



# The Herpetological Bulletin

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# THE HERPETOLOGICAL BULLETIN

The Herpetological Bulletin is produced quarterly and publishes, in English, a range of articles concerned with herpetology. These include society news, full-length papers, new methodologies, natural history notes, book reviews, letters from readers and other items of general herpetological interest. Emphasis is placed on natural history, conservation, captive breeding and husbandry, veterinary and behavioural aspects. Articles reporting the results of experimental research, descriptions of new taxa, or taxonomic revisions should be submitted to The Herpetological Journal (see inside back cover for Editor's address).

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**Front Cover:** A female specimen of the yellow-bellied toad, *Bombina variegata scabra* (Küster, 1843) from the only known locality in Turkey (Karacahasan Mt., Enez district, Edirne province, Turkish Thrace). See article on page 25.

# Zoological Society of London: contributions towards advancing the field of herpetology through conservation, research, captive management and education

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## INTRODUCTION

Founded in 1826, the Zoological Society of London (ZSL) is a world-renowned centre of excellence for conservation science and applied conservation. ZSL's mission is to promote and achieve the worldwide conservation of animals and their habitats and this is realised by carrying out field conservation and research in over 50 countries, conducting original scientific research, and through education and awareness at its two zoos, London Zoo and Whipsnade Zoo, inspiring people to take conservation action. ZSL has always had a strong herpetological focus and the emphasis on reptiles and amphibians is perhaps now stronger than ever before with many different aspects of herpetology being advanced through close collaboration between the different departments within ZSL.

## LIVING COLLECTIONS

ZSL's reptile and amphibian house was opened in 1927. The current collection is composed of 70 reptile and 29 amphibian species. The collection focuses on displaying a diverse array of reptiles and amphibians to an audience of 1.3 million visitors per year, educating and inspiring our visitors, and supports applied research on aspects of species biology. ZSL's herpetology team has a strong focus on developing evidence-based husbandry practices and advancing the field of herpetoculture, focusing especially on the management of venomous reptiles (Gill, 2014), training (Bryant et al., 2015), enrichment (Bryant & Kother, 2014; Januszczak et al., 2016), water quality (Michaels et al., 2015a), and UVB provision (Baines et al., 2015; Tapley et al., 2015a).

Zoos are in a unique position to undertake research that may underpin conservation efforts (e.g. Michaels et al., 2016a). Key areas of research include the development of husbandry techniques, which are frequently subtle,

complex and highly specific, and elucidation of species biology, which is often difficult, if not impossible, to observe in the field. ZSL's herpetology team recently bred the Critically Endangered Lake Oku clawed frog (*Xenopus longipes*) by replicating in captivity environmental data collected from the field (Michaels et al., 2015), and used this success to document the reproductive biology and larvae of the species (Tapley et al., 2015b). ZSL has a large collection of caecilians, a group of amphibians about which little is known. Through partnership with the Natural History Museum, London, UK, husbandry protocols have been developed (Tapley et al., 2014a) and significant advances in disease diagnosis and subsequent treatment have been made (Gower et al., 2013; Rendle et al., 2015). These findings have direct relevance to the conservation of caecilians in the wild.

ZSL's herpetology team is directly involved in ZSL's wider conservation and research projects, most notably the long-term conservation programmes for mountain chicken frog (*Leptodactylus fallax*) (coordinated by Durrell Wildlife Conservation Trust, see Adams et al., 2014; Tapley et al., 2014b), Mallorcan midwife toad (*Alytes muletensis*, Fig. 1) and Chinese giant salamander (*Andrias davidianus*, Fig. 2) (Tapley et al., 2015c). The team is currently assisting in the development of initiatives focusing on gharial (*Gavialis gangeticus*) in Nepal (Fig. 3), amphibians (in partnership with the Australian Museum and Hoang Lien National Park (Tapley et al., 2017), freshwater turtles in Vietnam (in partnership with The Asian Turtle Program), and amphibians in the Western Ghats of India (Harpalini et al., 2015). Capacity building is another vital component of our work, which is achieved through the mentoring of EDGE Fellows (EDGE of Existence, 2016) and other conservationists, the hosting of interns and students from a variety of institutions and countries, and the delivery of training courses addressing topics as varied as venomous reptile management and amphibian conservation (Tapley et al., 2015d). Thus, the team invest in the future of





**Figure 1.** The Mallorcan midwife toad (*A. muletensis*) has been the focus of field interventions to mitigate the impacts of chytridiomycosis



**Figure 2.** A Chinese giant salamander (*A. davidianus*) that was caught and later released as part of our range wide ecological surveys in China

herpetological conservation around the world while delivering direct conservation outputs in the present.

The ZSL veterinary department, consisting of five veterinary clinicians, six veterinary nurses, a veterinary pathologist and assistant, microbiologist, animal welfare officer, administrator and animal keeper, works closely with the ZSL herpetology team to promote excellence in the health and welfare of amphibians and reptiles. In addition to providing health care for the reptiles and amphibians at our zoos, the veterinary team is a key component of ZSL's *in situ* conservation programmes. Preventive medicine forms an important part of the veterinary department's role in ZSL's captive breeding and reintroduction programmes through development of quarantine and health screening procedures and protocols. In conjunction with the herpetology team, the veterinary department also helps to further knowledge of best husbandry practices for captive amphibians and reptiles; recent outputs include understanding UV-B radiation requirements for the Critically Endangered mountain chicken frog (Tapley et al., 2015a) and substrate preference in the caecilian *Geotrypetes seraphini* (Tapley et al., 2014a). Advancing the detection and management of disease forms an important area of research, and recent examples include the first reported diagnosis of intestinal adenocarcinoma in the mountain chicken frog (Jaffe et al., 2015), the first case of lethal chytridiomycosis in caecilian amphibians (Gower et al., 2013), the first report of *Batrachochytrium dendrobatidis* (Bd) infection in a wild neotropical caecilian (Rendle et al., 2015), and the effective use of itraconazole for treatment of Bd infection in captive caecilians (Rendle et al., 2015). In association with ZSL's Institute of Zoology, the veterinary team has assisted with development of disease risk analysis tools for herpetofauna translocations. Several collaborative research projects between the ZSL veterinary department and herpetology team are either planned or currently underway, spanning a range of herpetological taxa and health aspects, from anaesthesia and nutrition to pathology and reproduction.

## AMPHIBIAN AND REPTILE DISEASE

Infectious diseases are now accepted as a serious and global threat to the conservation of amphibians, but this was not always the case. Researchers at ZSL played pivotal roles in proving infectious agents were responsible for amphibian mass mortality and population declines, including the first identification of ranavirus infection as a cause of wild amphibian mortality in Europe (Cunningham et al., 1996), and led the international multidisciplinary team that first identified the chytrid fungus, (Bd), as a cause of amphibian population declines (Berger et al., 1998; Cunningham et al., 1998). This work has stood the test of time: these two pathogen groups (chytridiomycete fungi of the genus *Batrachochytrium* and ranaviruses) are consistently identified as the pathogens causing amphibian mass mortalities and declines. Input from ZSL, amongst others, led to these being listed by the World Organisation for Animal Health (OIE), becoming the first, and to date only, notifiable amphibian pathogens (Schloegel et al., 2010).

ZSL has taken a role at the forefront of global research to understand the spread and impact of amphibian disease, focussing mainly on chytridiomycosis and ranaviral disease. This work has ranged from identifying at-risk species (e.g. Bielby et al., 2008), understanding the emergence and spread of these diseases (e.g. Fisher et al., 2009; Price et al., 2016), their impact on populations and species (e.g. Soto-Azat et al., 2013; Price et al., 2014; Hudson et al., 2016a), and their infection dynamics (e.g. Hudson, 2016). Together, results from these studies help identify how to be reactive and proactive to minimise disease impacts on amphibians, but also inform methods to minimise impacts of other diseases of wildlife more generally.

ZSL's Institute of Zoology has undertaken national scanning surveillance of amphibian disease since the early 1990s in partnership with Froglife, UK and other conservation organisations. A citizen science approach is employed, appealing to members of the public to report sightings of sick or dead amphibians: this has transitioned from telephone reporting to the Frog Mortality Project



**Figure 3.** This gharial (*G. gangeticus*) was captured on the Chambal River for radio telemetry tag attachment



**Figure 4.** The pool frog (*P. lessonae*) at one of the reintroduction sites in the England; disease risk analysis has been an important component of this project.

in the 1990s to online reporting as part of the Garden Wildlife Health project which began in 2013. The latter also includes wild reptile disease investigation, a neglected topic, for the first time in Great Britain (i.e. England, Scotland and Wales). The established network facilitates horizon scanning and provides an early warning system for novel threats, such as the potential risk of incursion of *Batrachochytrium salamandrivorans* into wild amphibians, and reports findings directly to government and the OIE.

To increase the application and conservation impact of our research, ZSL staff have developed and promoted biosecurity guidelines for fieldworkers (<http://www.arguk.org/info-advice/advice-notes>) and keepers ([http://www.gardenwildlifehealth.org/files/2013/06/Amphibian-disease-alert\\_June-2015.pdf](http://www.gardenwildlifehealth.org/files/2013/06/Amphibian-disease-alert_June-2015.pdf)) and developed a library of amphibian disease factsheets for members of the public. In order to characterise distributions of the emerging pathogen Bd, track its spread and identify amphibian species at risk, ZSL staff have collaborated with others to develop a global mapping project for this pathogen (Olson et al., 2013). By combining collaborative field studies with fundamental scientific studies on pathogen genomic architecture and experimental studies on pathogen infectivity and virulence, we have described how the invasion process and disease dynamics are regulated by evolutionary as well as ecological processes. The expeditious use of experimental studies is extremely important for conservation research, being used to identify the causes of newly recognised amphibian diseases, inform on their likely impact, and develop mitigation strategies. At ZSL, we use these findings to develop and improve disease interventions in both captive and wild settings. Recently, we have trialled field interventions to mitigate the impacts of chytridiomycosis on highly threatened amphibian species (Bosch et al., 2015; Garner et al., 2016; Hudson et al., 2016b). These interventions have provided short-term and multi-year respite from threatening disease dynamics (Hudson et al., 2016b).

The Disease Risk Analysis and Health Surveillance (DRAHS) team at ZSL's Institute of Zoology has been

investigating methods to assess and minimise the risk of disease in interventions, such as reintroductions, undertaken for conservation purposes. Working with Natural England on its Species Recovery Programme since 1989, we conduct disease risk analysis and post-release health surveillance on a number of conservation priority native species across a broad taxonomic range. Species we have worked with include pool frogs (*Pelophylax lessonae*, Fig. 4) (Sainsbury et al., 2016), sand lizards (*Lacerta agilis*), smooth snakes (*Coronella austriaca*) and northern viper (*Vipera berus*).

Conducting a disease risk analysis (DRA) prior to wild animal translocation is now advised by the IUCN (IUCN / SSC, 2013). A DRA involves describing the translocation pathway and any ecological or geographic barriers crossed during translocation, followed by conducting a comprehensive literature review and liaising with experts to build an understanding of the parasites in the source and destination populations (Sainsbury & Vaughan-Higgins, 2012). A good DRA should also build an understanding of which parasites or non-infectious hazards, such as toxins, may pose a threat to the translocated animals at the release site. In some cases, if little is known about a species' parasites, then a field survey may be undertaken to carry out diagnostic testing for parasites. One of the key objectives is to minimise the risk of the translocated animals releasing pathogens into the ecosystem at the release site. Once a hazard list has been created, a disease risk assessment is conducted by examining the probability of the hazard being released, the likelihood that the population of concern may become exposed to it, and the potential magnitude of these consequences on wider biodiversity.

Importantly, the DRAHS team conducts post-release health surveillance to determine what effects, if any, translocation has had on the health of both translocated animals and naturally-occurring populations at the release site. Surveillance includes health examinations at the destination site as well as post-mortem examination of any animals found dead, and is undertaken over the long-term, in combination with longitudinal population monitoring, to assess the effectiveness of the translocation and to improve future translocation techniques.



## EDGE

Launched in 2007, ZSL's EDGE of Existence programme highlights the world's most Evolutionarily Distinct and Globally Endangered species, many of which are receiving little or no conservation attention. The 'top 100' priority EDGE amphibian list was launched in 2008 (Isaac et al., 2012). As well as raising the profile of these extraordinary species, EDGE supports early-career conservationists, called EDGE Fellows, around the globe to develop and implement conservation projects focusing on EDGE species in their native country. To date, the EDGE of Existence programme has supported 68 fellows from 36 countries working on 63 species of EDGE mammals, amphibians, birds and corals; EDGE has supported 19 fellows from 10 countries working on 18 amphibian species. Long-term monitoring programmes have been established for species previously overlooked by conservationists, including the sooglossid frogs of Seychelles, the olm (*Proteus anguinus*) of Croatia, and ambystomatid salamanders of Mexico. Research supported by ZSL has provided insights into the role of chytridiomycosis in the decline of Darwin's frogs (*Rhinoderma*), and the apparent extinction of the Northern Darwin's frog (*R. rufum*) (Soto-Azat et al., 2013b). Current projects include three fellows working on three Mexican ambystomatid salamanders (*Ambystoma granulosum*, *A. lermaense*, and *A. taylori*). The *Ambystoma* project has screened populations for Bd, discovered new populations of *A. lermaense* (Fig. 4), recorded fatal parasitic infection (Michaels et al., 2016b), and established community conservation initiatives to reduce pollution in critical habitats. EDGE also highlights the conservation of the Critically Endangered Chinese giant salamander (Fig. 2), an EDGE priority species, through the support of four EDGE Fellows working on this species. Activities have included assessing the distribution of the remaining wild populations through field surveys (Tapley et al., 2015c) and local ecological knowledge (Pan et al., 2015), investigating the effects of the salamander farming industry (Cunningham et al., 2015), assessing the genetic composition of wild and captive salamanders and developing a community, education and public awareness (CEPA) programme. Plans are also underway to finalise and launch the first EDGE reptile list, with new projects on the West African slender-snouted crocodile (*Mecistops cataphractus*) and Round Island keel-scaled boa (*Casarea dussumieri*).

## TRACKING STATUS AND TRENDS OF REPTILES

ZSL's Indicators and Assessments Unit (IAU) is leading the development of global biodiversity indicators to track status and trends of biodiversity over time. The Sampled Red List Index (SRLI) relies on IUCN Red List assessments to track species' extinction risk over time, while the Living Planet Index (LPI), in collaboration with WWF, integrates vertebrate population time series to track population changes over time. Reptiles represent an important component of biodiversity but have previously been overlooked in conservation decision-making; the SRLI assessment included the assessment of 1,500 species

of reptile, randomly drawn from the global species list, to give a first-ever representative and global picture of reptile extinction risk. One in five species of reptile was estimated to be threatened with extinction, with extinction risk highest in freshwater systems and tropical regions and amongst turtles and tortoises (Böhm et al., 2013). ZSL's IAU has been directly responsible for Red List assessments for more than 1,000 of the 1,500 SRLI reptile species, in collaboration with over 250 species experts. Work is continuing within the IAU and with collaborators outside ZSL to establish trends in extinction risk over time for this sample, and to develop modelling and machine-learning techniques to aid the assessment process for reptiles and other species groups. For example, identifying associations between extinction risk and biological traits or external environmental factors can help to improve the assessment process for species and estimate the true status of species currently assessed as Data Deficient (and hence potentially threatened with extinction) (Bland & Böhm, 2016; Böhm et al. 2016a). Trials of resulting models as predictors of extinction risk are currently underway as part of the Global Reptile Assessment's Australian squamate assessments, in collaboration with IAU. Increasing the availability of reptile population time series data in the Living Planet Database will add to our knowledge on reptile status and trends and a first-look Living Planet Index for reptiles showing population trends over time is currently in preparation (Saha et al. in prep).

Climate change presents an emerging and often slow-acting threat to species, which is more difficult to capture on the IUCN Red List than faster-acting threats such as habitat loss. A trait-based climate change vulnerability assessment for the same random sample of reptile species has been carried out to complement extinction risk assessments, in collaboration with the IUCN Climate Change Unit (Böhm et al., 2016b). This provides the starting point for future work on refining the process and methods of climate change vulnerability assessment and the elucidation of reptile vulnerability to climate change.

The goal of completely mitigating or managing the greater problems that arise due to synergies between existing threats to amphibians and reptiles is still well beyond our grasp. ZSL staff and students will continue to work towards this objective along with an international network of scientists, wildlife managers, educators, veterinarians, conservationists, taxonomists and the *ex situ* community, as such a complex conservation issue requires collaborative approaches and will likely require a range of complex solutions. ZSL has a long history of being a convening institution, bringing scientists together to address important research and conservation issues, and as such it is uniquely positioned to catalyse these efforts. ZSL staff are committed to leading the effort to translate their research outputs into real-world solutions for the benefit of amphibian and reptile conservation.

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# Captive husbandry and breeding of *Gonyosoma boulengeri*

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**ABSTRACT** - The rhino rat snake *Gonyosoma boulengeri* is a medium-sized arboreal colubrid snake from southern China and northern Vietnam. Captive specimens maintained at the Zoological Society of London presented little difficulty in husbandry and were found to breed between March and June. A clutch of 9 eggs were laid on the 16 May 2008 and a clutch of 8 eggs were laid on 4 July 2013, following manipulation of the captive environment to reflect natural seasonality for this species. Post-laying incubation temperature was maintained at a constant 28.0 °C and lasted 52 days for the 2008 clutch and 57 days for the 2013 clutch. All individuals from the 2013 clutch had sloughed their skin for the first time by 10 days post-hatching, and five of the six fed, subsequent to sloughing, by 20 days post-hatching.

## INTRODUCTION

The rhino rat snake *Gonyosoma boulengeri* is a medium-sized arboreal colubrid snake from southern China and northern Vietnam (Hecht et al., 2013; Nguyen et al., 2009; Zhao, 2006). On the dorsal surface adult *G. boulengeri* are coloured lime to olive-green with white and black stippling around some scales; ventrally this species is off-white. Juveniles are grey-brown on the dorsal surface and off-white on the ventral surface. With increasing maturity this colour shifts from brown to green, potentially reflecting a shift in habitat preference as shown in other species (Wilson et al., 2007). Both adults and juveniles have a dark stripe running along the upper labial scales from the snout through the eye to the angle of the jaw. A notable feature of this species is the elongated, pointed, rostral appendage (Fig. 1).

Throughout its range this species is closely associated with riparian forested areas and often found close to water in mountainous regions (Orlov et al., 2000; Hecht, et al., 2013; Schuchmann, 2011). Both subtropical and tropical lowland and montane forest are known to be inhabited (Hecht et al., 2013). From non peer-reviewed documents written by private keepers, captive *G. boulengeri* are reported to spend large amounts of time in water, especially as juveniles. This species is not commonly encountered in nature but is known to occur in several protected areas within Vietnam (Hecht et al., 2013; Miskovic & Ziegler, 2013). *G. boulengeri* is known to be both diurnal and nocturnal, and is known to feed on small mammals and birds in the wild (Hecht et al., 2013). This species was assessed by the IUCN as Least Concern in 2012 (Rao et al., 2012).

From a search on the Zoological Information Management System (ZIMS) and several online interest groups *G. boulengeri* was assessed to be relatively common in captivity, both in the private and professional sectors. However, so far, published peer-reviewed data on



**Figure 1:** Adult male *G. boulengeri* at ZSL London Zoo  
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husbandry and reproduction are lacking. The first reported breeding in a European zoo took place in 2008 in ZSL London Zoo. This was followed five years later in 2013 by this institution's second breeding of the species. The primary purpose of this paper is to provide information on captive care and breeding of this species at ZSL London Zoo. Additionally there are no published records of juvenile growth rates which this study does provide. Knowledge of captive requirements and breeding triggers is essential to be able to maintain an *ex-situ* population over more than one generation, whether this is for conservation purposes, education, research or in a private collection. Therefore this report seeks to disseminate this information to the wider herpetological community.

## MATERIALS AND METHODS

### Study Individuals

Study animals consisted of two different pairs of *G. boulengeri*; one pair during 2008 and another pair during

2013. During the 2013 breeding the male snake was nine years and ten months old, of St Louis Zoo progeny, and weighed 299g at the time of breeding. The female snake was of unknown origin but had been in captivity four years and eleven months, and weighed 373g at the beginning of the time of breeding. Data on adult snake weights and ages are lacking for the pair from the 2008 breeding.

### Captive care

Records in 2008 do not allow for robust descriptions so data from the 2013 breeding at ZSL London Zoo has been used for description and analysis in this paper.

Both snakes were individually-housed, off-show, in neutrally-coloured plastic Herptek vivaria measuring 90 x 60 x 90 cm. The vivaria were furnished with chipped bark substrate and an arboreal environment was created with branches of varying diameter, texture, and thickness. This provided multiple spatial, thermo and photo-gradients for the animals to choose from. Additional cover was provided with both artificial and live plants, including palm fronds (*Sabal* sp.) and bromeliads (*Guzmania* sp.). On the floor of each enclosure a hide box containing damp sphagnum moss was provided to all *G. boulengeri*.

Servicing these vivaria consisted of removing soiled substrate and faecal matter, providing fresh water daily and spraying vivaria with water to create a daily humidity fluctuation from around 50% daily minimum to 99% daily maximum. Snakes were frequently observed drinking from the water sprayed onto their bodies and water bowls were generally around 6 cm in diameter and filled to 4 cm in depth. Throughout the active period of the year, including the months between March and December, each snake was offered small defrosted rodents warmed to room temperature every 7-10 days. The nest box was checked when the female displayed swelling in the mid-region of the body, which could indicate being gravid, or after she had spent extended periods of time within it. Checking of environmental parameters with a Precision Gold N85FR non-contact thermometer and a Solarmeter 6.5 UV meter was performed when necessary, such as after a lamp had been changed. UV basking lamps above each enclosure, including Arcadia 80W and Arcadia 12% T5, were provided throughout the year to allow the snakes to regulate their exposure to UV, visible light and heat, as is normal documented behaviour for many reptile species (Baines et al., 2016).

Inferences from climate data in this species' natural range were used to inform captive management. The temperature in the rooms the snakes were housed in was programmable and controlled by built-in air-conditioning units. Ambient temperature in the vivaria during summer 2012 ranged from 28.0 °C, dropping to 24.0 °C during the night. On 8 January 2013 the breeding pair of *G. boulengeri* was moved to a separate room for brumation. For the duration of the brumation period the snakes were not offered food. In this room the daytime maximum temperature was incrementally reduced over a seven-day period from 26.0 °C during the day down to 25.0 °C during the day, and from 25.0 °C overnight down

to 18.0 °C overnight. A small bask spot of around 30.0 °C to 34.0 °C was provided in each vivaria during this time which is where the highest level of UVB was available. The UV index, a unitless measure of UVB irradiation (see explanation in Michaels & Preziosi (2013)) provided to the snakes was in the region of 0-3 over the whole year. During the brumation period the time the basking lamps were on was reduced from 8am – 8pm to 10am – 4pm to mimic the snakes' natural annual photoperiod more closely. Additionally, light levels through the large windows where the snakes were cycled would fluctuate throughout the year which may have aided in the cycling of these animals.

To conclude the brumation period, on 5 March 2013, daytime temperatures in the rooms where *G. boulengeri* were housed were increased from 25.0 °C to 26.0 °C and night time temperatures were increased from 18.0 °C to 25.0 °C. Immediately after this date, whilst the two individuals were housed separately, they were each offered their first post-brumation mouse feed. The male *G. boulengeri* fed on 6 March and the female refused feed. The female did not feed until 25 April 2013. Specimens were mixed in an on-show exhibit on 30 March until 19 July, after the female had laid her eggs. This exhibit measured 160 x 145 x 180 cm and was furnished with branches and live plants, similar to the off-show set-up but on a larger scale. During this time mating behaviour was not observed between this pair and could have taken place during the day or overnight.

Outside the breeding period of March to July the snakes were housed singly. This housing system may lead to reduced levels of stress in female snakes once they are separated from amorous males. Males may also benefit from being housed singly, as males of certain species of snake will refuse food in the presence of an adult female of the same species around the breeding season (Daltry et al., 1998; Goiran et al., 2013; Tetzlaff et al., 2015).

## RESULTS

### Captive Breeding

Based on the husbandry regime outlined above, a pair of *G. boulengeri* were bred during 2013 at ZSL London Zoo. A single infertile egg was found in the enclosure on 1 July 2013; three days subsequent to this on 4 July 2013 a further 8 eggs were found in the nest box placed in the enclosure. The eggs were removed and artificially incubated in a plastic box half-filled with a 1:1 water:vermiculite medium by weight at a constant 28.0 °C. Five eggs had hatched by the morning of 30 August 2013, after 57 days, followed the next day by the 6th individual hatching after 58 days incubation. The two remaining eggs did not hatch.

Prior to this, on 16 May 2008, a clutch of nine eggs was laid by a female. Records held on ZIMS do not show any notes on environmental changes recorded in the male or female snakes' enclosure although they do state that possible mating was observed on 1 April 2008. However, as this pair was inside a hide box at the time they were not unduly disturbed. Eggs were incubated on a 1:1 water:vermiculite mix by weight, at a constant

28.0 °C. The first two eggs had pipped by 7 July 2008, 52 days after laying, and by 8 July 2008 a total of three snakes had emerged from their eggs.

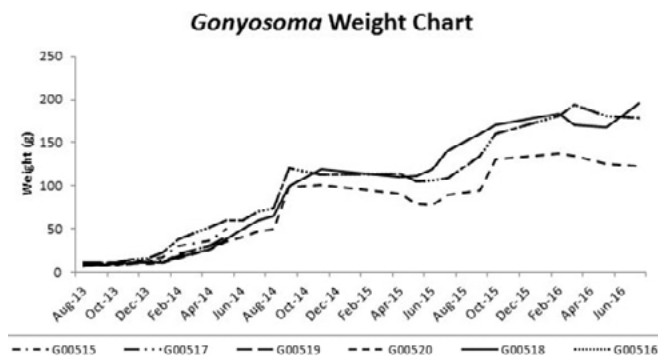
Mean average egg dimensions from the 2013 clutch were 42.5 x 21.0 mm. Mean average weight of the eggs in this clutch was 5.59 g. Eggs ranged from 48.0 x 21.0 mm to 38.0 x 22.0 mm. The mean average size of each egg in the 2008 clutch was 39.8 x 20.1 mm, and the mean average weight of the eggs in this clutch was 7.49 g. Eggs in this clutch ranged from 44.6 x 19.0 mm to 35.9 x 18.9 mm.

### Juvenile Care

Hatched specimens were set up in individual transparent plastic tanks with vented lids, which measured 37 x 22 x 25cm. A heat mat was placed underneath the back of each enclosure, covering approximately one third of the floor space, and an Arcadia 6% T5 UV-B emitting strip light was placed along the top. UV index reading of the tanks was 0-3.4 and temperature ranged from 25.2°C to 27.4°C. Each enclosure had a small water bowl and two plastic hides filled with moss to provide a warm and a cool retreat. Some branching with leaves was added to provide a more natural environment for the snakes. Small pinkie mice were offered on tweezers once every 7 – 10 days while the snakes were small. As the snakes grew the size of the food item offered was increased proportionately to the size of the snake, keeping roughly equal to the maximum width of the snake's body.

### Juvenile Growth Rates

Figure 2. below illustrates the weight changes of juvenile *G. boulengeri* bred at ZSL London Zoo. Three specimens are still maintained in the living collection of ZSL London Zoo, and the other three were transferred to a private individual which is why their zoo records only date as far as May 2014.



**Figure 2.** Growth rates for the six *G. boulengeri* hatched at ZSL London Zoo in 2013. Records for three animals stop in May 2014 as they were transferred out of ZSL's collection at this point.

## DISCUSSION

*G. boulengeri* have been found to be relatively hardy in captivity, with only a few individuals maintained in ZSL's

collection presenting difficulty. Two main issues were noted with this species; a reluctance to feed, and skin issues marked by discolouration and abnormal scale shape. Both issues were exhibited by only a few specimens in the collection and did not appear to be linked. The causes for both issues remain unknown. Regarding natural feeding behaviour of this species there is evidence from the wild that *G. boulengeri* are crepuscular or nocturnal and one animal has been recorded with a small bird in its stomach (Hecht et al., 2013). In ZSL's collection occasional night time feeds, for reluctant feeders, using warmed rodents or quail chicks were generally successful. In contrast to these few occasions the majority of snakes were consistent at accepting food presented during the day.

Wild data are lacking for longevity of *G. boulengeri* but in captivity this species is estimated to reach ages of 15-20 years typical of other medium-sized colubrids (Filippi & Luiselli, 2000). The oldest individual maintained at ZSL London Zoo during 2016 hatched in 2005, showing this species can reach ages of at least 11 years.

An analysis of institutional information from the ZIMS database, as of 29 July 2016, shows 47 institutions worldwide holding a total of 126 *G. boulengeri*. Worldwide, one European institution has bred this species in the last 12 months, producing 12 juveniles. From this analysis recent captive breeding in the zoo sector appears to be uncommon, as also generally seems to be the case in the private sector. It is unlikely that the lack of captive breeding of this species of snake is due to difficulty in doing so. Potentially, keeper time and zoo resources are being allocated to species assessed by the IUCN as being more threatened, or those species with a higher or more targeted conservation focus (Brooks et al., 2006). Ultimately, the reasons for the low-level of captive breeding of *G. boulengeri* are not fully understood.

The zoo sector should not be reliant on wild-caught animals, and therefore needs access to self-sustaining captive populations in order to not have a negative effect on wild populations if a chosen species is to be exhibited. A net decrease in population size has already been demonstrated in other southeast Asian reptiles, including monitors (Nijman & Shepherd, 2009) and green tree pythons (Lyons & Natusch, 2011; Lyons & Natusch, 2013). Despite this not being the case with *G. boulengeri*, as current knowledge suggests there is no direct threat to this species from wild collection at this point, an IUCN assessment of Least Concern and no detectable trade issue with a species should not be an excuse for collecting animals from the wild without thorough justification. On a regional level, focussed collection planning can enable the number of captive-bred juveniles to not exceed the holding capability of the local collections so as to sensibly manage this species. In the zoo sector this species is currently managed by the European Association of Zoos and Aquaria (EAZA).

There are currently no peer-reviewed published data on the breeding season for wild *G. boulengeri*. The species is presumed to be a vernal breeder, as captive animals have bred in the warmer time period following cooler



temperatures over the preceding couple of months. The relative increase in temperature, rainfall and photoperiod throughout April and May in areas of southern China and Vietnam, as detailed in Nguyen et al. (2014), may be a trigger for this species to begin breeding in nature. However, it could well be the case that this species is able to breed throughout much of the year, as has been demonstrated with other colubrid snakes (Brown & Shine, 2002).

From reading information published by online interest groups available to the public, female *G. boulengeri* are reported to lay a single clutch of between 5-16 eggs, ranging from infrequently to annually. This potential plasticity reported from captivity is believed to have more to do with the environmental conditions under which the snakes were kept, rather than the species' reproductive ecology. It is also possible the nutrition plays a role in the reproductive ecology of this species, as has been reported in a European colubrid snake (Santos et al., 2005). Many female snakes do not initiate reproduction until body condition exceeds a vital threshold value (Aubret et al., 2002; Al-Sadoon, Kandeal & Al-Otaibi, 2013). Therefore, this species could have an annual breeding cycle typical of other tropical and sub-tropical colubrids (Brown & Shine, 2002) provided the correct resources are available. Indeed both male and female *G. boulengeri* have been shown to be capable of annual breeding in captivity, according to the ZIMS database, at the Birmingham Zoo in Alabama. Provided individuals of this species can remain at adequate levels of body condition throughout the year it is conceivable that an annual breeding season may exist in nature.

In terms of egg incubation duration of *G. boulengeri* in comparison to another species in the genus, *G. oxycephala* was recorded as having a longer incubation period for its eggs at approximately 132 days (Pickersgill & Meek, 1988) versus 52-57 days for *G. boulengeri* (this study). The same authors report a larger hatchling size of between 15.6g to 19.5g with a mean average 17.8g, in comparison to the hatchling *G. boulengeri* reported in this study, which ranged from 7.75g to 10.5g with a mean average of 8.61g. The shorter time for *G. boulengeri* incubation relative to *G. oxycephala* is roughly proportionate to the smaller mean average weight of hatchlings of the former species. This is despite a similar clutch size reported for both species and broadly similar adult size, therefore appearing to indicate higher maternal investment in reproduction in *G. oxycephala*. It is beyond the available data sets to meaningfully investigate this trend further.

This study was limited by sample size, as detailed data are limited to just the 2013 breeding where only a single pair of *G. boulengeri* was bred and six neonates were produced. As there was just one other successful breeding of this species to compare with, and on both occasions a very similar temperature, thermal and photoperiod cycling regime was used, we are unable to comment on whether or not our method was the most successful it could have been. In intervening years this species has not bred at ZSL, most likely due to a lack of seasonality for the animals maintained in the captive collection. It is hoped to repeat

the breeding success of this species, under conditions more akin to those found in nature for this species, so that we are able to further our knowledge. Additionally, two of the eggs did not hatch; records indicate that one of the embryos died very late-term during development whereas there is no data on file regarding the other egg. It is possible that incubation conditions were not optimal for all eggs in the clutch despite the best available knowledge being followed (Köhler, 2005).

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# Observed localities for three endangered, endemic Mexican ambystomatids (*Ambystoma altamirani*, *A. leorae*, and *A. rivulare*) from central Mexico

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**ABSTRACT** - The populations of ambystomatid salamanders around Mexico City are subject to a variety of threats, and some populations may be in decline. Three *Ambystoma* species found around Mexico City and in central Mexico are *A. altamirani*, *A. leorae*, and *A. rivulare*, and these three species are subject to a variety of conservation threats. We compiled a database of localities for these ambystomatid salamanders. The compiled observations of these three species of endangered salamanders suggest several patterns: 1) most localities for all three species are in the Estado de México, including several for *A. altamirani* within the borders of Mexico City; 2) there is little, if any, geographical overlap among these three species; 3) the relatively few documented sites for *A. leorae* and *A. rivulare* highlight their tenuous conservation status. Our hope is that this presentation of a map of documented locations of these three Mexican *Ambystoma* has created a starting point for future studies on these salamanders.

## INTRODUCTION

The genus *Ambystoma* has several Mexican species that are of conservation concern (Frías-Alvarez et al., 2010; Wilson et al., 2013). The populations of *Ambystoma* located around Mexico City are subject to a variety of threats, and some populations may be in decline (e.g., *A. mexicanum*, Zambrano et al., 2007; Contreras et al., 2009). Many of these issues arise because of the expansion of Mexico City (Merlín-Urbe et al., 2013). This is particularly important because many of these species of *Ambystoma* are small, isolated, and show low genetic diversity (Parra-Olea et al., 2012; Sunny et al., 2014a,b), and thus may be prone to extinction. *Ambystoma* may also be subject to collection and removal for the international pet trade (e.g., Carpenter et al., 2014) or other uses (e.g., food, medicine; Griffiths et al., 2004).

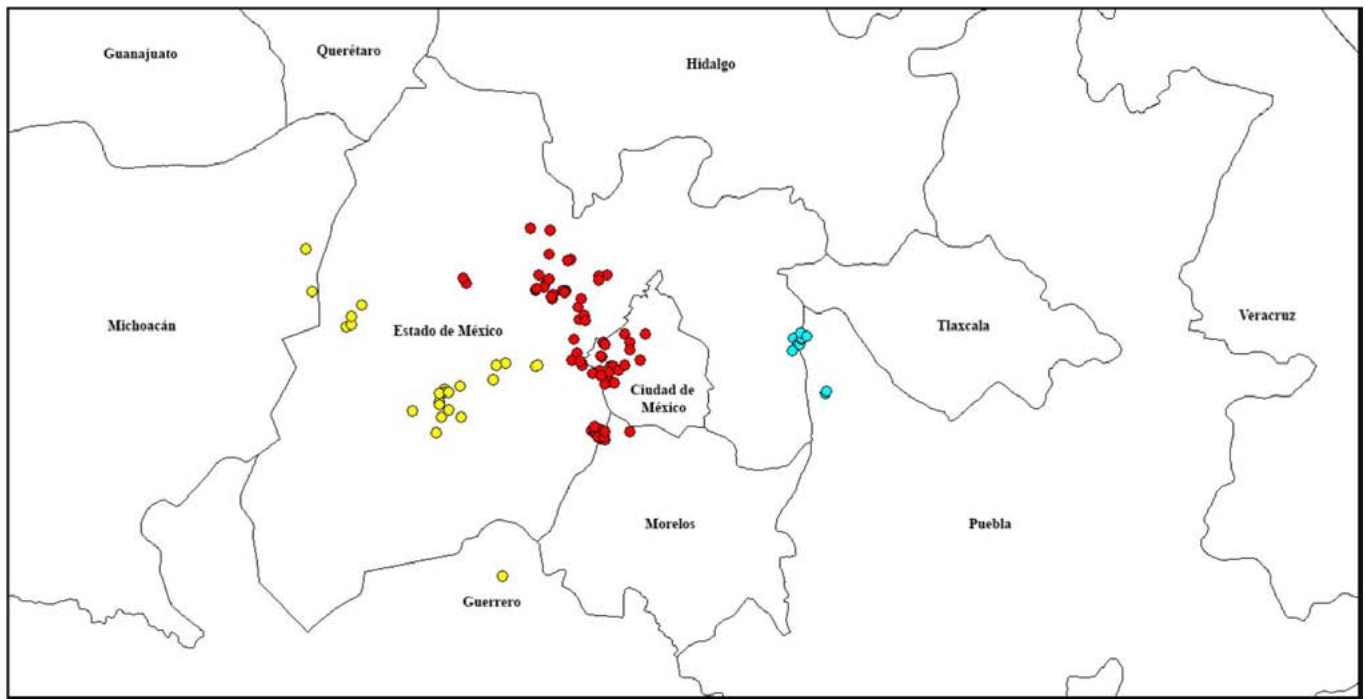
Three of the species of *Ambystoma* found around Mexico City and central Mexico are *A. altamirani*, *A. leorae*, and *A. rivulare*. These three species used to be considered part of a separate genus, *Rhyacosiredon*, but they are now considered to be *Ambystoma* (Brandon, 1977; Reilly & Brandon, 1994). They are also likely relatively closely related, at least *A. altamirani* and *A. rivulare* (*A. leorae* not sampled; Weisrock et al., 2006; Recuero et al., 2010). According to Wilson et al. (2013), the Environmental Vulnerability Score (EVS) of *A. altamirani* is 13, *A. leorae* is 15, and *A. rivulare* is 13, primarily due to the limited geographic and ecological

ranges. These are at the higher end of the intermediate vulnerability range (10-13) and in the high vulnerability range (> 14) (Wilson et al., 2013). According to the IUCN Red List the conservation status of *A. altamirani* is Endangered, *A. leorae* is Critically Endangered, and *A. rivulare* is Data Deficient (IUCN, 2015). According to the Mexican government (SEMARNAT, 2010), these three ambystomatid species are all classified as Threatened.

Populations of these three species of *Ambystoma* in central Mexico are endangered due to a variety of conservation threats. For example, *A. altamirani* and *A. rivulare* have both been shown to be infected with *Batrachochytrium dendrobatidis* (*A. leorae* was not tested; Frías-Alvarez et al., 2008). The introduction of fish to previously fishless habitats appears particularly damaging to *Ambystoma* populations in this region (e.g., Lemos-Espinal et al., 1999; Griffiths et al., 2004; Alcaraz et al., 2015). Pollution and lowered water quality is another potential threat to *Ambystoma* near Mexico City (e.g., Griffiths et al., 2004; Robles-Mendoza et al., 2009; Recuero et al., 2010), and many streams where these species are found are impacted by local residents (Lemos-Espinal et al., 1999).

Given these multiple threats to ambystomatid salamanders in central Mexico, it is important to establish and carefully document the locations of the existing populations. This is to both identify the types of habitats where they currently occur, but also to identify, via these locations, the specific types of threats they





**Figure 1.** Map of the localities where *A. altimirani* (red circles), *A. leorae* (blue circles), and *A. rivulare* (yellow circles) were found and documented

may be likely to face. We therefore set out to compile a list of all documented localities for these ambystomatid salamanders, *A. altimirani*, *A. leorae*, and *A. rivulare*.

Previous research has documented habitat use for a few populations of these three species. *A. leorae* are found in pools along streams with a variety of substrates and well oxygenated water (Vega-López & Álvarez S., 1992; Sunny et al., 2014b; Monroy-Vilchis et al., 2015). Lemos-Espinal et al. (1999) reported most *A. leorae* were first seen in shaded sites. *A. rivulare* are found in slow-moving streams or in pools along streams (Bille, 2009). In the Arroyo Los Axolotes, Mexico, *A. altimirani* tend to use portions of the stream with grassy vegetation, muddy or sandy substrates, more oxygenated and faster flowing water, as well as sites containing more water (Lemos-Espinal et al., 2016). They can also be found in well-oxygenated pools along streams (Maldonado Koerdell, 1947), and are frequently found under rocks along streams (Lemos-Espinal et al., 1999).

## MATERIALS AND METHODS

We collected locality records for these three species using the following sources: (1) by our own field work and using a GPS unit (Garmin eTrex Venture; accuracy < 15 meters) to record the location (n = 41); (2) literature records in Monroy-Vilchis et al. (2015) and Vega-López and Álvarez (1992) (n = 2); and (3) a data base provided by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (National Commission for the Understanding and Use of Biodiversity; CONABIO) (n = 90). Due to conservation concerns, we do not report specific locality data in this paper. Researchers with a legitimate need for specific locality data can contact the correspondence author. We used the locality data to draw a dot distributional map

showing each locality record for the three studied species using the program Biótica 5.0.4.1 by CONABIO which is a free access program found at <http://www.conabio.gob.mx/biotica5/documents/DescargaBiotica.php>.

## RESULTS

*A. altimirani* were found in 94 sites in the Distrito Federal (n = 20), Estado de México (n = 68), and Morelos (n = 6) (Fig. 1). The altitudinal range of sites where *A. altimirani* have been found is from 2450 m to 3487 m. *A. leorae* were found in 12 sites in the Estado de México (n = 7) and Puebla (n = 5) (Fig. 1). The altitudinal range of sites where we documented *A. leorae* ranged from 2525 m to 3750 m. We documented *A. rivulare* from 27 sites in Guerrero (n = 1), Estado de México (n = 25), and Michoacán (n = 1) (Fig. 1). *A. rivulare* were found at elevations between 2720 m and 3180 m.

## DISCUSSION

Our distributional observations of these three species of endangered salamanders suggests several patterns. First, most sites we documented for all three species are in the Estado de México, including several for *A. altimirani* within the borders of the Mexico City metropolitan area (see Fig. 1). This observation reinforces the concern about the potential effects of an expanding Mexico City on the long-term prognosis of these populations (see Monroy-Vilchis et al., 2015), especially in light of studies showing the continued loss of natural areas near Mexico City (García-Romero, 2002; Merlín-Urbe et al., 2013). Second, there does not appear to be much, if any, geographical overlap among these three species. This suggests that

while many of their habitat requirements may be similar (e.g., *A. altimirani*: Taylor & Smith, 1945, Maldonado Koerdell, 1947, Brandon & Altig, 1973, Lemos-Espinal et al., 1999, 2016; *A. leorae*: Sunny et al., 2014a, Monroy-Vilchis et al., 2015; *A. rivulare*: Brandon & Altig, 1973, Bille, 2009) and thus effective conservation efforts might be similar, they must be addressed in a species-by-species manner. For example, a single reserve or natural area is unlikely to provide coverage for all three species. The disjunctive species distributions also raise questions about why they do not co-occur given their use of relatively similar habitats. Answering such questions would be useful in helping to determine what might happen if these species are forced together by shifting habitats with climate change or with changes in habitats as urban and agricultural land use expands in central Mexico. Third, the relatively few sites where we documented *A. leorae* and *A. rivulare* highlight the tenuous conservation status of these species. This concern is especially high for *A. leorae* (see also Sunny et al., 2014a,b).

Finally, our hope is that by presenting a summary of the documented locations of these three Mexican *Ambystoma* we have created a starting point for future studies on these salamanders. In particular, we hope that others will continue to try to fill in additional locations so that we have a better and more complete understanding of the populations and distributions of these species. In addition, it is hoped that regular monitoring of these sites for salamanders will allow for the detection of any population or range declines, or loss of suitable habitat. We hope our results will also serve as a basis for exploring ways to conserve existing populations and localities by establishing where these salamanders are known to exist.

## ACKNOWLEDGMENTS

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# Repeated use of roadside tunnels of the European mole (*Talpa europea*) as a communal nesting area by grass snakes, *Natrix natrix*: are there thermal benefits?

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**ABSTRACT** - The presence of oviparous reptiles in relatively cool climates is mostly due to the ability of reproducing females to locate nest-sites with appropriate thermal regimes. In northern Europe the grass snake (*Natrix natrix*) is well known for exploiting anthropogenic derived heat sources to aid egg incubation. This paper documents, for the first time, *N. natrix* repeatedly exploiting roadside areas for egg-laying where females oviposited in tunnels of the mole *Talpa europea*. Temperatures in the tunnels were higher than dummy nest temperatures in shaded, partially shaded and sunlit areas around the nesting area but in good agreement with the temperatures of a dummy nest at a similar distance from the road edge. Hatching success in six clutches ranged from 87.5 – 100%. These results suggest a thermal benefit from heat from roadside tarmac for egg incubation.

## INTRODUCTION

Unlike viviparous reptiles that have the capacity to track shifting thermal zones to facilitate the development of embryos, in oviparous species the eggs, once deposited, are immobile and subject to changes in local climatic, which could depart significantly from optimum. In addition, the influence of thermal regimes on offspring fitness is known to impact on nest site selection (e.g. Burger & Zappallorti, 1986; Rhen & Lang, 1995; Deeming 2004; Blouin-Demers et al., 2004; Brown & Shine, 2004; Brown & Shine, 2005; Löwenborg et al., 2010; Refsnider & Jansen, 2010; Löwenborg et al., 2011). This is particularly important in temperate regions, where weather changes are frequent and incubation may last from between a few weeks to months. When oviparous reptiles repeatedly use the same nesting sites in natural conditions, this suggests that appropriate conditions for incubation are present and hence information from such sites provides insight into the conditions that females actually select (Refsnider & Jansen 2010; Löwenborg et al., 2010).

The European grass snake, *Natrix natrix*, is an oviparous snake with a distribution extending further into northern Europe than any other oviparous snake (Street, 1979). It is known to select sites with rotting vegetation for oviposition (e.g Street, 1979; Gregory, 2009; Lowenborg et al., 2010; Baker, 2011). These generate warmth and often retain moisture, important factors in enabling successful incubation of eggs, especially in northerly latitudes. In a human landscape, compost heaps frequently serve this function, especially if they also have exposure to sunshine (Löwenborg et al., 2010). This paper describes thermal conditions inside roadside nest sites that were repeatedly used by *N. natrix* at a location in western France during 2014 and 2016.

## METHODS

The nesting area was discovered during 2014 in sandy soil at a road-side grass verge 1.3km east of the village of Grues (46°39N;1°29W) in Department 85, Vendée, France. The verge was on a gradient of around 10 - 15° and faced south. The road was bordered by a hedgerow at a distance of around 1500mm from the road and small woodland surrounding a pond but most of the outer terrain was agricultural land (Fig. 1). The nests had apparently been raided by a species of small mammal (likely a mustelid based on scats found at the nesting area) with most egg remains scattered on the surface (Fig. 2). The eggs had been deposited in the tunnels of European mole (*Talpa europea*) that ran parallel to the road and hedgerow (Fig. 1). The absence of mole hills during summer suggests the tunnels were abandoned at this time. The nest location was marked (using paint at the road edge) and during 2016 a total of 63 visits made to the site from 17 May to 5 October, between 1130h and 1830h.

On each visit measurements of temperatures inside the tunnels were made using a *Koch Thermo-Hydrometer* (error  $\pm 0.2^{\circ}\text{C}$ ). To give insight into nest site selection several dummy nests around the nesting area were excavated within 20m of the nesting area. This method has value in describing the distribution of possible nest temperatures available and can be compared with the temperatures in the true nesting area. This included 1) a sunny position at the roadside 32.8m east from the nesting area (Nest<sub>road</sub>) 2) in full sun 19m from the roadside (Nest<sub>open</sub>) 3) partially shaded area 21.9m from the roadside (Nest<sub>partial/shade</sub>) and 4) at a shaded area 11.3m from the roadside (Nest<sub>shade</sub>). The locations are shown in Fig. 1. The measurements in the dummy nests were made at a similar depth into the soil as the real nests. Road surface temperatures next to the nesting area were recorded using a *Electronique Frontal infra red detector* (model TS112). This provided a faster



**Figure 1.** Aerial view of the study area showing localities of the nesting location (dotted line at roadside), artificial nests (Sr = roadside in sun; S = sunny; Ps = partial sun; Sh = shade), pond (P) and location of a road-killed gravid female *N. natrix*. See text for further details. In this Google Earth view the pond next to the nesting area appears dried out, which is usually from June onwards.



**Figure 2.** Example of a roadside nest, one of the two nests located in 2014, which has been excavated by a predator

reading than the Koch instrument for use with passing traffic.

**Statistical analysis.** Two gravid females were found road-killed close to the site in late June and it is assumed that egg deposition occurred sometime in July and hatching during September. Subsequently 24 visits to the site were made from 3 July to 5 October and statistics were applied only to data from this period. On each visit measurements of temperatures at the nesting area and 4 artificial egg-laying sites were made. In addition, road surface temperatures adjacent to the nesting area were also taken. Therefore sample sizes in the analysis were  $n = 24$

for each data set, i.e. egg laying site, artificial egg laying sites and road surface.

All  $\pm$  values represent standard deviations. Leven's test ( $L$ ) was applied to determine homogeneity of variances with the null hypothesis  $\sigma_1^2 / \sigma_2^2 = 1$  and  $\alpha = 0.05$ . Equal variances were found ( $L$  values 0.31 to 3.8,  $p$  from 0.06 to 0.58) in all data sets except between Nest<sub>1</sub> and Nest<sub>shade</sub>,  $L = 5.49$ ,  $p = 0.02$ . Temperatures were then compared using a non-parametric Friedmans repeated measures ANOVA, which employs a ranked procedure at  $\alpha = 0.01$  (Gotelli & Ellison, 2004). The null hypothesis is sum of ranks equality for all sample groups. Post-hoc analysis was with a two-tailed non-parametric Wilcoxon signed-rank test giving a  $z$ -score with  $\alpha = 0.01$ .

## RESULTS

A total of 38 empty egg shells were found in two clutches (N<sub>1</sub> and N<sub>2</sub>) on 28 09 2014. The eggs had been deposited approximately 120mm (nest<sub>1</sub>) and 90mm (nest<sub>2</sub>) into the soil situated 170 (nest<sub>1</sub>) and 140mm (nest<sub>2</sub>) from the road edge at around 8 metres apart (Fig. 1).

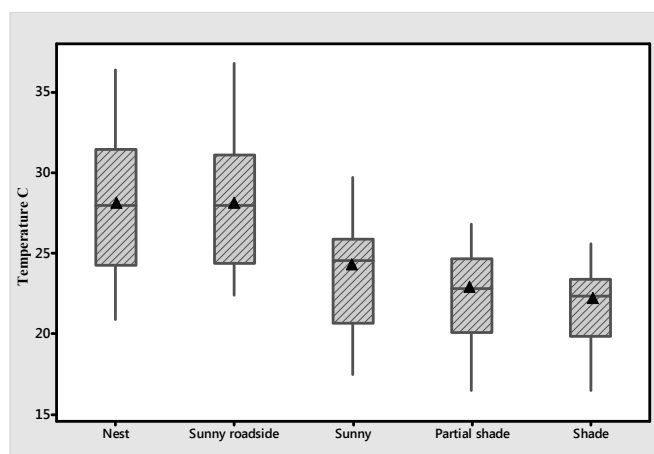
Between 04 09 16 and 22 09 16, four clutches totalling 74 egg shells were found at the same location at distances between 360 and 410mm from the road edge. They were at similar depths to the 2014 sites at around 120mm into the base of the tunnels. This gave a total, for both years (2014 and 2016), of 112 eggs. During 2016 numerous shed skins were found inside the tunnels alongside 2 emerged but dead fully formed hatchlings at the opening of N<sub>3</sub>, and 2 full term embryos still within the eggs in N<sub>4</sub>. Egg remains at two



additional nests were observed on 18/09/16 and 22/09/16 ( $N_5$  and  $N_6$ ) and indicated all hatchlings emerged successfully from these. The data are summarised in Table 1.

**Table 1.** Egg clutch information from 2014 and 2016; \* indicates 2 dead fully formed hatchlings outside shell and \*\* 2 full term hatchlings dead in the shell. Measurements are in mm.

Nest	Date	Clutch Size	Egg Length	Egg Width	Distance from road edge (mm)	Hatching success rate (%)
$N_1$	28/09/14	28	28	18	170	100
$N_2$	28/09/14	10	25	15	140	100
$N_3$	04/09/16	26	29	15	360	92.3*
$N_4$	04/09/16	16	31	20	410	87.5**
$N_5$	18/09/16	17	29	19	370	100
$N_6$	22/09/16	15	26	19	365	100



**Figure 3.** Boxplots of temperatures (°C) inside the nesting area and those of artificial nests. Triangles represent means, boxes the interquartile ranges, horizontal lines within the boxes medians and vertical lines the ranges.

During sunny weather road surface temperatures next to the nesting area ( $mean = 43.5 \pm 9.4^\circ\text{C}$ ) were higher than when overcast ( $mean = 37.5 \pm 8.5^\circ\text{C}$ ). The Friedman's test revealed significant differences between the data sets,  $\chi^2 = 28.45$ ,  $p < 0.001$ , d.f. = 4. Post-hoc analysis indicated no significant difference between the nest area ( $mean = 28.2 \pm 4.3^\circ\text{C}$ ) and Nest<sub>road</sub> ( $mean = 27.9 \pm 3.9^\circ\text{C}$ ;  $z = 0.36$ ,  $p = 0.72$ ) but nest area was significantly higher than Nest<sub>open</sub> ( $mean = 23.7 \pm 3.2^\circ\text{C}$ ;  $z = 3.8$ ,  $p < 0.01$ ), Nest<sub>partial/shade</sub> ( $mean = 22.5 \pm 2.7^\circ\text{C}$ ;  $z = 4.29$ ,  $p < 0.01$ ) and Nest<sub>shade</sub> ( $mean = 21.8 \pm 2.5^\circ\text{C}$ ;  $z = 4.28$ ,  $p < 0.01$ ). The data are shown in Fig. 3.

## DISCUSSION

Several oviparous snake species have been reported nesting adjacent to roads (e.g. *Pituophis melanoleucus*; Burger & Zappalorti, 1986; *Zamenis longissimus*; Kovar et al., 2016) but this is the first report of the behaviour in *N. natrix*. In *N. natrix* a likely explanation was the consistently higher temperatures present at the nesting area due to heat from roadside tarmac that produced

mean temperatures in the nesting area within the optimum of  $27 - 29^\circ\text{C}$  for development found for *N. natrix* in laboratory studies. These temperatures have produced early hatching and large offspring capable of enhanced locomotor performance (Löwenborg et al., 2010; Löwenborg et al., 2011; Hagman et al., 2012). However, the observed temperature range at the site was  $20.9 - 36.4^\circ\text{C}$ , and would have been much wider had there been nocturnal measurements. In laboratory studies fluctuating temperatures produced higher incidences of scale abnormalities in *N. natrix* compared to embryos incubated at constant temperatures and have been associated with low survivorship in adults (Löwenborg et al., 2012). Fluctuating temperatures are likely normal in natural nest sites but Löwenborg et al., (2012) suggested that the amount of time temperatures remain at either end of the thermal spectrum may be a key factor that impacts on embryonic development. Egg slit data (an indicator of hatching success; e.g. Doody et al., 2015), suggested high rates of hatching success under fluctuating temperatures (Table 1), but no data are available on scale abnormalities. It is worth noting that female viviparous snakes thermoregulate precisely when gravid, which produces larger fitter offspring and fewer stillborn. (e.g. Arnold & Peterson, 2002; Lourdaïs et al., 2004) but at a cost of increasing risk to predation as a consequence (Lourdaïs et al., 2004).

Communal nesting and repeated use of nest sites has been frequently reported in snakes (e.g. Foley, 1971; Covacevich & Limpus, 1972; Brown & Shine, 2005; Braz et al., 2008) with the presence of egg shells from previous nestings cited as the main cue for nesting area location (e.g. Plummer, 1981; Brown & Shine, 2005). Temperatures in communal nests have found to be higher than in solitary nests, which enhances hatchling fitness (e.g. Blouin-Demers et al., 2004) but other benefits such as predator satiation may reduce egg mortality. However, many species of snake may be unable to construct a nest and rely on pre-existing sites for oviposition (Packard & Packard, 1988). In this respect the tunnels of *T. europaea* in sandy soils present several potential advantages as nesting environments as *T. europaea* normally avoid tunneling in soils that flood or are excessively dry (Funmilayo, 1977). Sandy soils heat up more rapidly than clay based soils (which are common in the area) and drain rapidly whilst also retaining moisture (Townsend, 1977). These are soil characteristics that facilitate reptile egg incubation. Soils with good moisture retention could be especially important where summers are hot and dry since humidity has been identified as an additional key factor influencing both hatching success and phenotypic traits in reptiles (e.g. Packard & Packard, 1988; Löwenborg et al., 2012) although may not be as important for reptiles as temperature (Warner & Andrews, 2002). Humidity in the nesting area between 3 July and 2 October ranged from  $27 - 85\%$  ( $mean = 61.7 \pm 17.5\%$ ) and gradually declined during the incubation period.

No nests were found in 2015 (this does not of course indicate there were none) but the two gravid females (TL 680mm and 810mm) found road killed close to the site (170 and 480m) during June 2015 could have been migrating towards the nesting area. If the snakes originated



from wetlands due east and north of the nesting area the fragmented nature of the surrounding landscape, which consisted mostly of agricultural land, this would necessitate movement through hedgerow corridors (Fig. 1). This would also involve road crossings and mortality risk from road traffic and predators (Meek, 2015) but presumably the benefit of reaching the nesting area outweigh the risks.

## ACKNOWLEDGMENTS

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# Natural history observations of a dwarf ‘green’ gecko, *Lygodactylus conraui* in Rivers State (Southern Nigeria)

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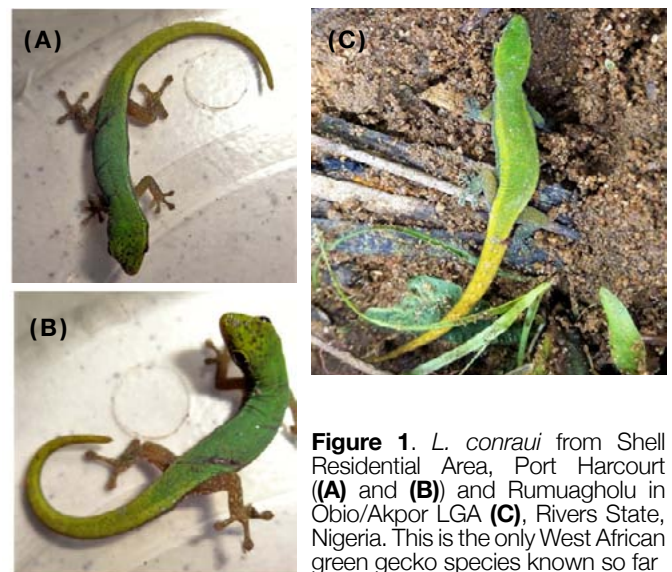
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**ABSTRACT** - *Lygodactylus conraui* is the only green gecko species occurring in West Africa, and is also one of the smallest gecko species of the African continent. Here, some aspects of the natural history of this species are documented for Rivers State, southern Nigeria. In total, 108 *L. conraui* individuals were observed in 1996-2016 in southern Nigeria. Individuals of this species were mainly observed at originally forested sites that were heavily altered by human inclusion. In southern Nigeria, *L. conraui* may be a pioneer species that quickly colonises microhabitats at ecotonal sites of recently deforested or rapidly re-growing forest areas. In each site, the number of observed lizards was significantly positively influenced by the percent of available shade within each habitat type. These geckos were found most commonly at 0.8-3.2 m height. Their activity was especially concentrated during the wet season months. Mean distance between individuals in each demes was 1.35 m (range 0.70-3.2 m).

## INTRODUCTION

Geckos are among the most speciose groups of lizards worldwide (Rösler, 2000). They have explored a wide variety of ecological niches in both temperate and tropical ecosystems, but most of them are nocturnal and dull in coloration (Rösler, 1995; Bauer, 2013). Nonetheless, there are several mostly tropical species, belonging to different groups of geckos, that are diurnal, and with green dorsal coloration (Rösler, 1995). *Lygodactylus conraui*, the only West African ‘green’ gecko, inhabits the West African Guinea savannahs and open forests (Trape et al., 2012) and also occurs in swamped and dry forests of southeastern Nigeria (Akani et al., 1999; Luiselli et al., 2007) and Cameroon (Chirio & LeBreton, 2007). The ecology of this species in the field is very little known (but see Luiselli et al., 2007; Rugiero et al., 2007), with studies showing that it is mainly diurnal and that it is found in different types of forest in Nigeria, where however it is one of the apparently least common of the forest zone geckos (Luiselli et al., 2007). The species appears to be relatively uncommon in other parts of its range: for instance, in Togo it has been reported for the first time just recently (Bauer et al., 2006) and no other data have been collected during devoted long-term field studies on the lizards of this country (Segniagbeto et al., 2015). In the rainforest zone of Nigeria, this species appears to be less generalist in dietary habits than sympatric geckos, its elective prey being adult lepidopterans of very small size (Rugiero et al., 2007). Considerable anthropochoristic habits have been observed in Benin (Manners & Goergen, 2015). The rest of the data concerning the biology of this species comes



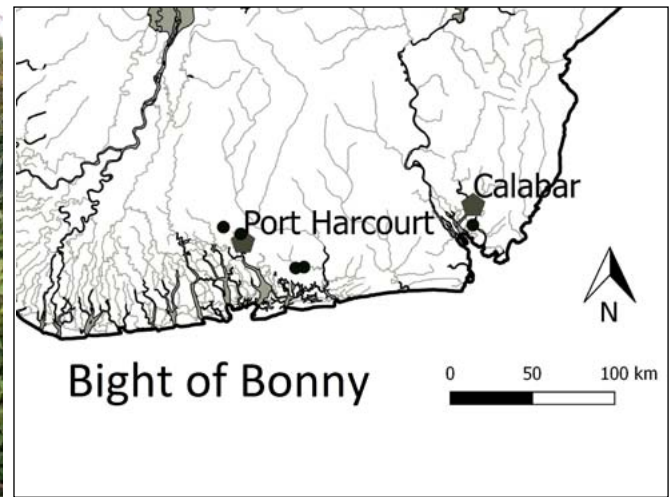
**Figure 1.** *L. conraui* from Shell Residential Area, Port Harcourt ((A) and (B)) and Rumuagholu in Obio/Akpor LGA (C), Rivers State, Nigeria. This is the only West African green gecko species known so far

essentially from captive specimens (e.g., Hofman, 2011) or from mostly distributional accounts (e.g., Hoogmoed, 1979, 1980; Bauer et al., 2006; Segniagbeto et al., 2015). In this note, we report additional natural history data on *L. conraui* in southern Nigeria, with emphasis on habitat use and climbing heights, seasonal activity patterns and inter-individual distances. We also include original data on the local distribution of the species on the basis of original field surveys. Although preliminary, our field data may be useful to enhance our general knowledge on the ecological strategies of tropical diurnal green geckos.





**Figure 2.** Habitat characteristics of the study sites where individuals of *L. conraui* were observed during the present study



**Figure 3.** Map of southeastern Nigeria showing the localities of capture/observation for *L. conraui* during our field investigations, years 1996-2016. In dark grey, the area subjected to seasonal inundations. Only original data (not bibliographic compilation of presence sites) is presented in this map.

## STUDY AREAS

This study is based on (i) opportunistic presence records obtained during field surveys for reptiles conducted between 1996 and 2016 in southern Nigeria, and (ii) ecological observations, specifically focused on *L. conraui*, that were carried out at two distinct study sites.

The main study sites (Fig. 2) were two distantly separated stations in Rivers State, Nigeria, namely at Shell Residential Area, PH (04°51.022'N; 07°4.143'E) (site 1) and Taabaa (04°39.881'N; 07°22.193'E) (site 2). The two stations in Rivers State are approx. 43 km apart and lie on the north eastern flank of Port Harcourt, the capital city of Rivers State of Nigeria. These sites lie in the humid equatorial bioclimate, with an annual rainfall well over 2500mm annually, and with rainy season concentrated between April and September.

The various habitat types available at the study sites were categorised as follows:

**Site 1 (9.5 ha area)** – (a) Orchards (2 ha); this habitat was dominated by shade-producing trees (*Roystonea regia*, *Delonix regia*, *Anthocleista vogelii*, *Terminalia catappa*), with grassy ground, and about 60% of the area was shady at midday. (b) Ornamental garden (3.5 ha); this habitat was predominantly characterised by plants of *Roystonea regia* and *Pseudotsuga menziesii*. Wide pedestrian paths occurred in this habitat, and were bordered on both sides with oil palms *Elaeis guineensis* and with *Hylocereus undatus* and *Terminalia superba*. This habitat was less shaded than the previous one (about 30% at midday). (c) Shaded forest (2.5 ha); this habitat was characterised by a remnant secondary forest and bush fallow, including trees of *Elaeis guineensis*, *Ficus* spp., *Psidium guajava*, bush orange trees, and *Persia americana*. This is the most shaded habitat available at the site (about 80% at midday). Despite being the most natural place (although with many nonnative species), it was the habitat type closest (less than 500 m distance) to human settlement. (d) Human settlement (1.5 Ha); this habitat

was characterised by houses and compounds, with just a few ornamental flower plants and trees (*Terminalia* spp). Shade was about 40%.

**Site 2 (9.5 ha)** – (i) Bamboo hut (0.5 ha); this was a small bush-house made of mud, with the roof made of palm fronds. This place was situated at the border of Taabaa village. This is the most shady habitat of site 2 (about 70% by midday). (ii) Bush fallow (2.5 ha); this habitat type was an abandoned farmland with weeds, rhizomes, bulbs and corms, plus stems of uprooted cassava and plantains. Shade was not much (about 15%). (iii) Freshwater swamp (6.5 ha); this habitat type was more elaborated than the previous ones, with *Raphia* palms, umbrella trees (*Musanga cecropoides*), *Harungana madagascariensis*, and weeds such as *Chromolaena odorata*, *Urena lobata* and *Aspilia Africana*. Shade was high (60% at midday).

## METHODS

A total of 31 days were spent in the field at site 1 (in 2011) and 21 days at site 2 (in 2015). Out of the 52 field days, 29 were spent during the dry season and 23 during the wet season. In each field day, an effort was made to cover regularly the whole study areas during different parts of the day, and at least 8 hours were spent in the field. Overall, the search effort was 251 man-hours at site 1 and 172 man-hours at site 2. Geckos were captured by hand or were noosed. For each specimen we also recorded the time of activity, its habitat type (see above for the description of habitat types in the two study areas) and its height of climbing from the soil. Once a lizard was seen and its exact spot was noticed, we also searched visually all throughout in order to find other conspecifics. And when another individual was seen, its linear distance (cm) from the other individual was measured with a ruler (precision 5 cm).

The percentage of shade, related to a vertical plane, was also recorded at each spot where a given gecko was



observed. Measuring the percentage of shade along a vertical plane was necessary because the lizards are living on tree trunks and other vertical or near vertical surfaces above ground.

In order to evaluate the use by lizards of different height levels, we classified all sightings into four categories of height at which each individuals was observed: (i) < 0.80 cm from the ground; (ii) 0.81 – 1.60 m (iii) 1.61 – 3.2m and (iv) > 3.21m. The statistical differences in terms of utilisation of the four categories of heights by lizards and by site were analysed by  $\chi^2$  test. The relationship between the number of observed lizards and the percent of shade within each habitat type was tested by Pearson's correlation coefficient, after having (log + 1) transformed the variables in order to achieve normality. Inter-seasonal difference in the frequencies of observation of the study species was analysed by  $\chi^2$  test. All statistical analyses were performed with a PAST software, with alpha set at 5% and all tests being two-tailed.

## RESULTS AND DISCUSSION

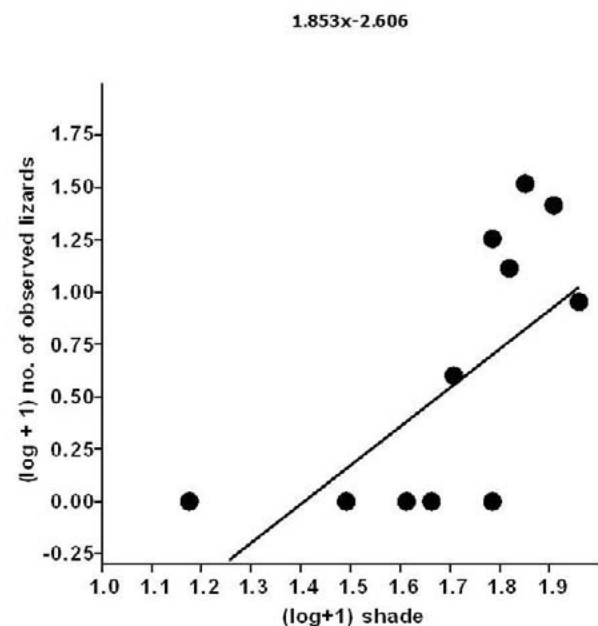
### Local distribution and general habitat characteristics

In total, 108 *L. conraui* individuals were observed in 1996–2016 in southern Nigeria, with 25 individuals captured at study site 1, 32 at study site 2, and the rest (n = 51) in other few localities (Fig. 3). As a general pattern, individuals of this species were mainly observed at originally forested sites that were heavily altered by human inclusion. For instance, the species reached apparently high density of individuals at Rumuagholu community in Obio/Akpor Local Government Area (LGA) and at Wilyaakara community in Khana LGA, in recently deforested sites (Fig. 2). However, *L. conraui* appears generally uncommon, and indeed it has not been observed during careful field investigations in protected forests such as the Upper Orashi Forest Reserve (Akani et al., 2014a), Taylor Creek Forest Reserve (Akani et al., 2014b), and Edumanom Forest Reserve (Akani et al., 2014c), all situated in the Niger Delta region. In addition, the species was also not observed in coastal forests (such as at Bonny island, Akani & Luiselli, 2009, and Brass, see Akani et al., 2010) and in eight distinct types of plantation situated in the surroundings of Yenagoa (Bayelsa State), Port Harcourt (Rivers State) and Eket (Akwa-Ibom State) (Akani et al., 2014d). The apparent rarity of this species in Nigeria may be a reason for that it was wrongly considered absent from the country by some available literature (e.g., the 'Reptile Database', available at: <http://reptile-database.reptarium.cz/species?genus=Lygodactylus&species=conraui>, lastly accessed on 5th February 2017). The bulk of our observations suggests that, in southern Nigeria, *L. conraui* may be a pioneer species that quickly colonises microhabitats at ecotonal sites of recently deforested or rapidly re-growing forest areas (when shade is dominant, see below; and see also Scott, 1982), becoming rarer or even absent from more stable habitats such as mature forests as well as extended/intensive plantations. Apparently, Nigerian populations of *L. conraui* are not at all linked to rocks and stony places, differently from other *Lygodactylus*

species from South Africa (e.g. *L. lawrencei*, *L. ocellatus*, see Branch, 1988).

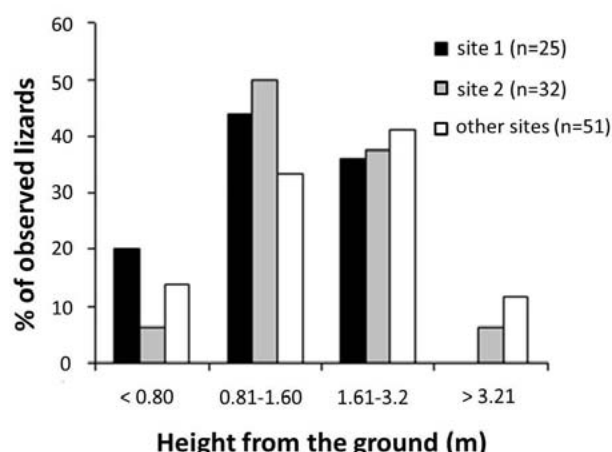
### Ecological observations

At site 1, 100% of the lizards (n = 25) were observed in habitat (c), and at site 2, 100% (n = 32) were observed in habitat (i). Thus, these lizards appeared to be habitat specialists in both the study sites, despite the two preferred habitats were quite different each from the other (i.e. a shaded re-growing secondary forest versus the surroundings of a bamboo hut). Adding the other 51 individuals observed by us in south-eastern Nigeria to the sample sizes from sites 1 and 2, it resulted that the number of observed lizards was significantly positively influenced by the percent of shade within each habitat type ( $r=0.640$ ,  $n = 13$ ,  $r^2=0.410$ ,  $P < 0.02$ ) (Fig. 4). Thus, it is likely that an important correlate of their habitat selection within each presence site would be the relative availability of shade. Indeed, these lizards were often observed while moving in the shade: in Tabaa, for instance, these geckos preferred a shaded bush near a hut made of bamboo, and they were often observed to move from this bush to the hut to forage on insects (especially termites) they find in the bamboo hut. Pooling all the data recorded by us in southeastern Nigeria



**Figure 4.** Relationships between percent of shade and number of observed individuals of *L. conraui* in each habitat in the various presence sites in southern Nigeria. For statistical details, see the text.

(n = 108 geckos), there was a significant difference in terms of utilisation of the four categories of heights of climbing by lizards ( $\chi^2 = 10.89$ ,  $df = 3$ ,  $P < 0.05$ ), with at all sites geckos being found most commonly at 0.8–3.2 m height (Fig. 5). Although it is possible that the decreasing frequency of observations of geckos at over 3.2 m height may be due to observational biases (due to small size of the



**Figure 5.** Perpendicular height (m) from ground of *L. conraui* individuals (total n = 108) observed at the various study sites. Most of the individuals were observed on trees, but occasionally also on palm fronds, houses and other walls. For the statistical comparisons, see the text.

animals and their cryptic colouration), their low frequency of occurrence at ground level and at low heights was surely not biased. Our quantitative observations confirmed that *L. conraui* is clearly a climbing, mostly arboreal species, as the great majority of the about 60 species of *Lygodactylus* worldwide (an exception being *L. gravis* from Tanzania; Msuya et al., 2014).

Despite the higher survey effort in the dry season (see methods), 81 out of 108 (75%) individuals (data from all sites being pooled) were observed in the wet season (inter-seasonal difference:  $\chi^2 = 14.4$ ,  $df = 1$ ,  $P < 0.0001$ ). The statistically higher activity of these geckos during wet season mirrors more general data on the seasonal activity of reptiles in the Niger Delta, that consistently revealed wet season activity peaks for these animals (e.g., Akani et al., 2014a).

Distance between individuals (m) was measured in 17 cases, and was 1.35 m (range 0.70-3.2 m) on average, thus suggesting that the demographic structure of these reptiles may be characterised by small groups of individuals that live very close each other, probably concentrating in sites with suitably high prey density and appropriate microclimatic conditions. Another possibility is limited retreat and/or egg-laying sites, and if this is the case, certainly this would dictate local distribution and spacing in at least some other geckos. Both these hypotheses remain merely conjectures in absence of larger data samples and will require further investigations.

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# Genetic confirmation of the occurrence and notes on the ecology of the yellow-bellied toad, *Bombina variegata* (L., 1758) (Amphibia: Bombinatoridae) in the European part of Turkey

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**ABSTRACT** - We confirmed the occurrence and report some ecological traits of the yellow-bellied toad, *Bombina variegata* from Turkish Thrace. The specimens were caught in April 2016 from ponds and several streams of Karacahasan Mountain, Enez District, Edirne Province. In order to determine and confirm its systematic position, we genetically analysed the tissue samples of caught specimens. We also present some behavioural data of the species.

## INTRODUCTION

The yellow-bellied toad, *Bombina variegata* has a wide distribution in Europe and is represented by three subspecies: nominotypic subspecies *B. v. variegata* in Western Europe and Carpathian Mountains, *B. v. pachypus* in Apennine Mountains, *B. v. scabra* in Balkans (Vukov et al., 2006; Fijarczyk et al., 2011). *B. v. pachypus* is suggested as full species by Canestrelli et al., (2006). *B. v. kolombatovici* is found identical to the nominal subspecies, which is supported by mtDNA analyses (Fromhage et al., 2004).

Recently, a first record of *B. variegata* was reported from Turkey; it was affiliated to *B. v. scabra*, which is the geographically nearest subspecies and well known from the Balkans (Bülbül et al., 2016). However, the very first record of the species in Turkey was given by Boulenger (1897) as *Bombinator pachypus* (actually a subspecies of *B. variegata*) from Adrianople (ancient Roman name of Edirne Province, Turkey) without the exact location, and then mentioned by Atatürk & Yılmaz (1986) who considered Boulenger's record as irrelevant. In the Bülbül et al. (2016)'s paper these two aforementioned references were ignored.

In April 2016, during a herpetological trip to the European part of Turkey, we encountered a yellow-bellied toad population in Karacahasan Mountain, Enez District, Edirne Province, which was morphologically assigned to *Bombina variegata scabra* (Küster, 1843) by Bülbül et al. (2016). The main aim of this study was confirmation of the occurrence, to genetically determine the systematic position of the found *Bombina variegata* population, and to reveal some ecological traits of the species in Turkey.

## MATERIALS AND METHODS

We observed about 50 specimens (adults and juveniles) of which 19 adults were captured (9 males, 10 females). Adult males were recognised by the presence of nuptial

pads on the fore limbs. We photographed all caught specimens individually and took tissue samples from the toes of all individuals. We stored the tissue samples in 96% ethanol. We afterwards released all specimens to their natural habitat.

The authors had received special permission for the fieldwork from the Republic of Turkey, Ministry of Forestry and Water Affairs, Directorate of Nature Conservation and National Parks (permit number: 2014-51946). Also, ethical permission for tissue samples from Ege University Animal Experiments Ethics Committee (permit number: 2014-002) was received.

DNA was isolated from five samples using the Qiagen Blood and Tissue Kit following the manufacturer's instructions. We sequenced a ca. 500 bp fraction of the 16SrRNA gene [primers 16SAL and 16SBH of Palumbi et al. (2002); initial melting for 120 s at 94 °C, 33 cycles of denaturation for 30 s at 94 °C, primer annealing for 30 s at 51 °C, extension for 60 s at 65 °C, final step at 65 °C for 10 min]. PCR reactions were prepared using the 5Prime Master Mix. PCR products were purified using the High Pure PCR Product Purification Kit of Roche. Sanger reactions were run using the Big Dye Terminator (ABI) with initial melting for 60 s at 96 °C, 25 cycles of denaturation for 10 s at 96 °C, primer annealing for 5 s at 50 °C, extension for 240 s at 60 °C. Single stranded fragments from both directions were sequenced on an ABI 3500 Genetic Analyzer Series 2 automatic sequencer using standard protocols.

Phylogenetic analyses were done with Mega (version 7, Kumar et al., 2016). We added homologous reference sequences from complete mitochondrial genomes of known *B. variegata* and *B. bombina* lineages (GenBank accession numbers JX893172, JX893174, JX893176, JX893177, JX893178, JX893179, EU115993 and AY971143; Pabijan et al. 2008, 2013) as well as from two *Alytes* species (GenBank accession numbers AY333709 and AY333710; Fromhage et al., 2004) as outgroups. Sequences were aligned with ClustalW (Thompson et al., 1994). Our

intention was not to produce a robust phylogeny of selected *B. variegata* haplotypes, rather we wanted to just barcode the new samples. We therefore only performed a reduced phylogenetic analysis. The best fitting out of 24 substitution models were selected with Mega 7 (Kumar et al., 2016) based on AICc values. For phylogenetic reconstruction we used Maximum-Likelihood (ML), with the selected substitution model (GTR+G) and 2,000 bootstrap replicates.

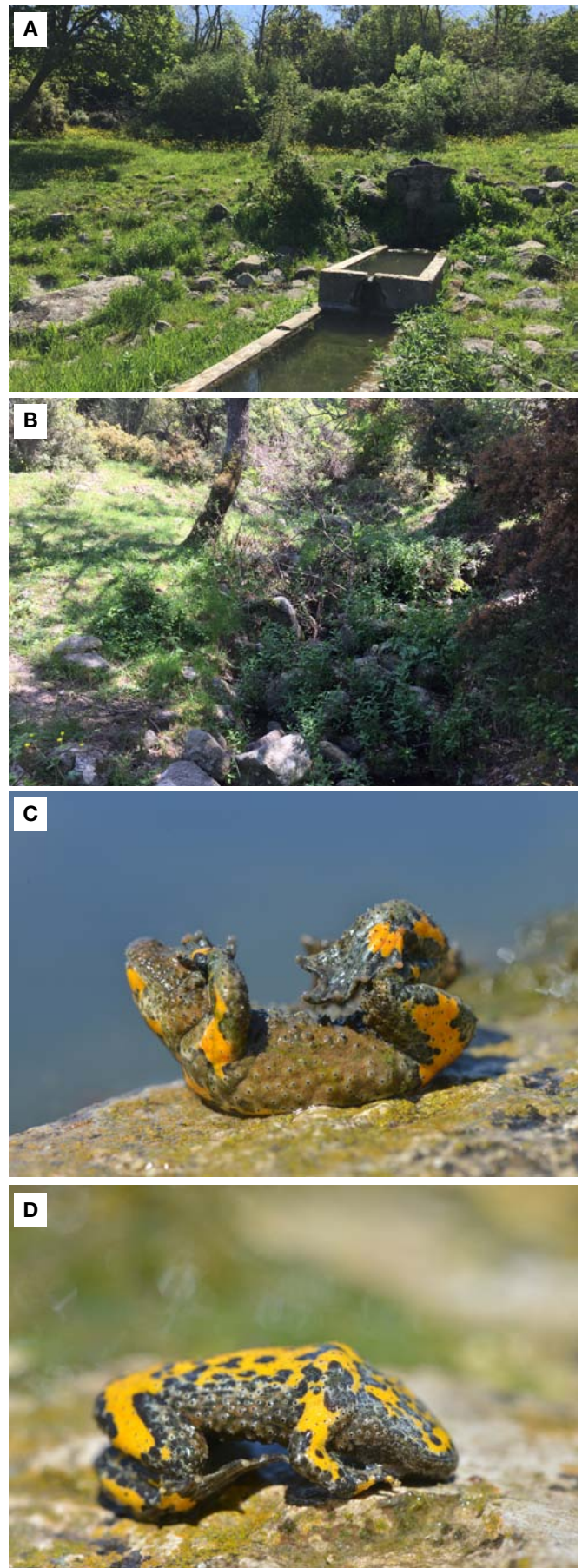
## RESULTS AND DISCUSSION

Morphological comparison of live specimens from our study and previously published results (Radojičić et al., 2002; Vukov et al., 2006; Bülbül et al., 2016) do not differ much, so we did not present any measurements or colouration-pattern characteristics.

We observed about 50 specimens in almost all ponds and streams of the north-western parts of Karacahasan Mt., i.e., the south-eastern of Gala Lake (Fig. 1-a, b). The first record was given from one pond (181 m asl) by Bülbül et al. (2016), we restated the altitude range of the species as 85-185 m asl on Karacahasan Mountain. Specimens were found in the pond at the highest elevation (185 m asl; 40° 45' N, 26° 13' E) and on the stream side at the lowest elevation (85 m asl; 40° 45' N, 26° 13' E). The exact coordinates (second parts of the GPS) are not given for conservation purposes. Air temperature was 19 °C, while the water was colder at range between 4-7 °C. During the photography session of the individuals, some specimens showed defensive postures (unken reflex) (Fig. 1-c, d). One specimen (Fig. 1-d) did the ventral unken reflex behaviour spontaneously while we tried to catch the specimen from the pond. We moved the specimen from the water to rock surface for better photography. Also some pairs of specimens were on inguinal amplexus behaviour while they were stored together in containers before photography session and tissue sampling. Nuptial pads of the male specimens were also very distinct, so we can indicate the mating season starts from April. Habitat and behavioural photographs are given in Fig. 1.

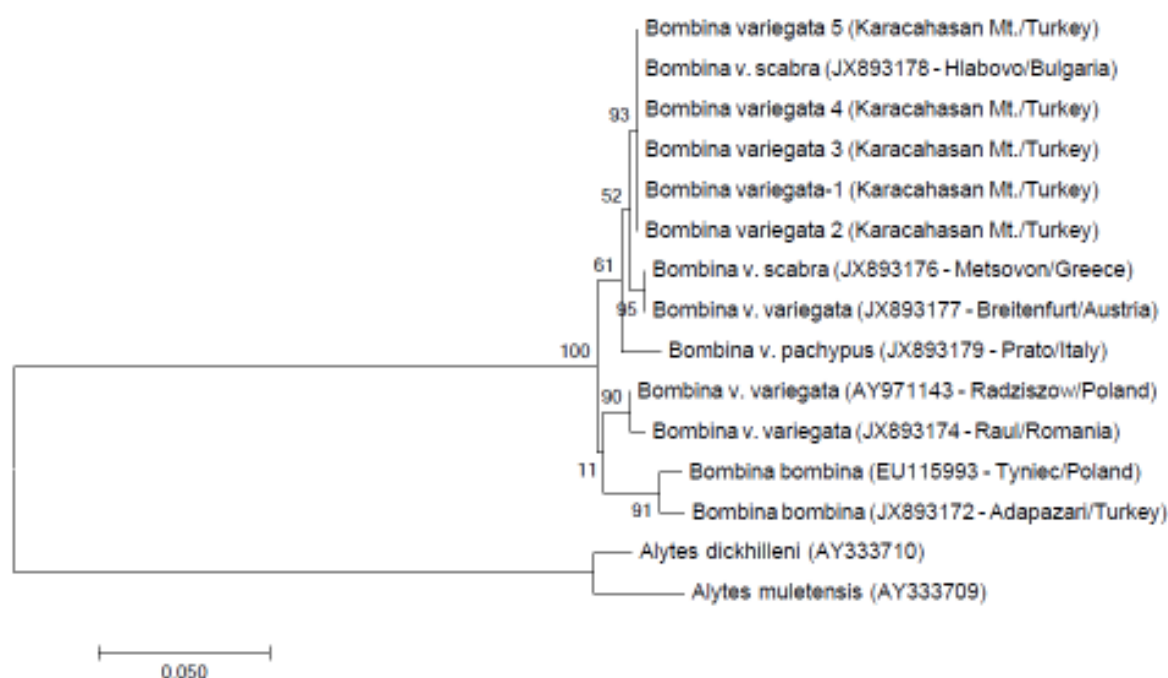
The sympatric herptiles, *Emys orbicularis* (Linnaeus, 1758), *Lissotriton vulgaris* (Linnaeus, 1758), *Natrix natrix* (Linnaeus, 1758), *Podarcis tauricus* (Pallas, 1814), *Pelophylax ridibundus* (Pallas, 1771), *Bufoles variabilis* (Pallas, 1769) were observed.

All samples showed the same haplotype (GenBank accession numbers KY496588-KY496592). According to the results of the phylogenetic analysis, our *Bombina variegata* haplotypes and *B. v. scabra* from Bulgaria (B3 in Pabijan et al., 2013), which is geographically closest to the Turkish samples, form a clade with 93% bootstrap support [Fig. 2; topological discordance to the tree shown by Pabijan et al. (2013) is due to the reduced data set]. According to their molecular dating this lineage must have diverged from the second Balkan lineage of *B. variegata* during the Early Pleistocene.



**Figure 1.** Pond habitat (A), stream habitat (B), dorsal unken reflex [boating] (C) and ventral unken reflex (D) of the yellow-bellied toad, *B. variegata*





**Figure 2.** Maximum likelihood tree based on the GTR+G substitution model; numbers at nodes indicate bootstrap values for 2,000 replicates

## ACKNOWLEDGEMENTS

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# Geographical distribution and conservation biology of the Mesopotamian spiny-tailed lizard *Saara loricata* in Bushehr Province, southern Iran

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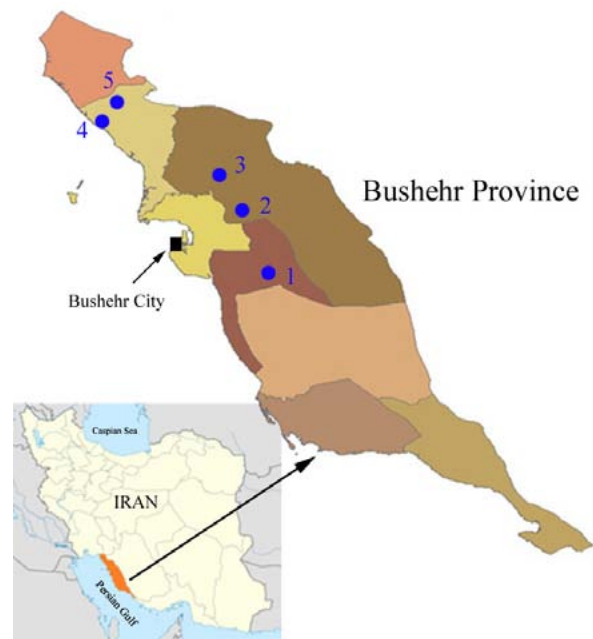
## INTRODUCTION

Spiny-tailed lizards are inhabitants of the deserts and semi-deserts of North Africa, Arabia, and the Middle East (Wilms et al., 2009). Three species are found in Iran: *Uromastyx aegyptius*, *Saara asmussi* and *Saara loricata* (Anderson, 1963, 1974, 1999; Firouz, 2000; Wilms, 2005; Sindaco and Jeremcenko, 2008; Šmíd et al., 2014). The Mesopotamian spiny-tailed lizard (*S. loricata*) is confined to the Mesopotamian plain and Zagros foothills of Iraq and southwestern Iran, and the Gulf coastal plain of southwestern Iran (Leviton et al., 1992; Anderson, 1999). In Iran, this includes the provinces of Kordestan, Kermanshah, Khuzestan, Lorestan, Bushehr and Fars (Anderson, 1974; Wilms, 2005). The species is possibly present in Kuwait, however this needs to be confirmed (Papenfuss et al., 2008).

Most studies of the Mesopotamian spiny-tailed lizard have concerned the taxonomic status of the species and its affinities (Haas and Werner, 1969; Joger, 1986; Moody, 1987; Anderson, 1999; Wilms et al., 2009). Investigation of conservation ecology has been limited to a handful of papers (Frynta, 1997) and there has been no recent information on the distribution and abundance of the species in Iran for assessment of conservation issues. The species is categorised as Least Concern (LC) in the IUCN Red List (Papenfuss et al., 2008), in Appendices II of CITES (CITES, 2009). It is protected by wildlife conservation laws of Iran (Department of the Environment, 2006). The aim of the present study is to provide up-to-date data on the geographic distribution and conservation biology of *Saara loricata* in Bushehr Province, Iran.

## MATERIAL AND METHODS

Field work was carried out in Bushehr Province, southern Iran. We listed all the areas in which we observed *S. loricata*. We then made a more detailed survey of the Genaveh area (Fig. 1). We delineated the potential range of the lizards based on previous reports and interviews with local people. Then we looked for lizards 8.00 and 10.00 a.m. local time and looked for signs (scats, feeding or resting signs and tracks) by hiking along trails and sometimes by using pseudo-random transects. We tried to visit all villages and interviewed more than 40 elderly local-born people,



**Figure 1.** Map showing the location of Bushehr Province and the locations of the detailed areas (numbered 1-5) mentioned in the text

including farmers, for information about the species. We asked them about existence and relative abundance in the past and present at different localities. We asked whether they had ever seen the Mesopotamian spiny-tailed lizards, when they had seen them and how many they had seen. We also enquired as to problems with the lizards and whether they had seen or heard of them being eaten.

## RESULTS AND DISCUSSION

### Distribution

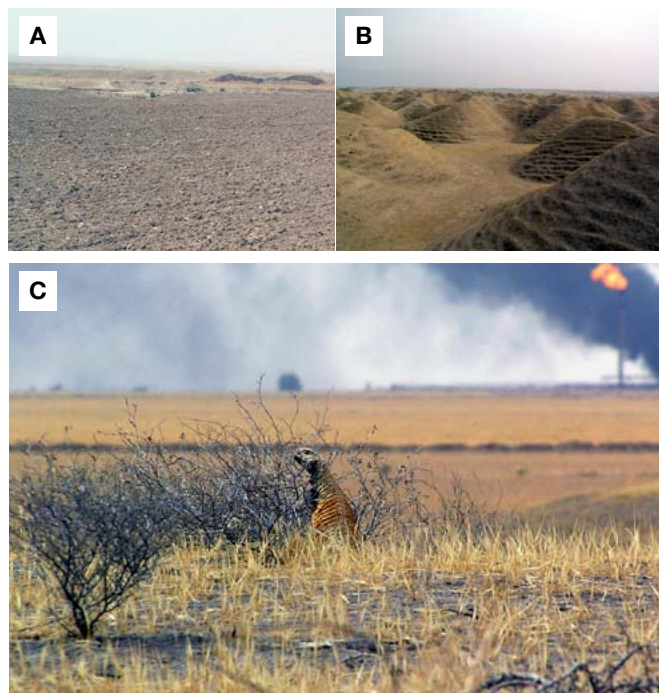
Based on our field observations, the geographic distribution of *Saara loricata* in Bushehr County is as follows: (1) Hillsides by the road from Tangestan to Khaeiz (28°49'37.3'' N, 51°20'02.2'' E); (2) Borazjon, Abol Firouz village, (29°8.00'07.6'' N, 51°11'31.9'' E); (3) Borazjan, Shul Village (29°29.00'26.1'' N, 51°09'51.3'' E); (4) Genaveh near to Shul-e Kuchek Village (29°48.00'13.8'' N, 50°30'42.4'' E); (5) Genaveh on the road to Deylam (29°40.00'43.7'' N, 50°24'01.7'' E). The locations of these five areas are shown in Fig. 1.

### Habitat

*S. loricata* inhabits three main habitats throughout its range in southern Iran: hilly areas, deserts and agricultural lands (Fig. 2). The most important need appears to be for compact soil to facilitate burrowing, although in rocky habitats in the Zagros Mountains the lizards select gravel soils and in the coastal Persian Gulf they are found in strips of wasteland between agricultural areas. Other reptile species often observed in the same habitats include *Trapelus agilis*, *Stenodactylus affinis*, *Bunopus crassicauda*, *Echis carinatus* and *Coluber* spp. The main stronghold of the species is the foothills of Genaveh plain.

The largest of three burrows that we measured was 240 cm in length with 3–4 bends. The burrows extended to about 70 cm under the ground surface (Fig. 3): they have an initial part which slopes at about 30–45° leading to a horizontal, more spacious, section which is used for resting at night and egg-laying (Fig. 3). If lizards are resting by day, this tends to be in the sloping part of the burrow. There may be subsidiary burrows connecting with the horizontal chamber, but these appear to be little used.

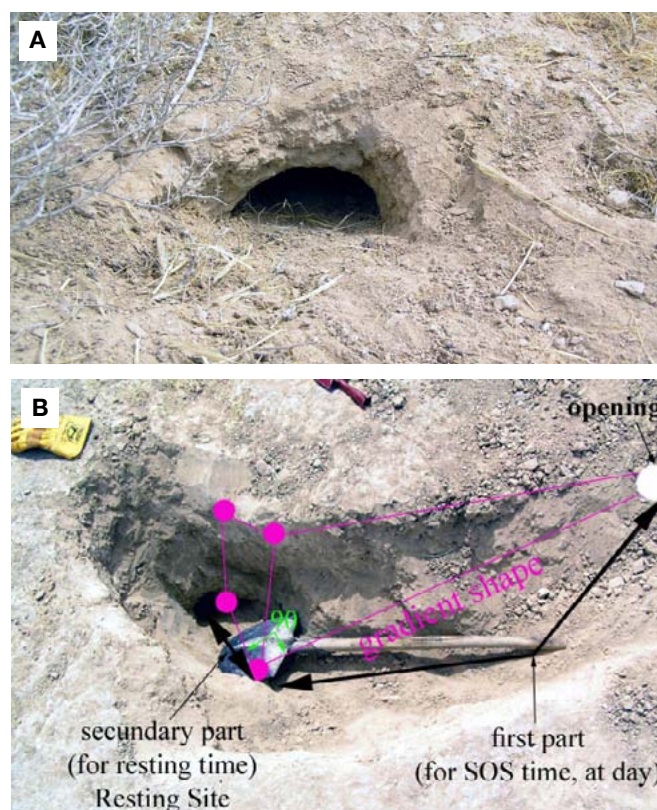
*S. loricata* are primarily herbivores, mostly feeding on leaves and twigs of shrubs in the genus *Prosopis* (which are closely related to North American mesquites). Wheat and barley plants may be consumed in agricultural areas. Occasionally the lizards pick up grasshoppers and other insects.



**Figure 2.** Some habitats of *S. loricata* in Bushehr Province (A) agriculture land, (B) hilly land, and (C) desert land

### Conservation: threats to the species.

The lizards are most abundant in the Khaeiz area (location 1 in Fig. 1), where they appear to be under no serious threat. This is a mountainous area and human activity is prohibited. Near to Borzjan, road building companies has destroyed some of the habitat, but substantial lizard



**Figure 3.** Shelter sites of *S. loricata*. (A) burrow opening, (B) interior of one burrow

populations remain. Around Shul village, however, where according to local people the species was abundant 20–30 years ago, destruction of the natural habitat by date palm plantations has severely reduced populations. The lizards are now found mostly in fragments of natural habitat at farm boundaries. General intensification of agriculture and recent droughts are the greatest threats in the Borozjan area. In the area around Genaveh oil and gas extraction have severely modified the habitat and result in considerable disturbance (Fig. 2C), and over-grazing by domestic animals is also problem.

The ideal solution to these problems would be the creation of special conservation areas where vehicular and agricultural activity should be prohibited. The data embodied in Fig. 1 show where such reserves would be most useful for the conservation of *S. loricata*. Since Bushehr Province is a biodiversity hotspot in the N.E. Persian Gulf, this would be a particularly appropriate area in which to set up reserves of this kind. In the meantime, as with most conservation needs, public education has an important role to play – spiny-tailed lizards are still feared by many people. Finally, the data presented here suggest that, at least so far as Iran is concerned, the “least concern” status of *S. loricata* should at least be re-examined.

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## *Teira dugesii* (Sauria: Lacertidae) – high aggregation

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The Madeiran wall lizard *Teira dugesii* (Milne-Edwards, 1829) is endemic to the Madeiran archipelago (Kwet, 2009), where it occurs in a wide range of habitats from sea level up to mountain tops (Sadek, 1981). However, several populations (probably introduced by human activity) have been also reported from the city of Lisbon, Portugal (Sá-Sousa, 1995), Selvagens islands (Bischoff et al., 1989) and several islands of the Azore archipelago (Malkmus, 1984). *T. dugesii* is omnivorous with a diet consisting mainly of insects, plant leaves, flowers, pollens, fruits and seeds. Plant residues (nectar, blooms, leaves) may even represent up to 60 % of the diet (Sadek, 1981). This species is also considered to be the first lizard reported to consume plant nectar (Elvers, 1977) and occasional predation on seabird's nestlings (*Calonectris diomedea*) has been reported (Matias et al., 2009). This paper reports on a high aggregation of individuals on a single site.

The observation was made on April 8th 2016 around 14:00h of local time on a rocky plate by a route leading from Pico Arieiro on Pico Ruivo, Madeira, Portugal (32°44'17"N, 16°55'57"W; 1600 m a. s. l.). One of us

(VV) noticed a very high aggregation of *T. dugesii* (>30, males and females) on a single spot (Fig. 1). Many more individuals (>100) were observed on cracked sun-exposed rocks in the surroundings. A steep rocky slope represented the general site with numerous loopholes providing potential shelters. Weather conditions at the time were rather cloudy with occasional sunshine and temperature between 15 - 18 °C. The aggregation possibly involved thermoregulation as the location was in a sunny locality and that also provided many potential retreat sites.

Aggregation behaviour is known in many lizard species; e.g. large numbers of marine iguanas *Amblyrhynchus cristatus* on small areas of Galapagos reefs (Eibl-Eibesfeldt, 1984). Explanation for such gatherings is complicated. Previous studies have reported benefits of reptile aggregations – for example increased chance for survival during wintering in *Xantusia* (Rabosky et al., 2012). Hofmann (2008) describes aggregations in the lacertid *Zootoca (Lacerta) vivipara* for thermoregulation and also predator avoidance. This behaviour is generally considered to present insight into lizard social interactions



Figure 1. High aggregation of *T. dugesii* individuals (Photo: VV)

(e.g. Duffield & Bull, 2002) but reports of specific observations are often missing. According to Gardner et al. (2015), such high aggregations have not been previously reported in *T. dugesii*. Therefore we believe that our report may provide value for future studies on social interactions within this species.

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# Observation of a *Varanus salvator* consuming potentially dangerous waste refuse in Karamjal, Bangladesh Sundarbans mangrove forest

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*Varanus salvator* Laurenti, 1768 (Asian water monitor) is the largest monitor lizard found in Bangladesh (Khan, 2008; Hasan et al., 2014) where it is considered Vulnerable (IUCN Bangladesh, 2015). Anthropocentric pressures, whether directly or indirectly, are considered the primary threats to biodiversity loss across Bangladesh with animal numbers decreasing from environmental change. However, some species, including monitor lizards, are more flexible in their behaviour which allows them to adapt to human altered ecosystems. Monitor lizards are well known scavengers and this note records potential problems for these lizards when scavenging around human habitations.

On 4 August 2016, 11:38 (Bangladesh Standard Time) an adult *V. salvator* was observed scavenging among aquatic vegetation in tidal water behind a building at Karamjal ecotourism center (N 22°25'43.19"/ E 89°35'24.96"), Bagerhat, Bangladesh. Here we observed the lizard consume a piece of polythene bag from an open garbage site near the ecotourism office kitchen (Fig. 1). The lizard tried to engulf the polythene bag by moving its head up and down in a sideways lashing movement. After about 10 minutes the lizard succeeded in consuming the whole section of polythene

This type of behaviour is clearly unusual and to the best of our knowledge has not been recorded previously. We assume that the lizard had mistaken the plastic for a natural food item. Consumption of such a man made products is likely very harmful for the lizards and in terms of conservation, action should be taken by companies to clean up the area and not discard dangerous waste materials into the environment.

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**Figure 1.** *V. salvator* from Karamjal, Bangladesh Sundarbans consuming a piece of polythene

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## Observation of a paedomorphic palmate newt (*Lissotriton helveticus*) in Scotland

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Paedomorphosis or neoteny is one of two developmental pathways that amphibians can follow (Denoël, 2006), and is characterised by the maintenance of juvenile features, such as external gills, through to adulthood (Whiteman, 1994). In newts, neoteny may be dependent on environmental determinants (Denoël, 2005) and is uncommon in Scotland (McInerny & Minting, 2016). Since 2012, the author has undertaken amphibian monitoring of up to 30 ponds in East Kilbride, South Lanarkshire, Scotland, utilising standard methods (Paterson, 2014). This note reports the presence of a single individual paedomorphic palmate newt, *Lissotriton helveticus*, in Central Scotland.

On the 20th April 2014 at 22:00 a paedomorphic palmate newt *L. helveticus* was captured in a hand net (Fig. 1). The animal was adult sized (c.75 mm), with light brown dorsal surface colouration. It had a low, smooth dorsal fin over the posterior two thirds of the torso continuing to the tip of the tail, which did not have a terminal filament. A pair of dark dorsolateral stripes posterior of the shoulder continued to the hip becoming less distinct on the tail. The lateral surfaces were mottled dark fading onto the ventral surface which was light tan to cream and unmarked. The head was the same light brown as the dorsal surface, with the external gills lighter tan-yellow. The cloaca was small, and the animal appeared to have a distended stomach suggested that it was a gravid female, though this was not confirmed.

The newt was found in a Sustainable Urban Drainage System (SUDs) pond located at NS 59444 54170, approximately 1500 m<sup>2</sup> in area, and up to 1 m in depth (Fig. 2). The pond is densely vegetated with aquatic plants, though dominated by reeds (*Phragmites* sp.), and subject to runoff water effluent from the adjacent A726 dual carriageway. In addition to palmate newts, both common frogs *Rana temporaria* and common toads *Bufo bufo* inhabit the pond, the nearest detected population of smooth newts *Lissotriton vulgaris* is 1.4 km away.

Denoël (2005) notes that paedomorphic populations of *L. helveticus* are particularly common on the Larzac plateau, France, mostly in man-made ponds surrounded by arid habitat, suggesting that paedomorphosis in these cases may be facultative. However, the East Kilbride SUDs pond is surrounded by non-arid habitat and so it is unlikely that avoiding unfavourable terrestrial conditions is an explanation for paedomorphosis here. An alteration of the chemical balance of water bodies are known to affect the development of amphibians (Mann et al., 2009) and has been suggested as a potential cause of paedomorphosis (Dodd &



**Figure 1.** Female neotenic palmate newt *L. helveticus*, East Kilbride, Scotland, April 2014



**Figure 2.** The East Kilbride, Scotland SUDs in which the neotenic newt was found, April 2013

Callan, 1955). The East Kilbride SUDs pond is subject to run-off effluent from a road which may have altered the chemical composition, though this is untested. However, O'Brien (2015) noted in 12 SUDs throughout Inverness, Scotland that the inorganic chemical concentrations were lower than would be sufficient to cause adverse effects in amphibians. It has also been suggested that an explanation

for the presence of neotenic newts in higher latitudes is due to the cooler climate and shorter summers causing slowed larval development resulting in failure to reach maturity before winter (Banks, 1985).

No other paedomorphic newts have been found at the site. However, paedomorphic palmate newts have been reported in Fife, Scotland (Dodd & Callan, 1955) though are considered to be rare in Scotland, with paedomorphic great crested newts *Triturus cristatus* even rarer (McInerny & Minting, 2016). In the UK, paedomorphic smooth newts are encountered most often (Banks, 1985; Leeke, 1990) with paedomorphic great crested newts less commonly reported (Baker, 2006).

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## Predation of *Feihyla hansenae* (Hansen's bush frog) eggs by a nursery web spider

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*Feihyla hansenae* (Cochran, 1927) is an arboreal-breeding frog distributed in Thailand, Cambodia, and Myanmar (Taylor, 1962; Aowphol et al., 2013). Eggs are laid in a gelatinous hemispherical clutch overhanging ponds and are cared for by female frogs until they hatch (Average egg stage = 5 days). Maternal care, viz. suppling water (Poo & Bickford, 2013) and deterring invertebrate predators (Poo et al., 2016a), is essential to the development and survival of eggs. The primary source of egg mortality is predation (Poo & Bickford, 2013). Known egg predators include ants, katydids, and snakes (Poo & Bickford, 2013; Poo et al., 2016b). In cases of partial clutch predation, threats from egg predators can lead to premature hatching in *F. hansenae* (Poo & Bickford, 2014), which can negatively affect the fitness and survival of hatchlings in subsequent life stages (Gomez-Mestre & Warkentin, 2007).

Here, we report the first observation of *F. hansenae* egg predation by a nursery web spider, *Nilus* cf. *albocinctus* (family Pisauridae, Fig. 1). Field observations were conducted at a dammed pond within the Sakaerat Biosphere Reserve in northeastern Thailand (14.5090° N, 101.9537° E, WGS 84). At 00.57 hrs on 24 July 2013, we observed an adult *N. cf. albocinctus* preying on a *F. hansenae* egg clutch (Fig. 1). The egg clutch was attached to a blade of grass and was located roughly 50 cm above the pond surface. Embryos within the egg clutch had not started neural fold development, and there was minimal egg jelly covering the outer surface of the clutch. Based on the developmental stage of embryos, the egg clutch was laid within 24 hours prior to the predation event. We concluded our observations five minutes later, with no change in spider behaviour. No female frog was observed to be caring for the egg clutch or present within the vicinity of the egg clutch during our observations.

Little is known about spider predation of frog eggs in Southeast Asia. However, in the Neotropics, spiders in the family Pisauridae are known to prey on adult and juvenile frogs (Menin et al., 2005). To our knowledge, *Nilus* species predation of frog eggs has not been reported for arboreal-breeding frogs in Southeast Asia thus far.



**Figure 1.** Predation of *F. hansenae* egg clutch by *N. cf. albocinctus*.

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## An egg mass of the spotted salamander, *Ambystoma maculatum*, in an unusual terrestrial location

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North American salamanders in the family Ambystomidae typically oviposit in aquatic situations, (reviewed by Petranks, 1998). Spotted salamanders, *Ambystoma maculatum*, a species native to the eastern United States and southeastern Canada, deposit egg masses in vernal pools, swamps, roadside ditches, other water filled depressions, ponds, lakes, and the backwater portions of streams (Petranks, 1998). However, Boyle & Moldowan (2013) recently reported two novel instances of terrestrial oviposition under moist logs by *A. maculatum*. On 25 March 2016 at 1000 h (U.S. Eastern Standard Time), in Columbia County, Pennsylvania, USA (40°50'10.5"N, 76°22'19.1"W, WGS 84 grid), I found an *A. maculatum* egg mass situated on a stone protruding approximately 25 cm above the ground within a wooded vernal pool/wetland area (Fig. 1). The egg mass appeared to be attached to the stone (Thompson & Gates, 1982), but no attempt was made to move or otherwise disturb the egg mass. Approximately 50 other *A. maculatum* egg masses were observed in vernal pools throughout the area. After three weeks, the egg mass was observed to be intact and in the same position on the stone but appeared to have desiccated since the prior observation.

This observation of an egg mass in a terrestrial microhabitat is highly unusual for *A. maculatum* (Petranks 1998; Boyle & Moldowan, 2013). A possible explanation, is the surface of the stone was underwater when the female selected the site to oviposit, and subsequent drying of water "stranded" the egg mass (Nyman, 1987). However, no other egg masses observed at the site were stranded terrestrially, and consistent rainfall occurred during the observation period (pers. obs.), suggesting this scenario is unlikely. The egg mass or ovipositing female may have been moved to the location by another organism (i.e., a predator); however, the jelly coating on egg masses and toxic cutaneous secretions of adult *A. maculatum* are deterrent to many predators (Petranks, 1998). Therefore, the egg mass, intact and attached to the stone, might have been intentionally oviposited in this location. Boyle & Moldowan (2013) hypothesized that terrestrial oviposition in *A. maculatum* may occur in response to stressors. Perhaps a stressor (e.g., injury, intraspecific competition for oviposition sites) might have stimulated the *A. maculatum* to oviposit on the surface of the stone. The top surface of the stone bore small depressions and pitting which might hold a small quantity of water during rainy weather (Fig. 1), potentially stimulating oviposition at this unusual and unsuccessful oviposition site.

Further observation of *A. maculatum* breeding sites might elucidate further data on the causative mechanisms of the unusual observation of egg masses in terrestrial locations.



**Figure 1.** *A. maculatum* egg mass situated on a large, terrestrial stone within a wetland/vernal pool area in eastern Pennsylvania

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## A case of xanthochromism in the common frog (*Rana temporaria*)

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Xanthochromism is a condition that is characterised by the lack of skin pigments except brown, orange and yellow (xanthophores, Mitchell, 1994). Xanthochromism, a form of amelanism, has been reported in all vertebrate classes with the most notable examples in wild animals include those in birds (Isted, 1985) and in fish (Schwartz, 1978). Chromatophores are responsible for producing the skin colour primarily in ectothermic animals; these specialised cells can be grouped based on the colour they reflect under white light. There are other groups of chromatophores which help to produce various colouration including erythrophores, leucophores and melanophores (Bagnara et al., 1968). The causes for xanthochromism and other abnormalities are rare and may be caused by environmental conditions as well as mutations (Jablonski et al., 2014).

During a routine survey at a man-made inlet in Chesterton, Cambridge, UK (TL 4647 5957) on 06 March 2014, we came across a xanthochromatic male common frog (*Rana temporaria*). At first, the frog (Fig. 1) returned a red eye shine from torchlight, so we believed the individual to be an albino. On closer inspection, the frog indeed had red eyes but he was yellow in colour. Over the course of a few evenings over April and May that year, the same individual was spotted twice more. On the first occasion the frog was in amplexus with a female. On the second occasion, we were able to capture the individual for closer inspection. The male's nuptial pads were noticeably lighter in colour than usual with a brown hue. The same individual was seen again at the site in April 2015 but was not seen during 2016.

Albinism and melanism have previously been reported in *R. temporaria* (Smallcombe 1949; Alho et al., 2010), however xanthochromism has yet to be reported. The opposite pigmentation abnormality to xanthochromism is axanthism, which is a domination of cyanophores giving the individuals that suffer from it an overall blue colour. This pigmentation abnormality is more common in frogs found in the USA compared to Europe (Berns & Uhler, 1966). Axanthism is yet to be reported in *R. temporaria* but it has been reported in the closely related *Pelophylax esculentus* and *P. lessonae* (Jablonski et al., 2014).

Williams (1959) describes a male common frog from Sanderstead, Surrey much like the one which we observed, although Williams refers to it as an albino. We believe that Williams (1959) misidentified the skin condition his frog



**Figure 1.** The xanthochromatic male common frog (*R. temporaria*) on our first encounter

was suffering from as it is most likely his frog was actually xanthochromatic and not a true albino. This is due to the yellow hue and the brown nuptial pads the frog is described as having – which is an almost identical description of the male discovered on our surveys. Unfortunately the level of detail given in the original report is limited, but through our own experience we believe that Williams (1959) is the first record of a wild xanthochromatic *R. temporaria* in the UK.

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# A coconut-eating monitor lizard? On an unusual case of frugivory in the melanistic Sulawesi water monitor (*Varanus togianus*)

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Among the top predators in many environments they inhabit, monitor lizards (Varanidae: *Varanus*) are known to be almost exclusively carnivorous (Losos & Greene, 1988). Only three out of nearly eighty currently recognised species are known to feed mainly on vegetarian items, for example on fruits, seeds, and leaves (Koch et al., 2013). These are *Varanus olivaceus*, *V. mabitang*, and the recently described *V. bitatawa* (Auffenberg, 1988; Struck et al., 2002). All three nominal species are endemics of the Philippine Islands and seem to represent an adaptive radiation (Welton et al., 2010). Asian water monitor lizards of the *V. salvator* complex are the most wide-spread monitor lizard group, inhabiting most of mainland and insular southeast Asia reaching their easternmost distribution in the northern Moluccas (Koch & Böhme, 2010). Accordingly, these euryoecious lizards are carnivorous feeding on a wide variety of prey including carrion, garbage, and even human corpses (Traeholt, 1994; Uyeda, 2010; Gunawardena, 2016).

During field work on Selayar Island off the coast of southwest Sulawesi, an adult male specimen of the melanistic Sulawesi water monitor (*V. togianus*) was found dead on a road south of the village of Benteng (6°7'10.73" S, 120°27'59.88" E) on 6 June 2006 (Fig. 1). The specimen had a snout-vent-length of 56.5 cm with the tail measuring

79.3 cm. Its head exhibited clear evidence from a lethal accident with a vehicle, while the remaining body showed no injuries. The specimen was collected to be examined in detail. In order to preserve and deposit it at the Museum Zoologicum Bogoriense (MZB Lac. 5951, field number AK208), the venter was opened to remove all stomach contents and prey items. Surprisingly, no prey remains were recovered from the digestive tract, but instead an unidentifiable mass comprised of countless small pieces of a whitish hard substance. After flushing and cleaning, closer examination revealed that these were small pieces of coconut flesh and had filled out the body cavity of the lizard (Fig. 2). Although the stomach contents of other voucher specimens of water monitor lizards collected from different localities on Sulawesi have not been investigated, some specimens disgorged the remains of scorpions, chicken, and sea turtle eggs when handled for examination. The coconut pieces of the Selayar specimen, however, represent the only case of frugivorous remains recorded.

Aside from reports of vegetarian nutrition in the three Philippine species mentioned above, only a few cases of frugivory in monitor lizards have been published so far. Mertens (1971), for instance, reported on banana-eating in the emerald tree monitor lizard (*V. prasinus*) in captivity. Among other unidentified items, Sprackland (1982) found



**Figure 1.** A road-kill specimen of the melanistic Sulawesi water monitor lizard (*V. togianus*) found dead on a road on Selayar Island, Southwest Sulawesi. To prevent physical decay the stomach contents were removed.



**Figure 2.** Emptying and cleaning the stomach contents revealed countless pieces of coconut flesh from the intestines of the monitor specimen depicted in Fig. 1

55% frogs, 17% geckos and 12% plant material in the feces of newly acquired (i.e. assumed wild-caught) *V. prasinus* specimens. Subsequently, this author fed bananas and cantaloupe melons in addition to various carnivorous items to his monitor lizards and noticed no digestive problems in the specimens. However, based on the examination of wild-caught museum specimens of *V. prasinus* Greene (1986) and Losos & Greene (1988) could not confirm Sprackland's (1982) observation.

Observations of frugivory from the wild are restricted to few anecdotal notes. Parry (1932), for instance, mentioned that water monitors in the Garo Hills of Assam, North-East India, are said to "(...) come into the fields to eat melons, cucumbers and the ears of paddy (...)". Vogel (1979) reported frugivory in *V. salvator bivittatus* from Java.

It is well known that monitor lizards, like snakes, use their tongues to trace potential prey by guiding odorous particles from the air to the olfactory receptors located in the Jacobson's organ on the palate (Smith, 1986). Consequently, a possible explanation for our unusual finding could be the fact that coconuts are widely used in the tropics to obtain coconut oil. For this purpose, the coconut flesh is cut into large pieces and spread on the ground to dry in the sun. This treatment with heat leads to fermentation processes that cause an offensive smell that is carried by the wind. Thus, it seems possible that the monitor lizard detected the odour of fermenting coconut flesh (called copra) when searching for food and regarded it as a rotting carcass. This assumption is supported from the observations of a keeper of monitor lizards who reported that specimens under his care ate pieces of banana or carrots after they had been in contact with crickets (K. Wesiak, pers. comm.). Our observation probably represents an exception since water monitors can only feed on coconut flesh, where coconut palms are cultivated and harvested by men, and also very few animals are able to open these hard-shelled fruits. Thus, although being probably one of the better-studied monitor lizard groups (i.e., Vogel, 1979; Gaulke, 1989), due to their wide distribution and ability to inhabit anthropogenic environments, further field studies are certainly needed to better understand the ecology of (Sulawesi) water monitor lizards.

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## Anophthalmia in adults of two Amazonian treefrogs (Anura: Hylidae)

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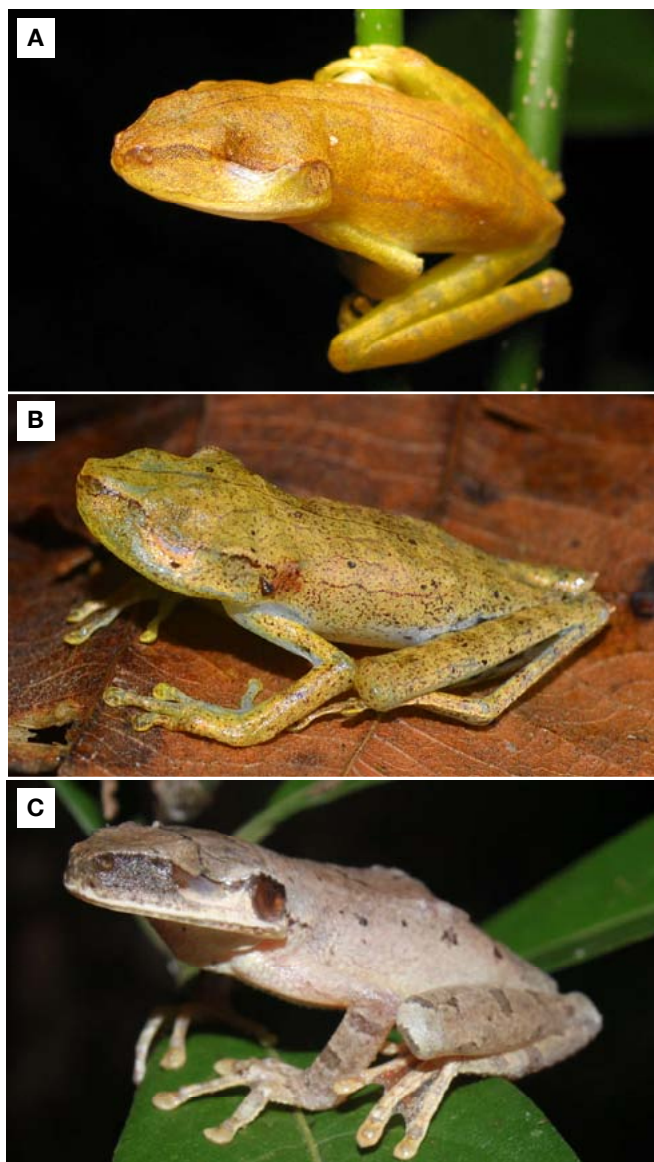
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**A**mong ocular deformities in anurans, anophthalmia is relatively common, characterised by the absence of one or both eyes (Meteyer, 2000). The absence of visual sense is highly detrimental, since sight is used for spatial perception, orientation, notion of depth, discrimination of barriers and surfaces, and location and selection of prey (Ingle, 1976). Some anurans also communicate by visual signaling, depending on vision during courtship, territorial and aggressive encounters (Toledo et al., 2007). Information about some abnormalities in frogs, as well as its causes are still little known and examples are rarely reported. In this note we report instances of two anuran species found without both eyes in different locations of the Brazilian Amazon.

On 19 December 2009, at 21:16 hrs, an eyeless adult *Hypsiboas fasciatus* (Fig. 1A) was recorded in an alluvial forest fragment (-5.205211°, -48.384711°) in Buriti do Tocantins, Tocantins state, Northern Brazil. The treefrog was found perched on a branch of a tree at 1.5 m above ground. This treefrog was not collected. On 5 January 2014, at 16:30 hrs, another eyeless adult *H. fasciatus* (Fig. 1B) was captured (ZUFG 8087) in a varzea forest (-8.632694°; -67.343083°) of the middle Purus River in Boca do Acre, Amazonas state, Northern Brazil. The treefrog was found perched on the branch of a bush at 1.0 m above ground. The identification of both *H. fasciatus* were performed according to the proposed by Caminer & Ron (2014). On 1 January 2016, at 18:51 hrs, an eyeless adult *Osteocephalus lepriurii* (Fig. 1C) was recorded in a riparian forest fragment (-10.267006°; -67.196667°) in Plácido de Castro, Acre state, Northern Brazil. The treefrog was perched on the branch of a bush at 0.4 m above ground. The frog was not collected. The identification of *O. lepriurii* was performed according to the proposed by Jungfer et al. (2013).

Examples of anophthalmia previously reported in anurans were believed to involve chemical pollutants in the environment originating from agriculture areas (Gurushankara et al., 2007; Guerra & Araújo, 2016), transgenerational and/or embryonic exposure to



**Figure 1.** Anurans with anophthalmia, missing both eyes, found in the Brazilian Amazon. **A** – *H. fasciatus* found in Buriti do Tocantins, Tocantins State; **B** – *H. fasciatus* found in Boca do Acre, Amazonas State; **C** – *O. lepriurii* found in Plácido de Castro, Acre State

petrochemical contaminants and metals (Bacon et al., 2013), trematode infection (Roberts & Dickinson, 2012), genetic incompatibility in hybrids (Smith et al., 2013), thyroid carcinomas (Cheong et al., 2000) and tyre debris (Mantecca et al., 2007). The absence of eyes can also be result of synergistic effects (Toledo & Ribeiro, 2009; Hayes et al., 2010; Bacon et al., 2013; Pizzatto et al., 2016). As multiple factors may interact to create anomalies, the causes of the conditions and survivorship of frogs in the wild state remain poorly understood.

Although vision has a fundamental role for anurans, frogs that are blind may orient themselves using the pineal complex, which responds to light stimuli (Cadusseau & Galand, 1980). However, although reaching the adult stage they appear unable to feed efficiently to maintain a good body condition (Pizzatto et al., 2016), which is especially important for arboreal anurans that may have sensory and neural capabilities that are not always found in terrestrial species (Wells, 2007). It is estimated, for example, that 88% of the prey of an arboreal species is obtained using visual detection and short pursuit (Freed, 1980). Therefore, we assume that to reach adulthood these blind individuals must have employed alternative feeding strategies.

The missing eyes in the three treefrogs recorded here seem to be a congenital feature but perhaps environmental conditions and biological characteristics of each species enabled the frogs to reach adulthood. However, abnormalities in anurans and their causes is not fully understood and further studies are required, in particular on to ascertain the possible effects of environmental degradation and disease in the decline of amphibian populations.

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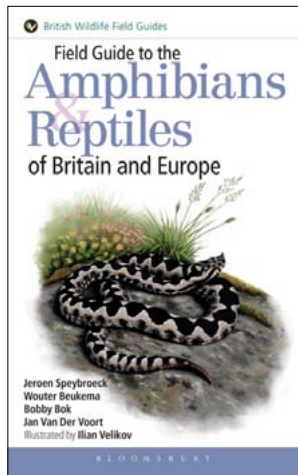
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# Field Guide to the Amphibians & Reptiles of Britain and Europe

Jeroen Speybroeck, Wouter Beukema, Bobby Bok, Jan van Der Voort, illustrated by Ilian Velikov

Bloomsbury, 432pp, ISBN PB 978-1-4081-5459-S (Paperback),  
ISBN HB 978-1-4729-3533-S (Hardback)



At large the 'field guide' is an interesting type of book. Good field guides have always been the cornerstone of an interest in any branch of natural history and many have become legendary publications in their own right. Such was the case with Nick Arnold and Denys Ovenden's, Collins Field Guide to the Reptiles and Amphibians of Britain and Europe published originally in 1978 (co-authored with John Burton) with a second edition in 2002.

Like all good field guides, the Collins publications provided the illustrations and descriptions to help naturalists with the considerable challenge of identifying the reptiles and amphibians they encountered during their travels around Europe. However the world has moved on and the first port of call is now the internet. How we find the places to look for wildlife and how we identify what we find has changed fundamentally. With Wi-Fi and mobile phones signals available almost everywhere, even though I'm a 'baby boomer' not a 'digital native' when I go looking for wildlife I find Google Earth the best way to locate and get to suitable habitat (in many areas much better than dodgy local maps) and I rely on the internet for the many excellent images of the animals I'm looking for to help with identification.

However books are special and the Field Guide to the Amphibians and Reptiles of Britain and Europe published by Bloomsbury under the British Wildlife brand is an exceptional field guide.

The layout of this guide is quite traditional with sort chapters up front on the diversity of reptiles and amphibians in Europe including species composition and conservation, how and where to find them, identification guides for amphibian early life stages and a checklist for all European amphibian and reptile species with tantalisingly empty tick boxes beside each species name.

The vast majority of space in the book however is taken up by the species accounts, colour coded into amphibians (which come first) and reptiles and organised into the obvious groups with simple identification keys that identify to a family level. The species are then grouped into families with identification keys to species level. Each species is described again quite traditionally; description, distribution, habitat and biology. The text in any field guide has to be quite succinct and clipped but throughout it is refreshingly readable with some nice detail to unravel similarities to related species. Based on the species with which I am most familiar, the accounts are extremely accurate and clearly based on real observation. Each species account is illustrated with an approximate distribution map and one or two photographs in addition to carefully prepared illustrations. Artist, Denys Ovenden's work in the Collins Field Guides is outstanding. The work of Ilian Velikov in this guide is breath-taking with all illustrations, but particularly the amphibians, incredibly life-like and accurate. I keep going back to the agile frog illustrations to check that they are actually not photographs.

This field guide describes 219 species and the accounts reflect up to date thinking on taxonomy which helps to unravel some of the more complex groups such as the Iberian wall lizards and the water frog group, *Pelophylax*. If you have not done so already, I urge you to buy this excellent field guide, it really is first class. It will rival the internet in its ability to help you in the field and it can happily sit alongside your Collins Field Guides (both editions) to help illustrate the worthy increase in knowledge as more and more field herpetologists share Jeroen Speybroeck's ambition (that he describes in his preface) to see all of the species of amphibian and reptile in Europe.

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# HERPETOLOGICAL BULLETIN REPORT

January 2017

All four issues of *Herpetological Bulletin* were published on time during 2016 with almost 100% of manuscripts published within 3 months of acceptance. The target for 2017 is a continuation of publication of papers in the next available issue once they have been accepted.

During 2016 we received 105 submissions with an additional 7 (not included in the table below) rejected without review. This represents a slight decrease in submissions compared to 2015. Full paper submissions remained high at around 40 % of total submitted manuscripts. Acceptance rate overall is now down to 31.4%. The reduced acceptance rate is mostly due the continuing high number of submissions.

The Table below provides the details with comparable figures for 2015 shown in parentheses.


	Submitted	Accepted	Percent accepted
<b>Full papers</b>	<b>42 (48)</b>	<b>13 (23)</b>	<b>30.9 (47.9)</b>
<b>Short Notes</b>	<b>11 (9)</b>	<b>7 (7)</b>	<b>63.6 (33.3)</b>
<b>Natural History Notes</b>	<b>52 (58)</b>	<b>13 (25)</b>	<b>25.0 (54.5)</b>
<b>Total</b>	<b>105 (115)</b>	<b>33 (51)</b>	<b>31.4 (44.3)</b>

Two reviews were published in 2016. Robert Jehle, Rachael Antwis and Richard Preziosi described herpetological activities at Greater Manchester Universities (issue 135) and Eleanor Drinkwater the threat to British amphibians from the amphibian pathogen, *Batrachochytrium salamandrivorans* (issue 137).

The following people gave their time and expertise reviewing manuscripts for *Herpetological Bulletin* during 2016:

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# THE HERPETOLOGICAL BULLETIN

Contents: Issue 139, Spring 2017

## REVIEW ARTICLE:

Zoological Society of London: contributions towards advancing the field of herpetology through conservation, research, captive management and education

BENJAMIN TAPLEY, JON BIELBY, MONIKA BÖHM, LOLA BROOKES, TYRONE CAPEL, JOE SMILEY CAPON-DOYLE, SHU CHEN, ALEX CLIFFE, LISA CLIFFORDE, OLIVIA COUCHMAN, ANDREW A. CUNNINGHAM, YEDRA FELTRER, AMANDA FERGUSON, EDMUND FLACH, MARTIN FRANKLIN, TRENTON W.J. GARNER, IRI GILL, CLAUDIA L. GRAY, RIKKI GUMBS, AMANDA GUTHRIE, JENNY JAFFE, STEPHANIE JAYSON, LUKE HARDING, MIKE HUDSON, DANIEL KANE, JO KORN, BECKI LAWSON, HEATHER MACINTOSH, NIC MASTERS, THOMAS MAUNDERS, FIEKE MOLENAAR, CHRISTOPHER J. MICHAELS, TREVOR MOXEY, CASSANDRA MURRAY, NISHA OWEN, PAUL PEARCE-KELLY, MATTHEW RENDLE, TONY SAINSBURY, CHRIS SERGEANT, FRANCESCA SERVINI, SOPHIE SPARROW, TAINA STRIKE, SARAH THOMAS & SAMUEL TURVEY.....

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