and lakes where they tend to perch on branches and rushes, near open water within a few metres of the surface (Fry & Fry, 2010). The majority of the diet of the Eurasian kingfisher is freshwater fish (Raven, 1986; Reynolds & Hinge, 1996). However, other prey items are occasionally taken including aquatic invertebrates and amphibians such as the pool frog *Rana esculenta*, fire-bellied toads *Bombina* sp., and newts (Čech & Čech, 2015; Novčić & Simonović, 2018), although such instances are apparently rare (Fry & Fry, 2010; Čech & Čech, 2015). When diving for prey, kingfishers usually reach depths of less than 25 cm, and on removal from water the prey is beaten head first on a hard surface until subdued, after which they are swallowed head first (Fry & Fry, 2010).

On 31 October 2022 at 10:44 h, a male Eurasian kingfisher was videoed by a trailcam (Browning Defender BTC 9-D) feeding on a smooth newt (Fig. 1) that it had caught in a pond at Cory Manor, West Putford, Devon. Throughout the next two days the kingfisher was videoed a number of times catching more smooth newts at the same location, and feeding upon them on a wooden jetty where they were subdued (BHS video, 2023). Before the trailcam was set up, the kingfisher had been observed to capture and consume 11 smooth newts in a single day, prompting further investigation of this behaviour.

The pond where the newts were caught was 180 cm at the deepest point, with the kingfisher catching them from a part of the pond that was approximately 120 cm deep, indicating that the newts were active within the water column. These observations provide the first evidence of smooth newts as more than purely occasional items in the diet of the Eurasian kingfisher.

Figure 1. Frame taken from video footage (slightly blurred) of a male Eurasian kingfisher *Alcedo atthis* feeding on a smooth newt *Lissotriton vulgaris* in the grounds of Cory Manor, West Putford, Devon, England (for more details see BHS video, 2023)

REFERENCES

Beebee, T.J.C. & Griffiths, R.A. (2000). *Amphibians and Reptiles*. London, HarperCollins. 270 pp.

BHS video (2023). A Eurasian kingfisher *Alcedo atthis* feeding on a smooth newt *Lissotriton vulgaris*. Recorded by Nigel Davies. https://youtu.be/a4j_j3G5LJs.

Čech, M. & Čech, P. (2015). Non-fish prey in the diet of an exclusive fish-eater: the Common Kingfisher *Alcedo atthis*. *Bird Study* 62: 457–465.

Fry, C.H. & Fry, K. (2010). *Kingfishers, bee-eaters and rollers*. A&C Black.

Novčić, I. & Simonović, P. (2018). Variation in the diet of the Common Kingfisher *Alcedo atthis* along a stream habitat. *Ornithological Science* 17: 79–85.

Raven, P. (1986). The size of minnow prey in the diet of young kingfishers *Alcedo atthis*. *Bird Study* 33: 6–11.

Reynolds, S.J. & Hinge, M.D.C. (1996). Foods brought to the nest by breeding Kingfishers *Alcedo atthis* in the New Forest of southern England. *Bird Study* 43: 96–102.

Speybroeck, J., Beukema, W., Bok, B. & Van Der Voort, J. (2016). *Field Guide to the Amphibians and Reptiles of Britain and Europe*. London, Bloomsbury Publishing. 432 pp.

Accepted: 9 December 2022

The Herpetological Bulletin 164, 2023: 44-45
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.4445

African Spurred Tortoise *Centrochelys sulcata*: Range extension to Algeria

BELKACEM AIMEN BOULAOUAD[1,2], BADIS BAKHOUCHE[1,3], BENHAFID FRIEL[3] & DANIEL ESCORIZA[4*]

[1]Laboratory of Biological Oceanography and the Marine Environment (LOBEM), Faculty of Biological Sciences, University of Science and Technology of Houari Boumediene (USTHB), BP 32, El Alia, Bab Ezzouar, 16111 Algiers, Algeria
[2]Université Mohamed El Bachir El Ibrahimi de Bordj Bou Arréridj, El-Anasser, 34030 Bordj Bou Arréridj, Algeria
[3]Laboratory of Eco-Biology Animals (L.E.B.A.), École Normale Supérieure de Kouba Bachir El Ibrahimi, BP 92, 6050 Algiers, Algeria
[4]GRECO, University of Girona, 17001 Girona, Spain

[*]Corresponding author e-mail: drdanielescoriza@gmail.com

The African spurred tortoise *Centrochelys sulcata* (Miller, 1779) is the largest extant continental tortoise and the largest tortoise in Africa (Petrozzi et al., 2021). This species extends throughout the Sahel, between Mauritania (where it reaches the Atlantic coast) to the coast of Eritrea (Red Sea) and in Yemen and Saudi Arabia, where it may now be extirpated (Petrozzi et al., 2021) (Fig. 1). In recent assessments its presence in Algeria has been considered Uncertain (IUCN Red List; Petrozzi et al., 2021) even though there are geographically close records from northern Mali and Niger (Petrozzi et al., 2020).

In October 2022 a female of this species (Fig. 2) was found by BAB & BB in the region of Timiaouine, close to the border of Mali (20° 27’29.59” N, 1° 51’29.35” E). The only species of tortoise previously reported from Algeria is the Mediterranean spur-thighed tortoise *Testudo graeca*, a small-sized tortoise present along the Mediterranean coast, Haut Plateaux and pre-Saharan Atlas, but not in the central Sahara and Sahel (Escoriza et al., 2022). Individuals of both species show pronounced thigh spurs, but the tortoise from Timiaouine differs from *T. graeca* by having sharp and triangular anal scutes (Fig. 2) (more rectangular in *T. graeca*), more sculptured plastron, larger cuspidal scales in forelimbs, and yellow claws on the foot (black in Algerian specimens of *T. graeca*; Escoriza et al., 2022).

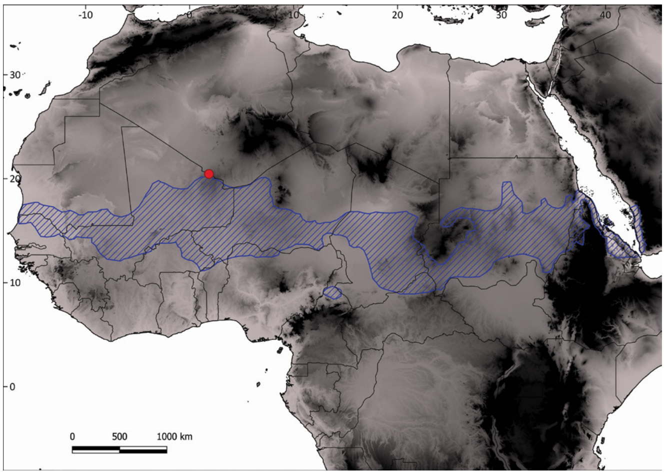


Figure 1. Map of north Africa showing the known range of *Centrochelys sulcata* based on IUCN (blue polygon) and the newly discovered population (red dot)

Figure 2. Female of the *Centrochelys sulcata* (dorsal, ventral), Timiaouine southern Algeria

In Timiaouine, *C. sulcata* was found in an Acacia savannah, which is the typical habitat for this species throughout the Sahel (Petrozzi et al., 2020). The south of Algeria has not been intensely surveyed and range extensions of several vertebrate species, including reptiles, have recently been reported from there (e.g. Bendjeddou et al., 2014; Bakhouche & Escoriza, 2017). The presence of *C. sulcata* in regions bordering Mali suggests that these Timiaouine and Malian populations could be contiguous. Future studies will allow us to assess the extent of the range of *C. sulcata* in southern Algeria and the genetic affinities of this newly discovered population.

ACKNOWLEDGEMENT

We thank Atouet Ismail for the two photographs shown in Figure 2.

REFERENCES

Bakhouche, B. & Escoriza, D. (2017). Genus *Malpolon*: New distribution area in Algeria. *The Herpetological Bulletin* 140: 35–36.

Bendjeddou, M.L., Bakhouche, B. & Bouslama, Z. (2014). A new locality for *Tadarida teniotis* (Rafinesque, 1814) (Mammalia, Chiroptera, Molossidae) in Algeria. *Natura Rerum* 3: 37–39.

Escoriza, D., Díaz-Paniagua, C., Andreu, A. & Ben Hassine, J. (2022). *Testudo graeca* Linnaeus 1758 (Western Subspecies Clade: *Testudo g. graeca, T. g. cyrenaica, T. g. marokkensis, T. g. nabeulensis, T. g. whitei*) – Mediterranean Spur-thighed Tortoise, Moorish Tortoise, Libyan Tortoise, Moroccan Tortoise, Tunisian Tortoise, Souss Valley Tortoise. *Chelonian Research Monographs* 5(16): 117.1–18.

Petrozzi, F., Hema, E.M., Demaya, G.S., Benansio, J.S., Eniang, E.A., Diagne, T., Segniagbeto, G.H. & Luiselli, L. (2020). *Centrochelys sulcata* (Miller 1779) – African Spurred Tortoise, Grooved Tortoise, Sahel Tortoise, Tortue Sillonnée. *Chelonian Research Monographs* 5(14): 110.1–16.

Petrozzi, F., Luiselli, L., Hema, E.M., Diagne, T., Segniagbeto, G.H., Eniang, E.A., Leuteritz, T.E.J. & Rhodin, A.G.J. (2021). *Centrochelys sulcata*. *The IUCN Red List of Threatened Species 2021*: e.T163423A1006958.

Accepted: 1 December 2022

The Herpetological Bulletin 164, 2023: 46
NATURAL HISTORY NOTE

https://doi.org/10.33256/hb164.46

Albinism in the southern spectacled salamander *Salamandrina terdigitata*

MASSIMO CAPULA[1*] & GAETANO ALOISE[2]

[1]Museo Civico di Zoologia, Via Aldrovandi 18, 00197 Roma, Italy
[2]Museo di Storia Naturale e Orto Botanico, Università della Calabria, Via Savinio, Edif. Polifunzionale, I-87036 Rende (Cosenza), Italy

[*]Corresponding author e-mail: massimo_capula@yahoo.it

We present here the first documented case of albinism in *Salamandrina terdigitata*. This is a tiny terrestrial salamander (maximum total length: females 88 mm; males 75 mm) endemic to southern Italy, where it occurs from Campania to Calabria (Angelini et al., 2007; Di Nicola et al., 2019).

The specimen (collection number MSNC 116) was identified in the herpetological collection of the Museo di Storia Naturale e Orto Botanico dell'Università della Calabria (Rende, Cosenza, southern Italy). It was an adult male (Fig. 1 A & B) 61 mm long (head-body length: 26 mm, tail length: 35 mm), weighing 3 g. Intergumentary pigment was completely lacking on the back, head, belly, limbs and tail (Fig. 1B). It was collected on 24 April 1992 in the Vallone del Gardo (municipality of Pietrapaola, Province of Cosenza, Calabria, southern Italy; 39° 29'17'' N, 16° 48'20'' E) during an entomological survey (Mazzei et al., 2006).

The genus *Salamandrina* is endemic to Peninsular Italy and includes two species: *Salamandrina perspicillata* and *S. terdigitata*. In the case of *S. perspicillata* there have been reports of individuals with partially depigmented backs (Lanza & Canestrelli, 2002; Crucitti et al., 2016) and partial leucism (Ramorino, 1863; Lanza, 1946). In the case of *S. terdigitata*, Angelini et al. (2007) reported a personal communication (without year and month) by S. Tripepi concerning an observation in the field (Pollino Mount, southern Italy) of an albino (leucistic?) individual *S. terdigitata* but gave no other relevant details. Thus to our knowledge the case we report here is the first documented record of complete albinism for both *Salamandrina terdigitata* and the genus *Salamandrina*.

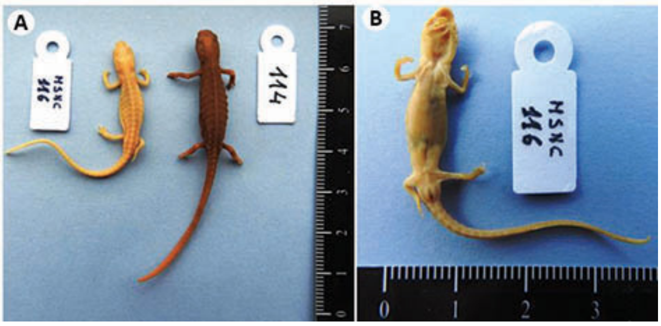


Figure 1. *Salamandrina terdigitata* from the Vallone del Gardo (municipality of Pietrapaola, Province of Cosenza, Calabria, southern Italy) - **A.** Albino male (left) and normally pigmented female (right), **B.** Ventral view of the albino male (centimetre scale)

REFERENCES

Angelini, C., Vanni, S. & Vignoli, L. (2007). *Salamandrina terdigitata* (Bonnaterre, 1789) *Salamandrina perspicillata* (Savi, 1821). In *Fauna d'Italia. Vol XLII. Amphibia*, 228–237 pp. Lanza, B., Andreone, F., Bologna, M.A., Corti, C. & Razzetti, E. (Eds.). Edizioni Calderini, Il Sole 24 ORE, Editoria specializzata S.r.l., Bologna.

Crucitti, P., Doglio, S. & Tringali, L. (2016). Depigmented phenotypes inside a *Salamandrina perspicillata* population of the Campagna Romana (Latium, Italy). *Natural History Sciences* 3(1): 15–20.

Di Nicola, M., Cavigioli, L., Luiselli, L. & Andreone, F. (2019). *Anfibi & Rettili d'Italia* Edizioni Belvedere, Latina. 576 pp.

Lanza, B. (1946). Un caso di semi albinismo in *Salamandrina terdigitata* (Lacépède). *Natura (Milano)* 37(1–2): 18–20.

Lanza, B. & Canestrelli, D. (2002). A typische Färbungbei *Salamandrina terdigitata* (Lacépède, 1788) und *Bufo viridisviridis* Laurenti, 1768. *Salamandra* 38(2): 105–108.

Mazzei, A., Novello, M.G., Bonacci, T. & Brandmayr, P. (2006). Comunità di Coleotteri Carabidi in habitat su suoli argillosi della formazione Gessoso-Solfifera in Calabria: conseguenze di una possibile 'desertificazione'. In *Ecologia. Atti del XV Congresso Nazionale della Società Italiana di Ecologia* (Torino, 12–14 settembre 2005, 1–7 pp. Comoglio, C., Comino, E. & Bona, F. (Eds.).

Ramorino, G. (1863). *Appunti sulla storia naturale della Salamandrina perspicillata*. Tipografia Sordo-Muti, Genova. 38 pp.

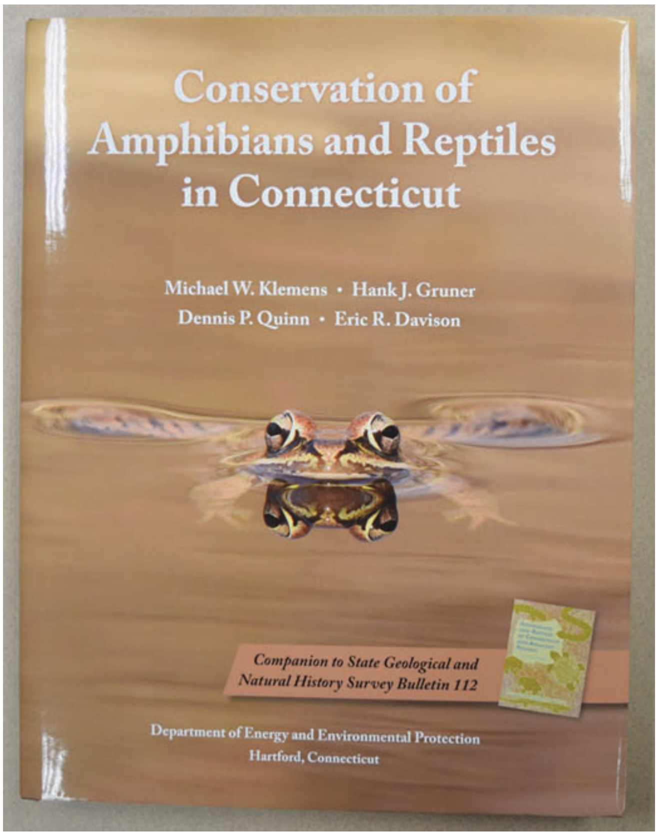
Accepted: 1 January 2023

The Herpetological Bulletin 164, 2023: 47–48
BOOK REVIEW

https://doi.org/10.33256/hb164.4748

Conservation of Amphibians and Reptiles in Connecticut

Michael W. Klemens, Hank J. Gruner, Dennis P. Quinn & Eric R. Davison
Connecticut Department of Energy and Environmental Protection, 2021
ISBN 978-0-578-87182-0, 302 pp.



Regional guides to herpetofauna are often labours of love by highly dedicated fieldworkers, who spend much of their lifetimes surveying wetlands, woodlands, mountains and moorlands in pursuit of the greater cause. Such publications can take many forms, ranging from a few distribution maps for a given state or county, to more comprehensive natural histories. Given that they often reflect the life's work of an individual (or a small group of individuals) regional accounts are frequently a snapshot in time. Consequently, it is unusual for regional surveys to be continued on a timeframe that spans decades, and therefore comprehensively document the changes that have occurred in the interim. This lavishly produced volume does all of this, and much more.

In 1993, Michael Klemens authored 'Amphibians and Reptiles of Connecticut and Adjacent Regions'. In over 300 pages of lucid natural history based on his PhD research as one of the very first herpetology students at the University of Kent, the author described the results of some 17 years of painstaking fieldwork on all of the amphibian and reptile species that occur in this region (Klemens, 1993). In this entirely new volume – and with the assistance of his co-authors – Dr Klemens provides an equally comprehensive update about the status and distribution of herpetofauna in Connecticut some thirty years after his original surveys.

An introductory chapter sets the scene and provides some background to Connecticut and its herpetological fauna. At 5543 square miles Connecticut is the third smallest state in the USA, but comprises a remarkable diversity of habitats and species. Indeed, the Introduction to the book lists 12 salamanders, 11 frogs, 8 (non-marine) turtles, 1 lizard and 14 snakes. In addition to these, there are two non-native turtles and the introduced Italian wall lizard *Podarcis siculus*. A concise table neatly summarises how conservation challenges have changed between 1993 and 2021. As in the United Kingdom and elsewhere, inconsistency in the scope, intensity and standardisation of surveys by consultant biologists has remained largely unchanged over recent decades. The authors cogently point out the limitations of reaching conclusions from mapped data. Point locations are not indicative of viable populations, an issue neatly illustrated by records of bog turtles. Many records of bog turtles were of individuals crossing roads while vacating sites that were no longer suitable, so sightings have increased while populations have declined.

Chapter 1 goes on to describe the biogeography of the State and the landscapes and habitats that these species occupy. Interestingly, power-line rights-of-way in rural areas can provide important areas for some species and can be managed sensitively, including the incorporation of wetlands. The implications of life history for conservation are introduced here, as are the principles of the 'conservation guilds' that underpin the conservation strategies that emerge later on in the book.

Chapter 2 moves on to discuss anthropogenic stressors. Connecticut has 65 % of its area comprising wildlife-urban interfaces, which is the highest of any state in the USA. Intriguingly, timber rattlesnakes are attracted to areas of fractured stones that cover gas pipelines. Because the pipelines are warm, the rattlesnakes have shifted their hibernation and birthing sites accordingly, but by doing so put themselves at risk of disturbance. Given ongoing discussions about how significant railway lines in Britain are as barriers to amphibians and reptiles, I was also intrigued to read that – with the possible exception of turtles – most amphibians and reptiles can traverse railroads in Connecticut. Railroads also seem to have provided a conduit for the dispersal of non-native Italian wall lizards. These are just examples of numerous nuggets of useful information in this chapter, contained within a lucid narrative with sections on habitat fragmentation, climate change, harvesting, disease and invasive species. Indeed, I would recommend this chapter to anyone seeking a synthetic and readable account of these threats to herpetofauna in general.

Some two-thirds of the book (Chapters 3–7) apply the concept of conservation guilds to classify the habitats, threats and management to the Connecticut herpetofauna. The authors point out that traditional conservation management tends to focus on individual species, threat or habitats. Given the complex interactions between these factors, a more cost-effective methodology may be to adopt an assemblage-based approach where the issues facing a 'guild' of species can be tackled collectively. Research has shown that the wider, ecological impact of a development may be an order of magnitude higher than that on the development footprint alone. Classifying species into one or more of 'Rare habitat dependent', 'Early successional habitat dependent' and 'Habitat mosaic dependent' or 'Long-lived/delayed sexual maturity/low fecundity' can inform the state-level listings and status of each species. Each species is assigned in this way along with an informative map of past and present records in relation to geographical features. A fascinating interlude at the end of Chapter 3 is a history of bounty hunting of rattlesnakes in the state. With habitat fragmentation and roadkills now taking their toll, the authors predict that just 8–10 % of the state's timber rattlesnake population may remain by 2050.

Chapters 4–7 systematically discuss the challenges facing each of the four conservation guilds that the species have been assigned to. These embrace ecology, habitat succession and resilience, and land-use planning. When it comes to mitigation banking, the authors rightfully point out the importance of distinguishing between remnant populations of long-lived species that are effectively the 'living dead', from more viable populations that have a chance of a long-term future.

Chapter 8 concludes the volume by focusing on local governance with an assessment of the issues concerning gaps in federal and state protection. In a densely populated state such as Connecticut, the key word here is 'fragmentation', and the chapter concludes with twelve strategies to improve land-use decision making. All of these recommendations (e.g. 'Avoid check-the-box conservation', 'Standardize assessment methods', 'Know your resources'), should resonate with anyone who has tried navigating the ecological, logistical, financial and political minefield that is land-use planning. Their relevance therefore goes way beyond the state boundaries of Connecticut.

It is refreshing – and indeed quite rare – to see long-term scholarly survey and research translated so effectively into a volume that is directly related to on-the-ground conservation. Apart from the maps, the whole book is lavishly illustrated with superb photographs of the species and the habitats and some colourful and informative diagrams of different habitats and landscapes and how they are used. There is a comprehensive glossary, list of acronyms and extensive reference list, but where these are incorporated into the text they do not detract from a readable and incisive narrative. This book is much more than a regional guide. Anyone seeking a general and well-informed introduction to herpetological conservation that uses Connecticut as a detailed case study would benefit from reading it.

All proceeds from the sale of the book go toward the conservation of reptiles and amphibians. This publication is available for purchase via the Connecticut Department of Energy and Environmental Protection's online bookstore: https://www.ctdeepstore.com/Wildlife_c24.htm. At $54.95, this is a quality publication at a very competitive price.

REFERENCE

Klemens, M.W. (1993). *Amphibians and Reptiles of Connecticut and Adjacent Regions*. State Geological and Natural History Survey of Connecticut, Bulletin No. 112.

RICHARD A. GRIFFITHS
Durrell Institute of Conservation and Ecology, University of Kent, Canterbury, Kent, CT2 7NR, UK
E-mail: R.A.Griffiths@kent.ac.uk

Received: 23 December 2022

BRITISH HERPETOLOGICAL SOCIETY COUNCIL 2022/2023

Society address: c/o Zoological Society of London, Regent's Park, London, NW1 4RY
Website: www.thebhs.org

President	Dr. Gerado Garcia	president@thebhs.org
Chairman:	Mr. Mark Hollowell (co-opted)	chair@thebhs.org
Treasurer:	Mr. Michael Wise (co-opted)	treasurer@thebhs.org
Secretary:	Mr. T. Rose (co-opted)	secretary@thebhs.org
Finance Officer:	Vacant	
The Herpetological Journal		
Scientific Editor:	Dr. Marcio R. Pie	bhsherpetologicaljournal@gmail.com
The Herpetological Bulletin		
Scientific Editor:	Prof. Rick Hodges	herpbulletineditor@gmail.com
The NatterJack		
Editor:	Mrs. Suzanne Simpson	suziesimpson0@gmail.com
Librarian:	Mr. David Bird	drbird.herp1@talktalk.net
Development Officer:	Mr. Mark Hollowell	mark22@btinternet.com
Website and Communications Officer:	Vacant	
UK Conservation Officer:	Dr. Steven Allain	steveallain@live.co.uk
Trade Officer:	Ms. Alexia Hesten	a.hesten@2022.ljmu.ac.uk
Meetings Organiser:	Mr. Paul Eversfield	paul.eversfield@btinternet.com
Head of International Conservation and Captive Breeding Projects	Dr. Simon Townson	s.townson@imperial.ac.uk
Education and YH Officer	Ms. Jennie Shea	education@thebhs.com
Research Officer	Dr. Chris Gleed-Owen	chris@cgoecology.com

Ordinary Members

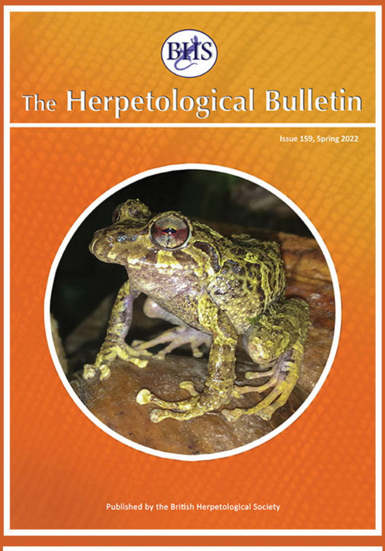
Mr. Stephen Copley	stephenccopley@btinternet.com (3rd year)
Mr. Vince Goodall	vincegoodall@gmail.com (1st year)
Ms. Catherine Whatley	catherinewhatley4@gmail.com (1st year)

Fellows of the British Herpetological Society

Prof. T.J.C. Beebee, Mr. D. Bird, Prof. J.E. Cooper, Mr. J. Coote, Prof. R.A. Griffiths, Mr. T. A. Rose, Mr. V. F. Taylor, Dr. S. Townson, Mr. R. Meek

Past Presidents (retiring date)

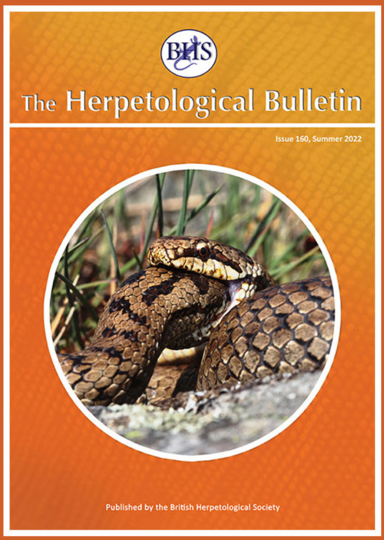
Dr. M. A. Smith (1956), Dr. J.F.D. Frazer (1981), The Earl of Cranbrook (1990), Prof. J.L. Cloudsley-Thompson (1996), Dr. R. Avery (1997), Dr. H. Robert Bustard (2005), Prof. T.J.C. Beebee (2013), Prof. R. A. Griffiths (2018)



The Herpetological Bulletin

Issue 159, Spring 2022

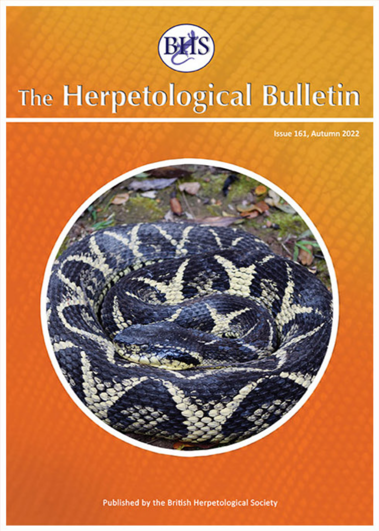
Published by the British Herpetological Society



The Herpetological Bulletin

Issue 160, Summer 2022

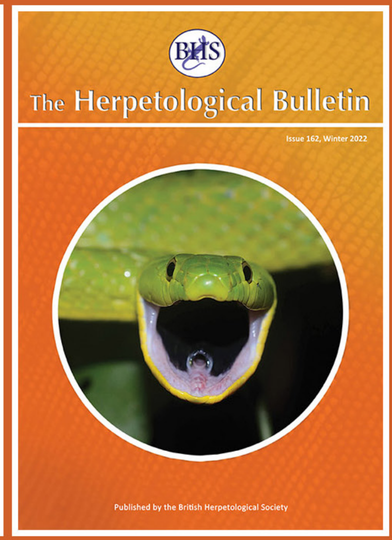
Published by the British Herpetological Society



The Herpetological Bulletin

Issue 161, Autumn 2022

Published by the British Herpetological Society



The Herpetological Bulletin

Issue 162, Winter 2022

Published by the British Herpetological Society