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Front Cover: A female northern viper (*Vipera berus*) photographed near Loch Lomond, Scotland. This individual has been observed for over six years at the same location, and observed during courtship and mating, successfully producing young twice. Photograph: Christopher McInerny. See article on page 6.

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Captive Husbandry and Breeding of the Tree-runner lizard (*Plica plica*) at ZSL London Zoo

LUKE HARDING[2,1], BENJAMIN TAPLEY[1], IRI GILL[1], DANIEL KANE[1], FRANCESCA SERVINI[1], INEZ S. JANUSZCZAK[1], JOE-SMILEY CAPON-DOYLE[1] & CHRISTOPHER J. MICHAELS[1*]

[1] ZSL London Zoo, Regent's Park, London. NW1 4RY, UK
[2] Paignton Zoo, Totnes Road, Paignton, Devon, TQ4 7EU
[*]Corresponding author Email: Christopher.michaels@zsl.org

ABSTRACT - Tree-runner lizards, *Plica plica* are neo-tropical ground lizards, native to South America. ZSL London Zoo has bred this species to the second generation (F2); and the 2.1 founder group has produced six clutches with a mean average of three eggs. The eggs were all removed for incubation, producing 11 viable hatchlings. The first F2 breeding took place in September 2015, and a clutch of two eggs were incubated producing two viable hatchlings.

This paper describes the captive husbandry and breeding of *Plica plica* at ZSL London Zoo, and serves to make some preliminary comparisons to wild data to suggest further areas of research and improvements for captive husbandry.

INTRODUCTION

Plica plica has a widespread distribution ranging across many areas to the east of the Andes and throughout much of Amazonia, the Guianas and eastern Venezuela.

Like many species of the lowland tropical rainforests of the Amazon, little is known about this species and much of its ecological data is anecdotal (Murphy & Jowers, 2013). Lizards referred to as this species have a widespread range through northern South America; however, the taxonomic identity of lizards found in the Amazonian Basin is uncertain and recent identification keys may not apply to this group of animals (Henderson & Murphy, 2012). In their study of tree runners, Murphy & Jowers (2013) identified *P. plica* as a cryptic species with a complex taxonomy. Their study showed that the species is distinguishable by the number of scale rows on the mid-body, the number of lamellae on the fourth toe, the number of sub-ocular plates and the arrangement, shape and ornamentation of the scales on the snout. *P. plica* is arboreal/scansorial, spending most of its time in the trees of the primary and secondary forests it inhabits. With a dorsoventrally flattened body shape, this species is frequently observed clinging to the side of tree trunks using its widely spread limbs and recurved claws, and rarely comes down from the trees except to lay eggs within the palm litter on the forest floor (Vitt, 1991).

This article presents methods developed at ZSL London Zoo for the maintenance and reproduction of *P. plica* in captivity, and data and observations regarding reproduction, fecundity and behaviour. The need to develop husbandry protocols was driven by the conservation needs of this species. As a group, reptiles are poorly represented on the IUCN Red List of Threatened Species and *P. plica* is not currently assessed. It is important that detailed life history information about a species is collected (in nature or as captives – there may be a difference) as such information can help inform extinction risk and conservation management (Bohm et al., 2016). Maintaining *P. plica* in captivity gave us an opportunity to build up a greater knowledge and understanding of this species to be better able to elucidate life history traits that are difficult to observe in nature.

METHODS

The animals held in the living collection at ZSL London Zoo are of unknown geographic origin, but are likely to originate from the Guiana Shield due to the prevalence of this source in the commercial trade in reptiles and the rarity of commercial animal exports from Brazil. Thus we have assigned the animals referred to in the present work as *P. plica* according to the key developed by Henderson and Murphy (2012).

Temperatures reported herein were measured using a digital thermometer (Precision Gold N85FR) and UVB radiation was measured in terms of UV index (UVi) using Solarmeter6.5 (Solartech). Lizards were weighed and digital callipers were used for all measurements.

Individual Identification

Each specimen was individually marked with non-toxic paint [Barry M Nail Pink Nail Polish], which was reapplied at intervals as it was lost through erosion and ecdysis. As animals began to develop adult colouration, photographic identification was possible and used alongside paint marking.

Sexing

Sexual dimorphism is apparent in adults of this species and a sexually mature male may be identified by their broader body width and their larger, broader heads (Vitt et al., 1997b). The males of the species also have more prominent femoral pores.

Basic Husbandry

A variety of enclosures were used with a variety of environmental parameters, stocking densities and

Table 1. Dimensions and environmental parameters of enclosures used for juvenile and adult *P. plica* lizards at ZSL London Zoo.

Enclosure type	Manufacturer	Dimensions (cm)	Life Stage	No. individuals	Lighting and heating array	Thermal gradient	UVi Gradient and method	Other information
Flexarium	ZooMed, USA	90x30x30	Adult	3			No records	
Exhibit	N/A	150x150x150	Adult	3	Osram Ultravitalux lamps; 2 basking areas	18-30; basking spot at 33˚C	0-4	Large filtered water body area (150x45x45cm); co-housed with sub-adult male *Dracaena guianensis*
Herptek	Herptek	Arboreal	Adult	3	12% D3+ T5 (Arcadia) (no reflector); 100W Mercury Vapour lamp in reflector dome (Arcadia)	22-26.6˚C; basking spot to 38.6˚C	0-3.3	-
Herptek	Herptek	Cube	Juvenile	Up to 3	6% T5 (with reflector); 80W mercury vapour lamp in reflector dome	22-25.4˚C; basking spot to 33.3˚C	0-3.5	-
Faunarium (PT 2265)	Exo Terra	37 x 22 x 25	Hatchlings (up to x cm)	Up to 2	6% T5 (no reflector)	22-28˚C; basking spot to 31.7˚C	0-2.5+/-0.1	-
Natural Terrarium (small/wide)	Exo Terra	45x45x45	Larger juveniles (between x and y cm)	Up to 3	6% T5, x3 T5s, 60W incandescent	22-25.5˚C; basking spot to 31˚C	3	-

dimensions, which are detailed in Table 1. In general, lizards were maintained with a temperature gradient of 22-28˚C during the daytime, with access to a basking site of 30-31˚C. At night, temperatures fell to around 22 ˚C across the enclosure. A UVi gradient of approximately 0-4 was maintained and UVB radiation was provided in gradients correlated with heat, UVA and visible light wavelengths to allow proper thermo- and photo-regulation.

Each of the different enclosures had a 5-7 cm base layer of substrate comprising horticultural bark chip topped with *Sphagnum* peat moss, which helps to hold moisture within the enclosures and aids with maintaining pockets of higher humidity and substrate saturation within the enclosure.

Young juvenile animals were kept in acrylic tanks (Faunarium 'Large', Exo-Terra; (see Table 1)) and here the substrate of choice was dry paper towels.

Various branches and palm fronds were used within the exhibits providing cover. Slabs of tree fern root, cork tile and cork bark were also provided for vertical climbing space. Lizards were also able to climb with ease on mesh and on rough concrete exhibit walls.

The enclosures housing the adult specimens were sprayed daily with tap water (alkalinity c. 280 mg/L) in order to increase relative humidity to around 70-80%, reflecting values frequently used for maintaining rainforest species, and to provide drinking opportunities from water accumulating on branches and plants. The substrates remained damp but not saturated. Standing water was also provided in small water dishes placed within the enclosures.

Body Temperature

The body temperature of lizards was measured on the dorsal surface using a non-contact infrared thermometer (Precision Gold N85FR), following the method described by Vitt (1991).

Diet

Vitt's (1991) study of *P. plica* in Amazonian Brazil suggests that their diet is predominantly made up of ants, which are abundant on the trunks of the trees on which *P. plica* live, although a significant proportion does comprise other invertebrates. Ants are not commercially available as a live food and so we offered insect prey including crickets (*Gryllus bimaculatus* and *Acheta domestica*), bean weevils (*Callosobruchus maculatus*), locusts (*Schistocerca gergaria*), and cockroaches (*Blaptica dubia*) as well as large fruit-flies (*Drosophila hydei*) for juveniles.

Food was offered three times per week. Lizards were fed in early afternoon to allow animals to warm up through basking before hunting, while still allowing time for digestive basking.

Prey insects were gut loaded for at least 48 hours on a diet of fresh fruit and vegetables, as well as chamomile and Chlorella powders. Prey items were dusted with Nutrobal multivitamin and mineral powder (Vetark, UK) and pure calcium carbonate powder immediately before being offered to lizards. Food was presented as a scatter feed in the enclosure; trials with slow-release enrichment devices proved less useful as enrichment than scatter feeding within a complex enclosure (Januszczak et al., 2016).

Table 2. Clutch data for F1 and F2 clutches

F generation of clutch	No. of clutches	Total no. of eggs	Clutch size (mean)	Egg width (mean; range)	Egg Length (mean; range)	Egg Weight (mean; range)
F1	6	18	3	15.2mm	25.2mm	2.88g
F2	1	2	2	13.5mm	26.5mm	2.5g

Incubation

Regular observations were carried out to monitor and visually determine if females appeared gravid. When oviposition was observed or eggs were discovered, they were removed from the enclosure for incubation. The orientation of the egg was maintained as found and the top of each egg was numbered with a pencil for identification purposes.

Each egg was then carefully removed to a plastic container, which had no ventilation panels and which was lined with a 2 cm layer of vermiculite soaked with an equal weight of reverse osmosis water.

Eggs were ¾ buried, leaving the numbered top protruding above the vermiculite. The eggs were held in a climate controlled incubator and heat was provided by thermostatically controlled heat pads to maintain a temperature of around 26.0˚C (+/- 2˚C).

The eggs and temperature were checked visually daily without opening the incubator. In addition, the incubation boxes were removed and checked weekly. At this point boxes were weighed and any weight loss corrected for by adding reverse osmosis water to the box to counteract evaporation. The hatchlings were removed from the incubator as soon as they had fully emerged from the egg.

RESULTS

Body Temperature

Body temperatures of two active lizards at an ambient temperature of 26 °C were measured at 29.5 °C and 30.1 °C.

Reproductive Data and Growth Rates

Under the conditions outlined above, we were able to breed this species to the second filial generation (F2). Our captive bred animals began to successfully reproduce when they reached a body mass of 45.0 g for the female and the males weighed 75.60 g and 116.0 g.

Reproductive and growth rate data from our observation in captivity are presented in Table 2 and Fig. 1. The 2.1 founder adults within the collection have bred and laid around two clutches per year. Over a three year period, six clutches were laid with a total of 18 eggs, 11 of which led to F1 viable hatchlings. The mean weight (SD) of all eggs was 2.71 g (±0.67 g), and they measured 14.43125 mm (±2.13 mm) in width and 25.56 mm (±1.79 mm) in length on or soon after oviposition. Viable eggs weighed 2.91 g (±0.62 g), and measured 15.41 mm (±1.41 mm) in width and 25.44 mm (±1.26 mm) in length. Non-viable eggs weighed 2.24 g (±0.41 g) and measured 12.50 mm (1.64 mm) in width and 26.0 mm (±2.47 mm).

The hatching process for all clutches was unremarkable and the mean (SD) hatchling weight was 2.57 g (±0.33 g) and the mean SVL for hatchlings was 43.41 mm (±2.68 mm). Juvenile lizards grew at broadly similar rates both within and between clutches (Fig. 1B); adult lizards also continued to grow noticeably after acquisition (Fig. 1A); however, although there are not enough data for formal analysis, clutch and egg size does not appear to have increased with the size of adults.

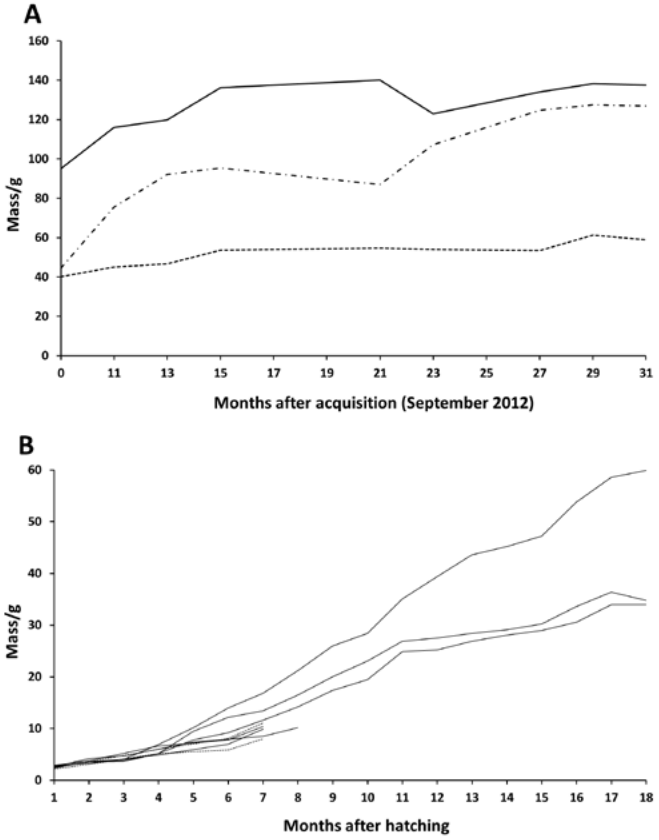

Figure 1. Changes in mass (g) of adult (A) and juvenile (B) *P. plica* lizards. In Pane A, the dashed line represents the single female founder (G00049); the other two lines represent male founder animals (Dot-dash = G00050; Dot = G00048). In Pane B, solid lines are F1 animals (long lines); dashed lines are F2 animals (short lines). Lines vary in length due to variation in age of juveniles.

DISCUSSION

P. plica proved to be adaptable and durable captives under the conditions we provided. There was little variation in egg weight and size and hatchling weight and size over the period of time and there does not appear to be any differences between generations, but there are not yet enough data for formal analysis.

There have been many changes in the management of

this species while it has been held at ZSL London Zoo. In particular, slender branches were initially used in enclosures, but subsequently these were replaced with cork panels, which provide a flatter surface favoured by this species, which is adapted for running over tree boles rather than along more slender branches (Vitt, 1991). We found that this species was quite sensitive to the amount of cover available for nest sites and where nest sites were not provided by keepers within the exhibits the female discarded eggs in the exhibit with no attempt to conceal them. Females preferred deep layers of leaf litter and organic material as oviposition sites. Where good leaf litter was provided for nesting, creching occurred with multiple clutches laid in one area of the exhibit. The adults and juveniles within the collection have not presented many health problems and no deformities have been identified in any of our captive bred specimens or founders with the exception of one incident of *Strongyloides* infection, identified through routine faecal screening, which was successfully treated using Ivermectin (Ivomec Spot-on). Seven out of 18 eggs failed to hatch; post mortem investigation of these eggs, where possible, revealed no evidence of development and so they are presumed to have been infertile.

The only published field data for *P. plica* refer to animals observed by Vitt (1997) in the Brazilian Amazon. The tree-runners in this region are of uncertain taxonomic identity (Henderson & Murphy, 2012) and, further, the locality of our animals is also uncertain. Therefore comparisons with these field data are tentative.

The environmental parameters maintained in our exhibits largely reflected wild temperature data, with ambient temperatures close to or slightly lower than the mean 27.43 ˚C recorded by Vitt (1991). More importantly, active lizards had a higher than ambient body temperature maintained by utilising basking sites and, even when ambient temperatures were slightly lower than those recorded in the field (26.0˚C), the body temperatures of our lizards (30 and 29.5 ˚C) were very close to the wild records of 30.67 ˚C (Vitt, 1991). This suggests that the enclosure design and heating and lighting arrangements used in our enclosures allowed lizards to thermoregulate effectively.

The nest sites preferred by females within the exhibits were similar to those documented in nature, with leaf litter provided, simulating the forest floor leaf litter (often rotten palm leaves) found in their natural habitat. This provides an opportunity for females to lay their eggs buried in substrate. The provision of suitable nesting materials and nesting sites has resulted in creching of eggs, which also occurs in nature (Vitt, 1991).

Two to three clutches per year were deposited by our captive animals and this is also the case in the wild. Our clutch size was similar to those in wild studies (circa three eggs per clutch), but eggs produced by our captive animals were on average smaller and lighter than those observed in nature (see Table 2). Similarly, our captive hatchlings were smaller and lighter than their wild born counterparts (Table 2). Eggs in nature were weighed at various stages of development (Vitt, 1991) and one clutch of eggs produced by our animals weighed later in development had a greater mass than those weighed at oviposition. Although this may explain some difference in mass between wild and captive laid eggs, our eggs were also smaller in dimensions and produced smaller hatchlings than reported from the field and so developmental stage cannot fully explain the differences between captive and wild eggs.

The adult animals in our collection were within the weight ranges described by Vitt (1991) in the field and eggs size in captivity did not increase as the female grew, so adult size is unlikely to explain the differences in egg and hatchling size in captivity.

Eggs were incubated somewhat cooler than nest temps recorded in natural conditions (Vitt, 1997) (26.0˚C rather than 28.6˚C). This may have contributed to smaller hatchling size as development of embryos may have been negatively affected by suboptimal incubation temperature (e.g. Van Damme et al., 1992). However, eggs were both smaller and lighter at oviposition than in nature and this is often associated with smaller hatchling size in lizards (e.g. Sinervo, 1990); hence the difference in hatchling size between wild and captive populations may be partly or wholly due to the effect of egg size and not incubation temperature. Moreover, both field temperatures and egg/hatchling size were only recorded at one instance and these limited data may or may not reflect optimal conditions or normal morphometric variation of neonates.

This study is limited by the small set of data available and further study is necessary with further pairs breeding and comparison of other hatchings and their subsequent development. Future studies to encompass other aspects of reproductive husbandry would also be interesting including investigation of any link between incubation temperature and hatchling performance.

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Co-existence of reptiles and humans: observations on a population of northern vipers on a Scottish golf course

CHRISTOPHER J. MCINERNY

School of Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK
Email: Chris.McInerny@glasgow.ac.uk

ABSTRACT - A population of northern vipers *Vipera berus* was studied for a five-year period, from 2012 to 2016, on a golf course in Scotland. Numbers, distribution, movements and habitat were monitored. Snakes were found predominately in three core areas, which contained stands of bracken *Pteridium* spp., gorse *Ulex* spp., bramble *Rubus fruticosus* agg. and heather *Calluna vulgaris*, adjacent to wet ground and streams. However, they were also occasionally found elsewhere on the golf course, even using artificial sites, such as golf tees. The reasons for the survival of this population at such a developed site were explored, with the implications of these observations for northern viper conservation discussed.

INTRODUCTION

Many reptile species are declining both in range and numbers in the UK and Europe, which has prompted research to reveal the reasons for these trends (Corbett, 1989; Beebee & Griffiths, 2000; Gleed-Owen & Langham, 2012). This has resulted in conservation measures, including some legal protection and, where necessary, habitat restoration and habitat management practices (Wild and Entwistle, 1997; Baker et al., 2004; Edgar et al., 2010).

Scotland has in some areas apparently healthy populations of northern vipers and other reptiles (McInerny & Minting, 2016). One such area is around Loch Lomond, where populations have been studied to provide information on reptile breeding biology, numbers, habitat preferences and conservation (McInerny, 2014a; McInerny, 2014b; McInerny, 2016a; McInerny, 2016b).

In western Scotland northern vipers are found in a wide range of habitats, from upland moors and forest clearings to more developed areas, such as roadside verges, embankments and golf courses, sometimes near to human habitation, suggesting that the species is versatile in its habitat requirements (McInerny, 2014b; McInerny & Minting, 2016). To understand how northern vipers survive in a developed area, a population was studied on a golf course near Loch Lomond for a five-year period. Northern vipers had been observed at the site for many years; these anecdotal observations prompted the study described here. This paper describes snake numbers, breeding biology and movements, along with the areas and habitat within the golf course where animals were found, and should inform conservation approaches to protect northern vipers at other developed sites in Scotland and elsewhere.

MATERIALS AND METHODS

Study site

The study site is described briefly in McInerny (2014b), and in more detail here; it is kept anonymous to protect both the reptiles and habitat, and to minimise interference to golfers using the course. The site is an 18-hole golf course near Loch Lomond of about 50 hectares, sloping to the south and ranging in altitude from 30-70 m (Fig. 1). A mature forestry plantation of spruce *Picea* spp. and an unsealed road forms the perimeter above, with a metalled road below.

The golf course contains large, managed areas of very short, cut grass on the tees, greens and fairways, areas of thicker cut grass along the sides of fairways, the 'rough', and artificial sand bunkers and golf tees (Fig. 1). These managed areas are interspersed with large sections of bracken, gorse, bramble and heather; areas of thicker tall grass are also present containing many mounds 1-3 m in size, these often covered with moss and grass. A number of small streams pass down the slope, and interspersed throughout the golf course are mature trees, predominately oak *Quercus* spp., with smaller numbers of birch *Betula* spp., ash *Fraxinus* spp. and rowan *Sorbus* spp.

The underlying geology is a mixture of glacial moraine and conglomerate rocks, with some exposed rock faces. A number of dry stonewalls cross and surround the course, and dry stonewalls have been created for some of the golf tees. Slow-worms *Anguis fragilis* and common lizards *Zootoca vivipara* are also found on the site.

Survey work

The survey methods used are described in McInerny (2014a) and McInerny (2016a), and followed published protocols (Sewell et al., 2013), although artificial refuges were not used to locate animals, due to public access at the site. The site was monitored from 2012 to 2016, with 10-20 visits each year from early February to late October, with each visit lasting 2-3 hours, covering a survey distance of 3-4 km. On each visit the location, number, maturity and gender of snakes were recorded, with the recorded day count noted. Juveniles were defined as snakes less than a year old, with adult males and females recognised through their background body colour, and snout scale edge colour.

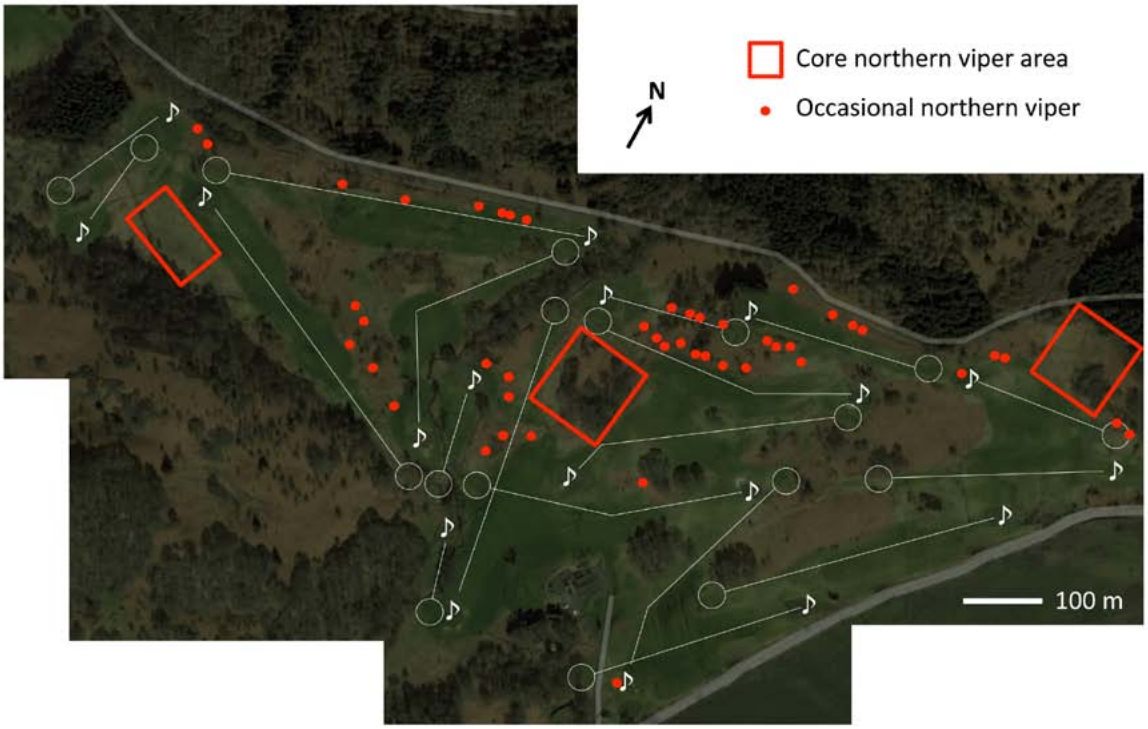

Figure 1. Distribution of northern vipers *V. berus*, on a Scottish golf course, 2012 to 2016. Core areas where vipers hibernated and were seen regularly are indicated by red boxes; locations of occasional sightings are shown by red dots. The layout of the golf course is mapped, with the 18 holes marked: open circles, tees; lines, fairways; ♪, greens and holes.

As artificial refuges were not used for monitoring, it is possible that sub-adults and juveniles were under-recorded (Hodges & Seabrook, 2016). Northern vipers were recognised through visual inspection and photographs of head-scale patterns, these being unique to individuals (Sheldon & Bradley, 1989; Benson, 1999). Thus, both day counts were recorded and minimum population numbers estimated. Habitat types were also surveyed, with these noted in relation to the location of snakes.

RESULTS

Northern viper numbers and annual cycle behaviour

The site held numbers of vipers, with 10 to 39 individuals counted each year (Table 1, Fig. 2). During the five-year period a minimum of 54 different snakes were recognised by head-scale patterns. Similar numbers of adult males and females were recorded, with a gender ratio of 1.0:1.1. Eight juveniles were observed.

Snakes first appeared in mid-February, with the majority emerging from underground hibernation sites in mid- to late March and early April (Fig. 2). Courtship and mating activity were observed in most years from early to late April, though in 2016 they were first seen in late March, on an unseasonably warm day. Many snakes were not observed through the summer, these either moving to feed elsewhere or remaining out of site under vegetation or underground, before being found again at hibernation areas from August. Gravid females instead remained visible at the study site throughout the summer, basking for extended periods near to hibernation areas, to aid gestation of developing young which are live born; young were first seen in August. Animals remained near hibernation sites to late October, when they returned underground to hibernate. These observations suggest that the snakes' annual cycle was unaffected by the managed habitat of the golf course, or by disturbance from the golfers, with feeding and reproduction uninterrupted.

Northern viper distribution in relation to habitat

Northern vipers were found at a number of places throughout the golf course, though they predominated in three core areas, near hibernation sites (Fig. 1). These were each about one hectare in size containing a mosaic of bracken, gorse, heather, tall grass and moss; in each a stream and wet ground were found nearby. Snakes were also observed basking in the core areas, and occasionally in the rough on the edges of adjacent fairways; one used an artificial tee as a basking site over two summers (Fig. 3). More rarely, adult males, adult females, and juveniles, were observed away from the core areas, on short grass on fairways and greens; such animals were always present temporarily. On a few occasions golfers also reported snakes on tees, fairways and greens. No evidence was

obtained that snakes moved between the three core areas, with it appearing that each core area contained a separate sub-population.

Management practice at the golf course

Table 1. Numbers of northern vipers *V. berus* on a Scottish golf course, 2012 to 2016. For each year, the number of visits, total number of day counts, and the minimum numbers of individual adult males, adult females and juveniles are shown. Individuals were identified through their head-scale patterns. During the five-year period a minimum of 54 different snakes were recognised.

Year	Visits	Day Counts	Individuals			
		Total	Total	Male	Females	Juvenile
2012	20	36	19	6	11	2
2013	17	37	26	14	10	2
2014	16	77	39	13	22	4
2015	10	21	10	6	4	-
2016	14	36	23	13	10	-

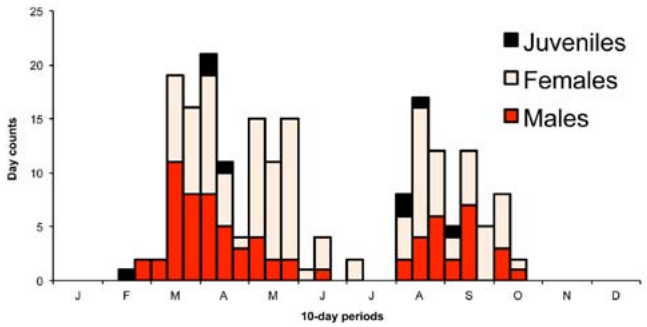

Figure 2. Total numbers of northern vipers *V. berus* on a Scottish golf course, 2012 to 2016. Total day counts for 10-day periods over the five-year period for adult males (red), adult females (pink) and juveniles (black) are plotted.

Figure 3. Northern viper *V. berus* basking on an artificial golf course tee. This male used the same tee as a sunning site during two consecutive summers, and was seen by many golfers.

The golf course was managed by at least two groundsmen working full-time from February to November. Large tractor-drawn grass cutters were used on the fairways and rough, with more specialised fine cutters on and around the greens. Areas of bracken and gorse were largely left unmanaged, though occasionally bracken, gorse, trees and bushes were pruned or removed.

The groundsmen rarely encountered snakes. Though northern vipers are shy and very well camouflaged, they can be tolerant of noise and activity (McInerny, 2016a). For example, on a number of occasions basking snakes were seen not to move in spite of grass-cutting equipment passing within 1-2 m. Perhaps surprisingly, therefore, no dead snakes were found by the author or groundsmen during the five-year study period.

Disturbance of snakes by golfers was minimal. Golfers largely remained on the fairways, rough and greens, rarely venturing into areas of bracken and gorse to find lost golf balls, being aware that snakes were present. This was demonstrated by the observation that at least 200 golf balls were found by the author while searching for reptiles in areas of bracken and gorse during the five-year study.

DISCUSSION

This study describes a population of northern vipers on a golf course near Loch Lomond, Scotland. It revealed up 54 individuals, with slow-worms and common lizards also present. Highest numbers were encountered in three core areas each about one hectare in size which contained areas of bracken, gorse, heather, tall grass, moss, wet ground and streams, where disturbance by golfers was minimal.

It appears that the golf course layout provides suitable habitat to allow reptiles to feed and breed, as annual cycle behaviour similar to that reported elsewhere was observed (Viitanen, 1967; Prestt, 1971; Neumeyer, 1987; Andersson, 2003; Phelps, 2004; Hodges & Seabrook, 2016; McInerny, 2016b). Furthermore, the management and use of the golf course did not appear to result in snake mortality, allowing the population to persist. This may also apply at other golf courses in Scotland, as northern vipers are present in at least three others (pers. obs.), which have similar habitat profiles of bracken, gorse and grass, interspersed between greens and fairways.

In the UK there are over 3,000 golf courses, with at least 100 of these recognised as environmentally important, being designated SSSIs (Gange et al., 2003). There is now an increased realisation that golf courses can, if managed sympathetically, be important areas for biodiversity (SGEG, 2004; Tanner & Gange, 2005; Mackey et al., 2014). In the context of reptiles, habitat management regimes have been introduced at the Royal Birkdale and Hesketh golf courses in Merseyside, England, to benefit sand lizards *Lacerta agilis* (Edgar et al., 2010). At another golf course in southern England log piles have been added to rough areas to provide cover and basking sites for northern vipers (Kent Reptile and Amphibian Group, pers. comm.). The observations presented here suggest that golf courses can sustain populations of northern vipers and other reptiles, if suitable habitat is present. Considering the general

decline in northern viper numbers and range in the UK, their presence in golf courses may become increasingly important, contributing to the species' conservation.

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Saurochory in the American crocodile
Are American crocodiles capable of dispersing viable seeds?

MARIANA GONZÁLEZ-SOLÓRZANO[1], ADAM E. ROSENBLATT[2], FABIO G. CUPUL-MAGAÑA[3], PABLO S. HERNÁNDEZ-HURTADO[3], FERNANDO VEGA-VILLASANTE[3], HELIOS HERNÁNDEZ-HURTADO[3], & ARMANDO H. ESCOBEDO-GALVÁN[3*]

[1]Universidad Politécnica del Centro, 22.5 km. carretera Villahermosa-Teapa, 86290 Villahermosa, Tabasco, México.
[2]School of Forestry and Environmental Studies, Yale University, New Haven, CT USA.
[3]Centro Universitario de la Costa, Universidad de Guadalajara, Av. Universidad 203, 48280 Puerto Vallarta, Jalisco, México.
[*]Corresponding author Email: elchorvis@gmail.com

ABSTRACT - Frugivory followed by seed dispersal is a mutualistic relationship between animals and plants. In the case of reptiles, this phenomenon has been reported in some lizards, turtles and iguanas. It has recently become apparent that frugivory is common across many species of crocodilians. However, it is unknown if crocodilians can effectively disperse seeds that are capable of germinating. To address this knowledge-gap, we tested the ability of *Leucaena lanceolata* seeds to germinate following consumption by *Crocodylus acutus*. We fed 100 seeds each to five juvenile crocodiles and then flushed each individual's stomach at specific intervals of time after feeding. We then planted the collected seeds and compared their germination rates to those of control seeds that had not been consumed by crocodiles. We found that crocodile consumption significantly lowered seed germination rates, suggesting that *C. acutus* is not an effective seed disperser.

INTRODUCTION

Crocodilian species play key roles in structuring food webs and maintaining ecological processes within tropical and sub-tropical aquatic ecosystems. For example, crocodilians can affect food web processes through predation (Bondavalli & Ulanowicz, 1999; Nifong & Silliman, 2013) and can affect population dynamics of lower trophic levels through ecosystem engineering (Craighead, 1968), as well as link multiple ecosystem types through their movement and feeding behaviours (Nifong et al., 2015; Rosenblatt & Heithaus, 2011). Traditionally, crocodilians had been thought of as strict carnivores (Grigg & Kirshner, 2015), but a recent review showed that crocodilians occasionally engage in frugivory (Platt et al., 2013a). That insight suggests that crocodilians could serve yet another role within ecosystems as seed dispersers, similar to other reptiles like lizards, turtles, and iguanas (Hnatiuk, 1978; Fialho, 1990; Moll & Jansen, 1995; Vázquez-Contreras & Ariano-Sánchez, 2016). However, the ability of crocodilians to disperse viable seeds remains largely untested.

Passage through the crocodilian digestive tract could be beneficial for seeds, as they would undergo physicochemical treatment that could enhance germination. Recently, Rosenblatt et al. (2014) tested this hypothesis with pond apple (*Annona glabra* Linnaeus) seeds consumed by American alligators (*Alligator mississippiensis* Daudin) within Everglades National Park in Florida, USA. They found that the seeds were rendered non-viable following consumption, likely because the porous nature of pond apple seed coats allowed the strong stomach acids of *A. mississippiensis* to damage the embryos. However, given that there has only been one test of the crocodilian saurochory hypothesis involving one crocodilian species and one type of seed, more research is needed to help determine if crocodilians are capable of acting as seed dispersers. Therefore, we tested the effect of seed consumption by American crocodiles (*Crocodylus acutus* Cuvier) on seed viability and germination under controlled conditions.

METHODS

For this study, we used five captive juvenile *C. acutus*, similar in body size (< 1 m) and healthy, kept individually in outdoor plastic containers (0.98 x 1.55 m, with capacity for 600 L) within the Cipactli Reptilarium facilities at Centro Universitatio de la Costa of the University of Guadalajara, Mexico. We collected mature fruits of lead trees (*Leucaena lanceolata* Watson; Family: Fabaceae) in El Salado Estuary (20°40'19.85" N, 105°14'11.35" W) located in Puerto Vallarta, Jalisco, Mexico. We used *L. lanceolata* because it has a wide distribution that overlaps with the distribution of *C. acutus* in the Pacific coastal environment of Mexico. *L. lanceolata* seeds have a semi-strong cuticle, which inhibits plant growth under natural conditions, and scarification processes increase seed germination rates (Roman-Miranda et al., 2013). *L. lanceolata* has not been reported in previous studies of the stomach contents of *C. acutus*, but some species from the family Fabaceae have been reported (see Platt et al. 2013a). In addition, the population density of *L. lanceolata* in El Salado Estuary is high (M. González-Solórzano, personal observation), therefore there is a high likelihood that *C. acutus* could consume *L. lanceolata* in this area either intentionally or accidentally.

We carried out the experiment over four weeks from October to November 2015. A total of 100 g of beef liver and fish, 40% and 60%, respectively (a detailed description in Hernández-Hurtado et al. 2012) were offered to each individual once per week. Twenty-five seeds of *L. lanceolata* were combined and amalgamated with the control diet each week, thus each individual had the opportunity to consume 100 *L. lanceolata* seeds in total. Each animal was fed ad libitum. After 24 h, the remaining food was removed and plastic containers were cleaned. The unconsumed seeds were planted in soil treated with compost and used as a control to evaluate the relative germination rate of the consumed seeds.

Stomach contents were collected from each individual using the hose-Heimlich lavage technique (Fitzgerald, 1989). In brief, this technique consists of threading a hose from the snout down to the stomach, pumping fresh water in, and then using the Heimlich maneuver to force the water and stomach contents out of the animal. The first stomach flushing was conducted at 24 hours, the second at 72 hours, and the third at 384 hours (16 days) post-ingestion. The seeds recovered from the stomachs were also planted in soil treated with compost. The germination rate of both consumed and unconsumed seeds were evaluated over 30 days. A Student's t-test was used to compare the relative amounts of consumed and unconsumed seeds among individuals. A Kruskal-Wallis ANOVA was used to compare the percentage of unrecovered and recovered seeds among individuals. A two-sample Mann-Whitney test was used to compare differences in germination rate between the unconsumed seeds and consumed seeds that were recovered by stomach flushing.

RESULTS AND DISCUSSION

Of the 500 *L. lanceolata* seeds supplied to all five individuals, 195 were consumed with a mean (± SD) of 39 ± 9.9 seeds per individual (range = 24 to 47 seeds). Of the 305 unconsumed seeds, the germination rate ranged from 7.9% to 28.3% (*mean* = 18.6 ± 8.2%). Of the 195 consumed seeds, 131 were recovered during stomach flushing, with 13 to 35 seeds recovered from each individual (*mean* = 26.2 ± 10.0). In contrast, only 8 to 16 seeds were digested or destroyed by each individual (*mean* = 12.8 ± 3.3). Thus, we found that significantly fewer seeds were digested or destroyed than those that remained intact (t = -2.85, d.f. = 8, P = 0.02). Surprisingly, there was no difference between the proportion of ingested seeds that were destroyed across stomach flushing events at different time intervals (Kruskal-Wallis test = 0.38, d.f. = 2, P = 0.82). Of the 131 seeds recovered from stomach contents, only four seeds germinated; the germination percentage ranged from 0.0% to 23.1% (*mean* = 5.2 ± 10.1%). Therefore, the two-sample Mann-Whitney test showed that a significantly lower percentage of consumed seeds germinated relative to unconsumed seeds (U = 15, P = 0.007).

In agreement with the results of Rosenblatt et al. (2014), our results provide further evidence that crocodilians may have a negative effect on seed viability. We suspect that the decrease in seed germination rates after consumption by *C. acutus* is at least in part caused by the low pH environment of *C. acutus* stomachs, which has been reported to be 3 or less (Grigg & Kirshner, 2015). We also observed that one third of the *L. lanceolata* seeds consumed by *C. acutus* were destroyed or digested, suggesting that it has a negative effect on seed viability in contrast with seed dispersers such as some birds and mammals (Castro et al., 1994; Krefting & Roe, 1949; Midgley et al., 2015), lizards (Fialho, 1990; Iverson, 1985) and both freshwater and terrestrial turtles (Hnatiuk, 1978; Moll & Jansen, 1995) which play a role as seed dispersers.

A potential issue with our study was the body size of crocodiles we used. However, although few studies have found seeds or fruits in the faeces or stomach contents of adult *C. acutus* (Alonso-Tabet et al., 2014; Alvarez del Toro, 1974; Beltrán-López, 2015; Casas-Andreu & Barrios-Quiroz, 2003; Cupul-Magaña et al., 2008; Platt et al., 2013b, 2013b, 2014; Villegas & Schmitter-Soto, 2008), seeds have been recorded in juveniles and subadults similar in size to the crocodiles in our study. This suggests that *C. acutus* may not frequently consume seeds or fruits, or that consumed seeds have a limited capacity to pass through the digestive tract. In fact, a secondary goal of this study was to estimate the excretion rate using *L. lanceolata* seeds. After feeding the crocodiles during two weeks, it was not possible to distinguish the seeds in the faeces, therefore we conducted the stomach flushing to recover the seeds and evaluate the germination rate based on the time that the seeds spent in the stomach.

Further laboratory and field tests, using different type of seeds and crocodiles of different body sizes, are needed to elucidate the functional importance of crocodilians as seed predators in tropical aquatic ecosystems.

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The defensive behaviour of *Naja oxiana*, with comments on the visual displays of cobras

ALIREZA NASOORI[1, 2], DELAVAR SHAHBAZZADEH[2], TOSHIO TSUBOTA[1] & BRUCE A. YOUNG[3*]

[1]Laboratory of Wildlife Biology and Medicine, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.
[2]Biotechnology Research Center, Medical Biotechnology Department, Venom and Biotherapeutics Molecules Lab, Pasteur Institute of Iran, Tehran 13164943551, Iran.
[3]Department of Anatomy, Kirksville College of Osteopathic Medicine, A.T. Still University, Kirksville, MO, 63501, USA
[*]Corresponding author Email: byoung@atsu.edu

ABSTRACT - The defensive behaviours of a group of freshly-caught central Asiatic cobras, *Naja oxiana*, were evoked by the proximity of an experimenter. The cobras responded by hooding and holding 13-22% of their total body length in an elevated or vertical posture. From this vertical posture the cobras would launch defensive sham (closed-mouth) strikes; these strikes were typically associated with a short "burst"-like hiss, and were more frequent in the smaller snakes studied. The presence of the experimenter provoked an increase in the cobra's ventilatory rib movements as well as the tongue flick rates; the latter metric was strongly correlated with the height of vertical posturing, strike frequency, and hissing frequency. Most of the observed behaviours result from the cobras' visual perception, are interpreted as a visual display intended as a deterrent. This interpretation raises interesting, and previously unexplored, questions about intra- and interspecific variation of these displays (within both *Naja* and the Hemibungarini), as well as the relationship between these defensive behaviours and (repeatedly evolved) ability to "spit" venom.

INTRODUCTION

Cobras are active-diurnal hunters that rely primarily on their visual system to locate both their prey and their potential predators (Kardong et al., 1997; Westhoff et al., 2010). As part of their predator deterrence strategy, cobras adopt what is perhaps the most iconic visual display of all snakes... the vertical elevation of the head coupled with the lateral spreading of the ribs to form the "hood" (Chiszar et al., 1983; Kardong, 1982; Kardong et al., 1997; Radcliffe et al., 1986; Young & Kardong, 2010). In many cobras vertical posturing and hooding are but two components of a defensive repertoire that may include striking, hissing, spitting venom, and death feigning (Radcliffe et al., 1986; Rasmussen et al., 1995; Young et al., 2004).

Despite the abundance of illustrations and descriptions of the defensive behaviours of cobras, there are some fundamental questions that have yet to be explored. The efficacy of vertical posturing and hooding as a visual deterrent presumably correlates with the prominence of the display. The naturally-occurring predators of snakes such as birds (Errington, 1932; Maklakov, 2002) and carnivorous mammals (Barchan et al., 1992; Vanderhaar & Hwang, 2003) usually target the head of snakes (Rasa, 1973; Francis et al., 1989; Langkilde et al., 2004). As such, any increase in the prominence of the defensive visual display likely comes at the cost of (direct) increase in vulnerability by moving the predator's target (the cobra's head) into a more elevated and prominent position.

The ability to "spit" venom has evolved multiple times in cobras (Wüster & Thorpe, 1992a). Spitting cobras exhibit a range of defensive behaviours and differ in their spitting performance (Chiszar et al., 1987; Rasmussen et al., 1995; Young et al., 2004; Westhoff et al., 2010). But in all spitting cobras the spat venom travels beyond the range of the snake's defensive strike, meaning that spitting cobras posses a form of "long distance" defense (when compared to non-spitting cobras). The efficacy of the spat venom in causing severe eye injury, loss of vision, and debilitating pain (Chu et al., 2010; Ang et al., 2014), suggests that hooding and vertical posturing may be more variable among spitting cobras than non-spitting cobras.

The Hemibungarini radiation of elapid snakes includes the true cobras (*Naja*) as well as several cobra-like taxa (including *Boulengerina*, *Ophiophagus*, *Pseudonaja*, and *Walterinesia*) (Castoe et al., 2007; Kelly et al., 2009). The genus *Naja* includes approximately 30 species, but previous behavioural and functional studies have disproportionately focused on five species: *Naja naja*, *N. haje*, *N. kaouthia*, as well as *N. nigricollis* and *N. pallida*, the latter two being spitting cobras. Herein we provide a description of the defensive repertoire of the central Asian cobra, *Naja oxiana* (Eichwald, 1831) which inhabits West Asia and Pakistan, with a limited distribution in India (Wüster & Thorpe, 1992b; Wallach et al., 2009). We chose to study *N. oxiana* in part because it has been rarely studied behaviourally, it is a non-spitting cobra, and is common in the Serpentarium of the Pasteur Institute of Iran. More fundamentally, *N. oxiana* is of a similar size (adult length of ~ 140 cm), general build, and colour pattern to the more commonly-studied black-neck spitting cobra, *N. nigricollis*. A more detailed comparison of these

two species may offer a unique opportunity to directly test the energetics, and behavioural effectiveness of venom spitting in cobras.

METHODS

This study used six *N. oxiana* which were wild-caught from the Khorasan province of Iran (Darvish & Rastegar-Pouyani, 2012; Nasoori et al., 2014) and had been kept for at least one month in the Serpentarium of the Pasteur Institute of Iran. The cobras were kept at 26°C under a 12:12 dark/light cycle, fed live and dead rodents, and given water ad libitum. All maintenance and experimental use of these animals conformed to both the internal regulations for keeping laboratory animals in our institute, and established international guidelines (ASIH, 2004). The animals were of roughly similar size (108.5 - 120.5 SVL) and all appeared to be healthy; at the time of the trials one of the cobras was approaching ecdysis and had opaque eyes.

Three days prior to the experiment, the cobras were immobilised with administration of an appropriate dose of diazepam (Chemidarou 10mg/2ml Amp, Iran). Each cobra was then weighed on a digital scale (A&D EK-1200 i NTEP 133 Jewelry Buyer Scale, USA), then both total length and snout-vent length were measured. Lastly, the snake's dorsal side was marked by small pieces of adhesive plaster placed in 5 cm intervals starting 5 cm from the snout.

For the behavioural trials, an individual cobra was placed on a wooden table (300 cm × 150 cm) within a room held at 26°C. The experimenter provoked the animal, by his physical presence, movement of his hands, and, in some instances, through direct contact with the end of a snake hook. The snakes' reactions were recorded with a digital camera (Handycam Video Camera, DCR-TRV265E, SONY, Japan) located 3 meters away from the table. Each behavioural trial lasted for a minimum of 2 minutes. Each snake was used for three trials, and the snake was always given a rest interval (during which it was removed from the table) between the trials.

The snake varied the height of the vertical posture regularly during the trials, so each trial [equal to 120 seconds] was divided into 3 parts of 40 seconds and the maximal height determined for each part. Vertical height was standardised by expressing it as a percentage of total body length. Other behavioural responses, including hissing, striking, tongue flicking and rib movement were assessed both by direct observation and via the video records. The data set was analysed using general linear model and the correlations between biometrics and vertical height were calculated and considered significant when $P \leq 0.05$.

RESULTS

Snake Biometrics - Four of the studied cobras had tail lengths that were longer, both absolutely (21-24 cm) and relative to total length (14.9-17.6%), than the other two (which had tail lengths of 10-14.5 cm which were 8.2-10.7% of their total length); The four snakes with longer tails are believed to be males (Nasoori et al., 2014). Body masses ranged from 307-432.5 g and increased slightly against total body length, while decreasing slightly against snout-vent length (Fig. 1).

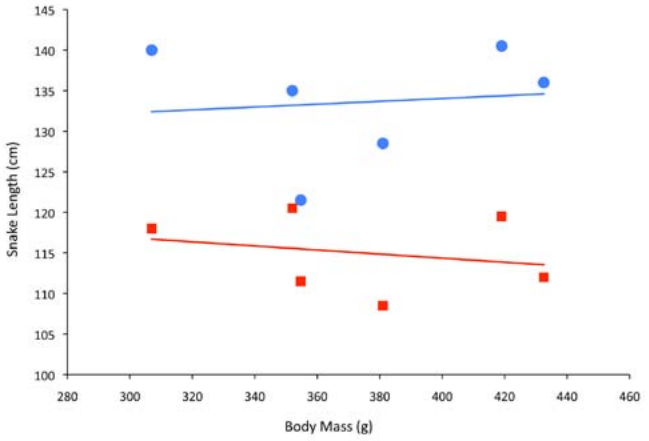

Figure 1. Biometric data for *N. oxiana* showing snake mass (X-axis) against snake length; blue circles are total length, red squares are snout-vent length.

Vertical Posturing - Every cobra held the forepart of its body vertically during every round of each behavioural trial; the average length of the body held vertically was 18.7% of the total length. The percent of body held vertically ranged from 13 - 22%; and did not vary significantly between rounds of the defensive trials (Fig. 2). No significant correlations were found between the height of vertical posturing and the biometric data collected. The snake nearing ecdysis consistently held less of its body vertically than most (or all) of the other snakes (Fig. 2) a difference that was significant (MANOVA, $F = 6.40$, $p=0.001$).

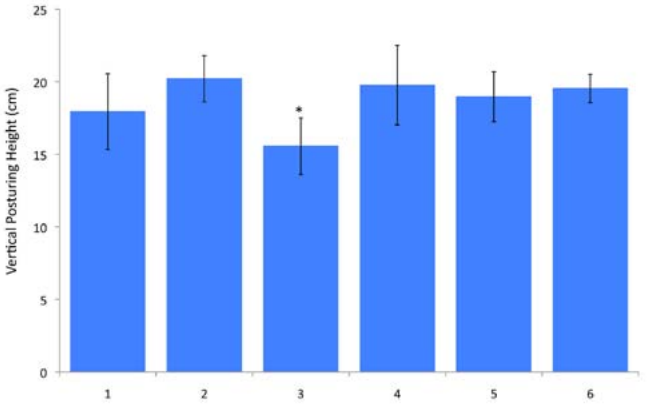

Figure 2. Mean and standard deviations for the vertical height achieved by the six specimens of *N. oxiana* during defensive posturing. Cobra 3 was in the early (eye opaque) stage of ecdysis and adopted a significantly lower vertical height while displaying.

Hooding - The hood of *N. oxiana* has an elongate, gradually tapering shape in contrast to the more ovoid hoods of some *Naja* species (Young and Kardong, 2010). Hooding was observed concurrent with all episodes of

vertical posturing. This species would also hood while the fore-body was horizontal, such as after a strike (see below), or immediately prior to adopting the vertical posture. We have worked with other (more relaxed) long-term captive specimens of *N. oxiana* in which the size of the hood was rapidly adjusted depending on the degree of stimulation provided; during the behavioural trials detailed herein the study snakes maintained a constant state of what appeared to be a maximally erect hood.

Striking - A total of 27 strikes were recorded during the behavioural trials. Of these, 26 (or 96%) were sham strikes during which the cobra's mouth remained closed. All of the strikes were launched while the forepart of the snake's body was being held vertically. During these strikes the cobra appeared to simply rotate the head and forepart of its body downward using the base of the vertically elevated segment as a pivot point. The cobras occasionally launched several strikes in quick succession, with each successive strike being launched from a less-elevated posture. The two smallest snakes accounted for 17 of the 27 strikes (or 63%), leading to a significant negative correlation between strike frequency and total length (r = -0.859, p = 0.028). Negative correlations were also found between strike frequency and both SVL and mass, but these were not significant.

Hissing - A total of 31 hisses were recorded during the 18 behavioural trials. Every strike was associated with a short, burst-like hiss. Additional, longer, hisses were produced while the animal was maintaining its vertical posture.

Tongue flicking - The tongue flick rate increased after the strike, while the cobra was in a more horizontal posture. Provocation of the cobra was consistently associated with an increased rate of tongue flicking. The tongue typically oscillated for about 1 second during each flick. However, this action was occasionally prolonged up to 5 seconds or even longer (maximum 10 seconds). The tongue flick rate showed a clear difference among the cobras, two of the snakes had tongue flick rates that were roughly 2-3x those of the other cobras.

Rib movements - Rib movement was divided into 4 groups including: no obvious movement, obvious movement, obvious and deep movement, and obvious and very deep movement. There was no indication that the body was being "inflated" as part of visual display. Rather, the costal movements seemed to be ventilatory, representing energetic demand and/or stress. The depth of breathing typically increased after the strike, and frequently increased following tongue flicking.

Behavioural integration - A cross-correlation analysis revealed significant ($p < 0.05$) correlations between four pairs of behaviours; the frequency of strikes and hisses (r = 0.97), the frequency of strikes and tongue flicks (r = 0.63), the frequency of hisses and tongue flicks (r = 0.52), and the tongue flick rate and the height of vertical posturing (r = 0.60). When we incorporate hooding (which was observed during every period of vertical posturing, the basic defensive repertoire is clear - *N. oxiana* elevates roughly 19% of the forepart of its body, while hooding, and increasing the tongue flick rate.... from this posture the snake is most likely to emit a short hiss while performing a closed-mouth "sham" strike.

DISCUSSION

When provoked these recently captured specimens of *N. oxiana* showed little tendency to flee or attack. Instead, this cobra consistently responded with what is herein interpreted as a visual display the primary function of which was to make the snake appear threatening or dangerous. This visual display had three components: vertical posturing of the fore-body, hooding, and sham strikes. We interpret this suite of three characters as a mechanism to bring attention to the head of the snake (by vertically posturing), while making the head and fore-body appear both larger (by hooding) and more menacing (by sham strikes).

A consideration of these three display components illustrates the complexity of cobra behaviour. In the present study the cobras held 13-22% of the body elevated while displaying. The body shape of these cobras is such that the center of mass is likely slightly in front of the cranial-caudal mid-point of the body; this suggests that the 22% max value recorded in the present study is a behavioural, not a functional or morphological limit. Presumably this 13-22% postural range would differ interspecifically (between cobras) and intraspecifically when *N. oxiana* was presented with different threats.

The vertical postures observed in this study were such that the entire cranial-caudal range of the hood was visible. *N. oxiana* has black horizontal bars on the ventral surface of the hood, which we interpret as a combination of aposematic colouration and a visual pattern that serves to draw attention to the head. The cobras kept the ventral surface of the hood directed at the experimenter; in contrast species with "eyespots" or other markings on the dorsal surface of the hood will often turn away from a threat presumably to display these visual patterns (Young & Kardong, 2010). If these interpretations of *N. oxiana* are correct, it would suggest that a comparative study of defensive posturing in *Naja* (or, more broadly, within the Hemibungarini radiation) would reveal correlations between traits like relative vertical elevation, hood size, and hood/neck pigmentation patterns.

The 18 trials performed yielded 27 defensive strikes, 26 of which were sham strikes performed with the mouth closed. Sham strikes are frequently observed in cobras (e.g., Rasmussen et al., 1995). Herein these are interpreted as a visual display intended to make the snake appear more dangerous and to draw attention to the head. The defensive strike kinematics involve the (elevated) head and fore-body rotating forward and down toward the substrate. Interestingly, in the present study though all the cobras used vertical posturing and displayed their hood, the larger snakes were significantly less likely to perform defensive

strikes than were the smaller snakes. Presumably these defensive strikes have the least deterrent value when the strike ends and the snake is more horizontal; if true, there may be a strong correlation between the relative scarcity of ground cover and the species tendencies to perform sham defensive strikes.

This suite of visual displays may seem to describe all cobras, but that is not the case. Even among non-spitting true cobras there are distinctions. The forest cobra, *N. melanoleuca*, has a similar shape and ventral banding pattern on the hood (Broadley, 1983). When provoked *N. melanoleuca* holds a considerable portion of its body vertical (some photos suggest almost 50%), tends to move toward the threat (or "attack"), and launch multiple true (open mouth) strikes (Young, pers. obs.; Spawls & Branch, 1995).

Associated with these three visual displays was a fourth behaviour, the production of an audible hiss. The hiss of *N. oxiana* is of relatively short duration, as opposed to long duration hisses frequently encountered in pythons and larger viperids (Young, 2003); and becomes almost "burst-like" when performed during a defensive strike (all strikes of *N. oxiana* were accompanied by a hiss). Brief "explosive" hisses associated with the strike are common in the cobra radiation; they are particularly prominent in Aspidelaps (Young, pers. obs.; Spawls & Branch, 1995), and were (incorrectly) postulated to propel the venom during "spitting" in cobras (Rasmussen et al., 1995). There are at least two, non-mutually exclusive, functions for these defensives hisses: as broadband acoustic warning signals (Young et al., 1999) or as another means of drawing the other organism's attention to the head of the cobra. In support of the latter, many cobras will "slap" the substrate with their erect hood at the end of a defensive strike.... a behaviour that blends acoustic and visual displays (Young, 2003). It would be interesting to explore the relationships between the elements of the visual display, the strike frequency, and the bioacoustic properties of the hiss within different cobras.

Two other behaviours were frequently observed during the trials of *N. oxiana*, an increase in tongue flick rate and an increase in costal movements. Herein these are both interpreted as signs of arousal or stress in the cobra (Young & Aguiar, 2002). Rib movements can be associated with defensive inflation of the snake's body (Young et al., 2000) or with the production of defensive sounds (Young et al., 1999), or both (Kinney et al., 1998). In *N. oxiana* we did not observe body inflation, and the hisses that were produced were not associated with pronounced body expansion (again, as is seen in the large viperids).

The close proximity of an experimenter created arousal/stress in *N. oxiana*. Interestingly, tongue flick rate proved to be an excellent metric of arousal in this species; the tongue flick was strongly correlated with the height of vertical posturing, strike frequency, and hissing frequency. The primary stimulus provided by the experimenter was visual, and it is interesting to note that the one snake in early ecdysis (when the visual acuity is reduced) responded with significant lower levels of vertical posturing.

There is considerable inter-specific variation in the effective distance of venom spitting (Rasmussen et al., 1995); however in all species the spat venom appears capable of traveling further than the length of the cobra's defensive strike, making this a long-distance form of defense. Given the ability of spitting cobras to precisely target a typically moving target (Young et al., 2009; Westhoff et al., 2010), and both the short- and long-term consequences of spat venom contacting the eye (Ang et al., 2014), the deterrent value of venom spitting (though never documented) seems clear. The physical act of "spitting" venom is rather quick; mean durations of 66 and 40 ms were reported in Young et al., (2004) and Westhoff et al., (2010), respectively. This speed, coupled with the nearly translucent nature of the spat venom, suggest that spitting venom is purely a chemical defense and, unlike the other defensive behaviours described above, does not function to draw attention to the cobra's head and/or make the cobra appear more dangerous.

The ability to spit venom appears to have evolved independently multiple times within the Hemibungarini radiation (e.g., Wuster & Thorpe, 1992; Keogh, 1998). Field and laboratory studies of the defensive behaviours of spitting and non-spitting cobras could explore the impact of the evolution of a long-distance chemical defense in species which rely primarily on visual display as a deterrent. Are there predator-specific defensive strategies in spitting cobras for the relative energy investment in visual display versus spitting? Could the visual display of a cobra actually lure a predator closer making the venom spitting more effective? This seemingly fruitful line of enquiry must begin with basic descriptions of spitting and non-spitting cobras which, if nothing else, will identify the shared features which can be quantified during comparative studies.

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Myiasis in the Neotropical amphibian *Hypsiboas caingua* (Anura: Hylidae) by *Megaselia scalaris* (Diptera: Phoridae)

CARLOS ARIEL LÓPEZ[1], THALLES PLATINY LAVINSCKY PEREIRA[2], MARCIO GERMÁN ANTÚNEZ[1] & MARÍA ELISA PEICHOTO[3,*]

[1]Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Neuquén y Jujuy s/n, 3370 Puerto Iguazú, Argentina.
[2]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, n. 101, 05508-900, Cidade Universitária, São Paulo, SP, Brazil.
[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ministerio de Ciencia Tecnología e Innovación Productiva; Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Neuquén y Jujuy s/n, 3370 Puerto Iguazú, Argentina.
[*]Corresponding author Email: mepeichoto@yahoo.com.ar

ABSTRACT - Host-parasite interactions between dipterans and anurans in the Neotropical region are still poorly known; we report here the first case of myiasis in the anuran *Hypsiboas caingua* (Hylidae). Three infecting larvae completed metamorphosis in laboratory conditions. The emerging three adult flies were morphologically identified as *Megaselia scalaris* (Phoridae). This is the first evidence of phorid flies parasitizing a South American wild anuran.

INTRODUCTION

Myiasis is defined as a condition caused by dipterous larvae that can invade a host to complete their development while feeding on its living or dead tissues (Zumpt, 1965). Amphibians are parasitized worldwide by larvae of numerous fly species, however, this is a poorly studied area of amphibian biology. Anuran myiasis in juvenile and adult individuals – in wild and captivity – has been reported recently from the Neotropical region (de Mello-Patiu & de Luna-Dias, 2010; Sousa Pinto et al., 2015). Although infestation by fly larvae could be one explanation for the decline of populations of several amphibians, examples of myiasis in wild amphibians are poorly documented, mainly due to the difficulty of recording the phenomenon, which may be due to the rapid action of parasites and consumption of small host carcasses (Sousa Pinto et al., 2015). The current study describes myiasis in a wild specimen of anuran from the Upper Parana Atlantic Forest of Argentina (province of Misiones).

OBSERVATIONS and DISCUSSION

In 16 November 2015, CAL and MGA were performing a preliminary inventory of anurans in the reserve inside the INMeT (National Institute of Tropical Medicine, Argentina) campus (25°38'29,89''S – 54°34'54,62''W, elev: 179 m. a. s. l.). Around 22 hrs, a medium-sized adult individual of *Hypsiboas caingua* Carrizo, 1991 was observed swimming awkwardly in a small pond with its right leg almost paralysed. This tree-frog (Hylidae) is widely distributed in Argentina, Brazil and Paraguay (Frost, 2016), and it was easily identified in the field by the characters detailed in its original description (Carrizo, 1990). Several males of *H. caingua* and some individuals of *Dendropsophus nanus* were vocalising from grass and bushes surrounding the water body. At closer examination of the injured specimen, we perceived a circular skin lesion on its thigh, next to the cloaca, infested with larvae (Fig. 1-A and B).

The individual was caught, placed into a plastic box and carried to the Herpetology Laboratory of the INMeT, where 8 spiracles of larvae were observed in the lesion. After 24 hours the frog died but the larvae remained alive inside the body cavity and continued feeding on the frog carcass, of which they left only the anuran skin. Forty-eight hours later, the larvae had reduced the frog body to bones (INMeT Herpetological Collection, code 058), and began to disperse and migrate to drier areas for pupation. One larva was preserved in 70% ethanol. For identification at species level of the imagos, we used the technique proposed by de Mello-Patiu and de Luna-Dias (2010). After 17 days in the pupal state, three larvae completed metamorphosis emerging adult flies, two males and one female.

Using morphological characteristics described in the key to Neotropical species of *Megaselia* given by Borgmeier (1962), such as anepisternum without setae (Fig. 1-C,D); two pair of supra-antennal setae of the same size (Fig. 1-E); scutellum with four setae, the two posterior being greater than the anterior ones (Fig. 1-F); hind tibia with one dorsal setal palisade (Fig. 1-G); female with tergite 6 short, extremely broad, extending laterally on segment (Fig. 1-G) (Brown & Horan, 2011), the dipterans were identified as *Megaselia scalaris* (Phoridae). The three specimens of dipterans were deposited together with the anuran bones in the Herpetological Collection of the National Institute of Tropical Medicine, Puerto Iguazú, Argentina (code INMeT 058).

Previously, myiasis has been reported for the following species of *Hypsiboas*: *H. atlanticus* (Oliveira et al., 2012), *H. beckeri* (de Mello-Patiu & de Luna-Dias, 2010), being both caused by Sarcophagidae flies, and *H. curupi* (López

& Nazer, 2009) caused by an unidentified fly. Herein, we present the first evidence of myiasis on *H. caingua* as well as the first evidence of phorid flies, *M. scalaris*, parasitising a South American wild anuran. Disney (2008) has indicated that the larvae of this species are generalised feeders, eventually able to infest invertebrates under laboratory conditions (Koch et al., 2013). Until this report, among the poikilothermic tetrapods, only snake (Vanin et al., 2013) and amphibian (Zwart et al., 2005) species from zoological collections have been associated as hosts for this fly species.

The infestation reported herein occurred in a neighbourhood with rapid anthropogenic change, next to the city of Puerto Iguazú, where the territory is organised into forest patches and inhabitant units. The fly *M. scalaris* has a cosmopolitan distribution, with records in a wide range of habitats, from tropical rainforests to urban buildings. This plasticity has been explained by the large diversity of niches of *M. scalaris* larvae. The latter can be found in natural habitats as scavengers in nests of social insects, and feed on invertebrate carrion, live insects, vertebrate eggs, fungi and living plants (Disney, 2008).

Coincidently with other authors (Bolek & Coggins, 2002), we observed a rapid death of the parasitised anuran (24 to 48 hours), with the consequent decomposition of the affected carcass in 48 to 72 hours (in laboratory conditions). It is probably that this process could be faster in natural conditions, which would jeopardise the observation of anuran myiasis in the wild. Nevertheless, Eaton et al. (2008) found differential mortality index in anurans from North America that were parasitised with *Lucilia silvarum*, and they demonstrated that it was inversely correlated with their body size. Surveys on either infection prevalence or virulence profile have not yet been delineated in anuran hosts of communities from south America. Thus, further studies on the host-parasite interactions between dipterans and anurans in the Neotropical region will be useful in understanding their impact on declining anuran populations in climate change scenarios.

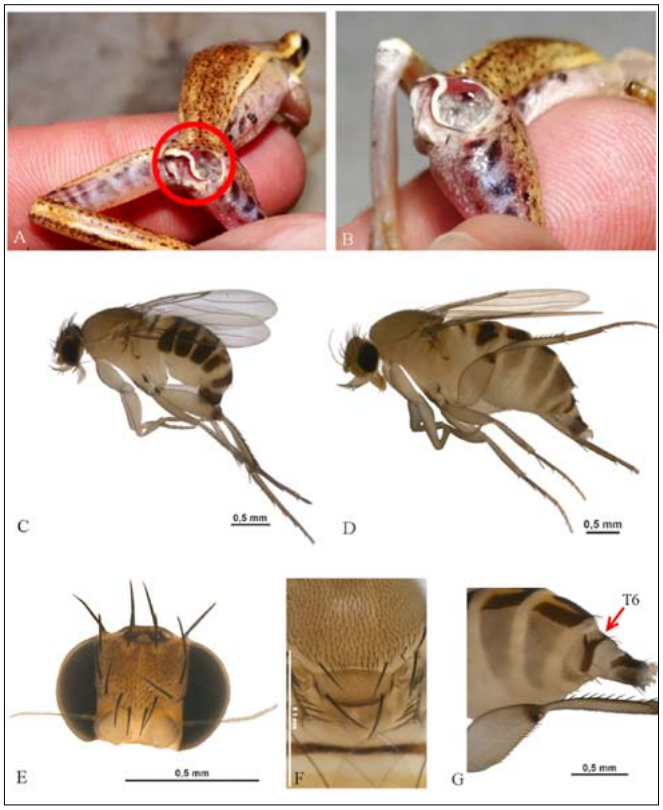

Fig.1 A- *Hypsiboas caingua* specimen infested with myiasis in right posterior limb. **B**- Close up of the lesion marked by red circle in Figure **A**. C-G- Specimens of *M. scalaris*. **C**- Male adult. **D**- Female adult. **E**- Head with emphasis on forehead. **F**- Scutellum with emphasis on setae. **G**- Hind tibia with one dorsal setal palisade (T6 - Tergite 6). The photographs were taken by the authors.

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A new population of European cave salamanders (genus *Hydromantes*) from west-central France: relict or introduction?

DANIELA LUCENTE[1*], JULIEN RENET[2], MIGUEL GAILLEDRAT[3], JOHAN TILLET[4], GIUSEPPE NASCETTI[1] & ROBERTA CIMMARUTA[1]

[1]Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università snc, 01100 Viterbo, Italy
[2]Conservatoire d'espaces naturels de Provence-Alpes-Côte d'Azur, Pôle Biodiversité régionale, Maison de la Crau, 2 place Léon Michaud, 13310 Saint-Martin-de-Crau, France
[3]Vienne Nature, 14 rue Jean Moulin, 86240 Fontaine-le-Comte, France
[4]50 rue de Saumur, 86440 Migné-Auxances, France
[*]Corresponding author Email: daniela.lucente@unitus.it

INTRODUCTION

European cave salamanders, all belonging to the genus *Hydromantes*, are the only members of the family Plethodontidae living in Europe and are furthermore characterised by a unique disjunct distribution, with three American species living in California and eight European ones occurring in France and Italy, including Sardinia (Wake, 2013). This odd range is likely the result of Europe colonisation by *Hydromantes* from North America through the Beringia land bridge (Shen et al., 2015) and it is probably a relict of a past widest distribution, as witnessed also by fossil records from Slovakia (Venczel & Sanchiz, 2005) and by the recent discovery of the only known Asian plethodontid salamander, *Karsenia coreana* (Min et al., 2005).

Hydromantes salamanders miss an aquatic larval stage, having direct-developing larvae, and lack lungs, so needing moist and fresh retreats warranting suitable conditions for their skin-mediated respiration. They are therefore characterised by poor dispersal ability, subtroglophile habits and a mainly nocturnal activity, making hard the finding of new populations without a great sampling effort. Accordingly, several new sites inhabited by *H. strinatii* have been recently discovered in France, showing a wider and more continuous distribution than previously thought, despite this was a widely studied species (Renet et al., 2012). Due to their cryptic nature, the discovery of a new population of H*ydromantes* in France, in March 2015, would be relatively unsurprising unless this finding has some peculiar features that makes it worth of in depth studies. The site is located in west-central France in the Vienne County, at Angles-sur-l'Anglin, more than five hundreds kilometres outside the known range of the French Cave Salamander *H. strinatii* (Fig.1), whose range goes from north-western Italy to south-eastern France (Lanza et al., 2005). This is a limestone area of the Poitou-Charentes region hosting many caves, providing suitable natural habitats for European cave salamanders and so suggesting the finding of a possible relict population. On the other hand, it is known that in the past decades some experiments of translocation/introduction were made throughout the mainland part of European *Hydromantes* range. These experiments were only seldom publicised in the scientific literature, indicating that further unknown introductions may have occurred. Therefore, we decided to investigate the genetic structure of the population from Angles-sur-l'Anglin to discriminate between the hypotheses of having found a new relict in a suitable area or, more likely, an introduced population and, in this last case, to assess from which species and area the founders come from.

METHODS

Nine individuals were sampled in August 2015 in a natural cavity located on a Natura 2000 area ("Vallée de l'Anglin" – FR5400535) from Angles-sur-l'Anglin (AsA). Permissions were issued by order of the prefect [2015-DRCLAJ/BUPPE-173], according to French law. Tissue samples were obtained cutting the tail tip from each individual and total DNA was isolated using the CTAB protocol slightly modified from Doyle and Doyle (1987). Two mitochondrial regions comprised within the genes cytochrome-b (cytb) and NADH dehydrogenase subunit 2 (ND2) were amplified using primers and PCR conditions as reported in Cimmaruta et al. (2015). PCR products were purified and sequenced by Macrogen Inc. (www.macrogen.com). After visual electropherograms checking, the sequences were deposited in GenBank (KX347904 and KX347905 for Cytb; KX347911and KX347912 for ND2). To assign the obtained sequences to a species of *Hydromantes*, they were compared to representative sequences of the three mainland species: six *H. strinatii* sequences were recovered from Cimmaruta et al. (2015) while 4 specimens of *H. italicus* (from Florence, Lucca and Reggio Emilia) and 2 of *H. ambrosii ambrosii* (from the environs of La Spezia) were specifically sequenced for cytb and ND2 (KX347898-KX347903 and KX347906-KX347910 respectively). These sequences were used to build a maximum likelihood (ML) tree in RAxML GUI v.1.3 (Silvestro and Michalak, 2011). We set a GTRGAMMA model with partitions by gene, one thousand bootstrap replicates and ten independent runs. A median-joining network was then built on a concatenated alignment consisting of the whole *H. strinatii* mitochondrial dataset from Cimmaruta et al. (2015) and the nine sequences from AsA, using Network v.4.6.1.2 (http://www.fluxus-engineering.com) under Greedy FHP criterion (Bandelt et al., 1999; Foulds et al., 1976).

RESULTS AND DISCUSSION

The fragments obtained from the nine individuals from AsA were 638 bp long for cytb and 676 bp for ND2, providing a final concatenated alignment of 1314 bp. The 9 specimens from AsA provided three haplotypes (named 31, 32, 33), which differed for one or two mutations from each other.

ML analysis strongly supported the clusterisation of AsA haplotypes within the *H. strinatii* clade, where they grouped with the samples from the French Maritime Alps (Fig.2a). The same result was obtained by the outcome of the median-joining network reported in Fig.2b, where 30 haplotypes from the entire species range were included. The three haplotypes from AsA resulted directly connected to the haplogroup from Col de Tende (haplotypes 29 and 30), from which they are separated by 3 (haplotype 31) or 4 (haplotypes 32 and 33) mutational steps.

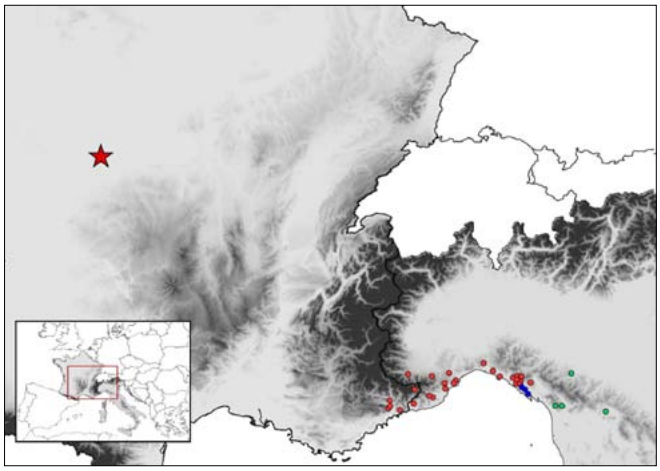

Figure. 1 Distribution map of the samples considered in this study. Green circles are *H. italicus*, blue circles are *H. ambrosii*, red circles are *H. strinatii*. The red star spots the location of the new population from Angles-sur-l'Anglin.

These data showed that the three haplotypes recovered from Angles-sur-l'Anglin tightly nested within *H. strinatii*, assigning to this species the newly discovered locality. In particular, the three haplotypes showed a greater affinity with *H. strinatii* populations from the French Maritime Alps in the Col de Tende area, as shown by the network analysis. The comparison between the high geographical distance and the low genetic differentiation between Angles-sur-l'Anglin and Col de Tende, indicates that the discovered population is the result of a human introduction, rather than a relict. In fact, such a geographical distance is far superior to that between populations from the two main lineages found within *H. strinatii*, living respectively in the western (French Maritime and Ligurian Alps) and eastern (eastern Liguria) parts of its range. These are indeed separated by an amount of 62 mutational steps over a geographic distance of tens of kilometres (Cimmaruta et al., 2015), while Angles-sur-l'Anglin and Col de Tende are hundreds of kilometres distant but show only 3 to 4 mutational steps from each other. The haplotypes shown by the nine individuals from Angles-sur-l'Anglin were however unique, never observed in other previously examined French sites, preventing

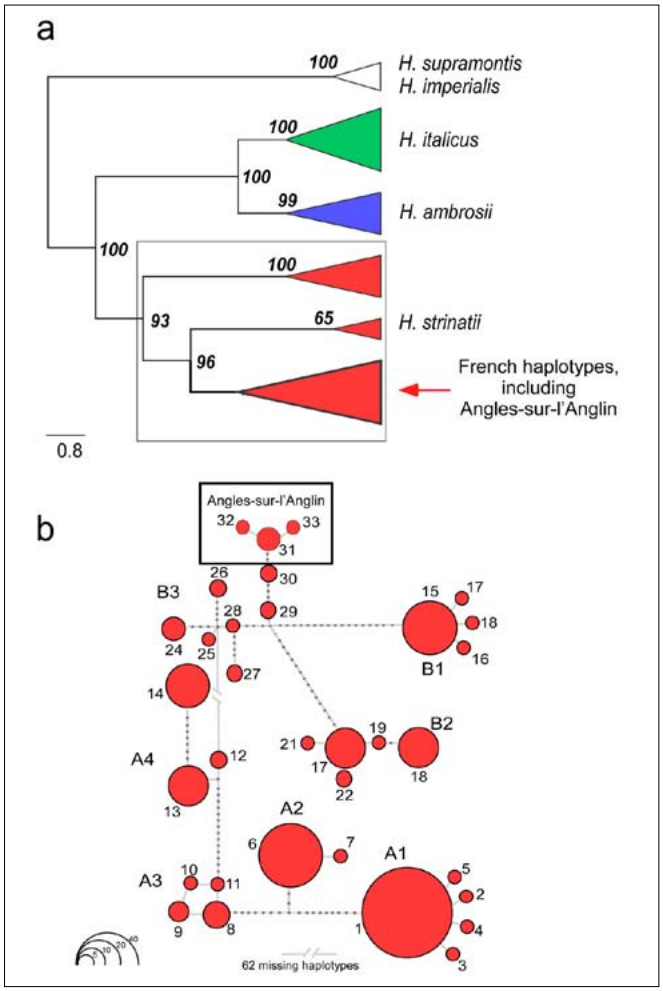

Figure. 2. a) Maximum likelihood tree built on concatenated sequences of cytb and ND2 of *H. italicus* (green), *H. ambrosii* (blue) and *H. strinatii* (red). Bootstrap values are showed on each node. The Sardinian species *H. supramontis* and *H. imperialis* represent the outgroups. **b)** Median-joining network based on concatenated sequences of cytb and ND2 including 194 sequences of *H. strinatii* from Cimmaruta et al. (2015) and the nine sequences from Angles-sur-l'Anglin of this study (squared). Haplogroups A1-4 and B1-3 are named as in Cimmaruta et al. (2015).

the identification of the exact source of the introduced population. This finding evidences the need of expanding the genetic survey of *H. strinatii*, with particular regard to the Maritime Alps, an area with a peculiar paleoclimatic history resulting in particularly high levels of biodiversity (Schönswetter et al., 2005) and in highly fragmented and genetically differentiated populations of *H. strinatii*, each one characterised by private haplotypes in this zone (Cimmaruta et al., 2015 and Fig. 2b).

The discovery of an introduced population is not surprising since other introductions of *Hydromantes* took place in the past, either within or outside the range of the genus and with different aims. For example, the parapatric species *H. strinatii* and *H. a. ambrosii* were put in syntopy in the southernmost part of *H. strinatii* range to demonstrate the role of competitive interactions in generating replacement patterns (Cimmaruta et al., 1999). An artificial syntopy between *H. italicus* and *H. a. ambrosii* was carried out outside the genus range, in Tuscany, to test for their

reproductive isolation (Forti et al., 2005; Cimmaruta et al., 2013). Other introductions took place between 1965-70 in a mine gallery of the French Pyrenees (in the Salat Valley, Ariege) by Durand and in north-eastern Italy (Gorizia) where *H. strinatii* specimens were released (Lanza, 2005). In both cases the populations survived and settled in the sites. These data show that *Hydromantes* introductions may easily result in local viable populations, provided that the site has subterranean suitable retreats, which is also the case of Angles-sur-l'Anglin where, after the discovery of the site in March 2015, up to 28 individuals were recovered simultaneously including some juveniles, so suggesting that the population is reproducing and has permanently settled in the site.

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Record sizes for the Turkish house gecko, *Hemidactylus turcicus*, from Aegean islands, Greece

YUVAL ITESCU[1*], RACHEL SCHWARZ[1], MICHAEL MOSES[1], PANAYIOTIS PAFILIS[2] & SHAI MEIRI[1]

[1]Department of Zoology, Tel Aviv University, Tel Aviv 6997801, Israel
[2]Section of Zoology and Marine Biology, Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Ilissia, Athens 157-84, Greece
[*]Corresponding author Email: yuvitescu@gmail.com

ABSTRACT - Insular animals are thought to evolve extreme sizes compared to the mainland. The reported maximum body size of the Turkish house gecko, *Hemidactylus turcicus*, does not go beyond 61 mm in snout-vent length, 120 mm in total length and 3.6 g in mass, across its distribution. Here we report much higher size estimates for this species on the Aegean islands of Kassos, Sifnos and Anafi. The commonly perceived drivers of insular gigantism vary across these islands. Therefore, the reasons for the extreme size on these islands, while on other Aegean islands live "normal-sized" geckos, are unclear.

INTRODUCTION

The Turkish house gecko, *Hemidactylus turcicus*, is a nocturnal and insectivorous gecko (Durrell, 1956; Baha El Din, 2006; Bar & Haimovitch, 2012; authors' personal observations). It is a common edificarian house gecko that frequents rocks, boulders and house walls (the Hebrew origin of Proverbs 30:28 is more specific than common English translation in stating that unlike "a lizard", a gecko "is caught by hand, yet inhabits the king's palaces"). It is naturally distributed in Southern Europe, North Africa, the Middle East (Sindaco & Jeremcenko, 2008), Pakistan and India (Sharma, 2005; Khan, 2006). It is widely introduced in the New World, from Panama to the USA (Uetz, 2016). *H. turcicus* is a relatively small member of its genus (Feldman et al., 2016). The vast literature pertaining to its body size suggests it grows to no more than 61mm in SVL (maximum recorded for males: 60.2 mm, for females: 59.7 mm, Delaugerre, 1984). Our own measurements of 563 live individuals and museum specimens (Table 1; adults and juveniles of both sexes) suggest an overall size range of 17- 60.1 mm SVL. Mass data are less common for *H. turcicus*, as they are for lizards in general (Meiri, 2010). Literature data (Huey et al., 1989; Garland, 1994; Irschick et al., 1996; Van Damme & Vanhooydonck, 2001) suggest a maximum of 3.6 g. Our measurements (Table 1) suggest an overall mass range of 0.1- 6.5 g).

METHODS

During field work from May 2013 to May 2016 we searched for geckos on several Aegean islands (Fig. 1(a)), by looking under and into possible covers and surveying building, natural and dry-stone walls, both at day and at night. Snout-vent lengths (SVL) and tail lengths of animals were measured using a digital caliper to the 0.01 mm precision. Animals were also weighed to the 0.1 gram precision, and their sex was determined visually.

RESULTS

We found several specimens with SVL, total length and mass that were much larger than previously reported. On 27 May 2014, on Anafi Island, we found a very large female on a house wall (36°20'47.4"N 25°46'27.8"E) foraging for beetles under a street lamp around midnight. This female (60.1 mm SVL; Fig. 1(b)) was gravid with two eggs and weighed 7.9 g which is by far the heaviest specimen ever reported for this species (see above).

We found still longer (but lighter) lizards on Kassos Island, during 6-8 May 2016. Five specimens (three adults and two sub-adults) were found in a single location on this island, inactive under various objects in the western outskirts of the port Fri (35°24'59.4"N 26°55'13.4"E) at midday. While specimens from the nearby islands of

Table 1. Body size measurements of *H. turcicus* from Greece and Israel.

Source	Geographic origin	n	Size index	Size range	Size mean	Comments
Museum	Israel	140	SVL (mm)	17.0-60.0	39.7±9.9	Steinhardt Museum of Natural History, Tel Aviv University
		138			1.6±1.2	
Wild caught		78			43.1±8.6	Authors' measurements
		77			2.2±1.1	
Museum	Aegean Islands, Greece	128	SVL (mm)	29.4-58.1	47.1±5.5	42 islands; Zoologische Staatssammlung München; Zoologiche Forschungsmuseum Alexander Koenig in Bonn; Natural History Museum of Crete
Wild caught		217	SVL (mm)	28.9-60.1	47±6.4	22 islands, authors' measurements
			Mass (g)	0.5-6.5	3±1.1	

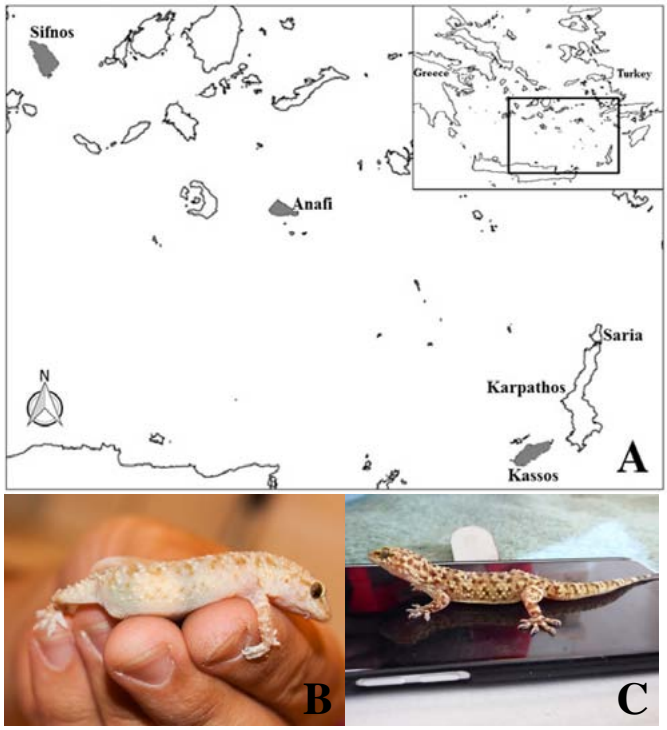

Figure 1. A. Islands with giant geckos: Sifnos, Anafi and Kassos; **B**. The heaviest individual ever reported (Anafi Island); **C**. The longest individual ever reported (Kassos Island) on a 5.5 inch phone.

Karpathos and Saria (first *H. turcicus* record from the later island) are of "normal" size, three of the Kassos ones were veritable giants: the largest specimen (Fig. 1(c); TAU #17870), a gravid female with two eggs, measured 64.7 mm (tail length 63.3 mm, mass=6.1 g). It is, to our knowledge, the longest specimen ever recorded of the species. The other specimens (including two adults measuring 58.4 and 58 mm SVL, 4.4 and 3.6 g, respectively) were released soon after capture. The mean SVL of adult Kassos Island *H. turcicus* (60.4 mm) is thus similar to the maximum SVL previously reported.

Potentially, an even larger gecko was found on Sifnos Island, Cyclades Archipelago, Greece, by Cattaneo (1984). This author reported, as part of a table of *H. turcicus* measurements an animal with a SVL of 65 mm (tail length 40 mm, no weight reported). Cattaneo (1984) rounded SVLs to the nearest 1 mm, thus this animal could have been any length from 64.5 to 65.4 mm. It may also be that this measurement represents a typo, because Cattaneo (1984) does not mention anything unusual about this gecko, and its tail is short. We do, however, have reasons to think the figure may be genuine: three weeks after we visited Kassos (on 23 May 2016) one of us (RS) found a very large female (SVL=63.3 mm, regenerated tail length=46.8 mm, mass=5.9 g) on Sifnos (36°58'38.6"N 24°44'25.8"E), supporting the possibility that individuals of this species could be very large there.

DISCUSSION

The reasons for the large sizes of the geckos from Kassos, Sifnos and Anafi are unclear. Insular lizards are often larger than their mainland conspecifics or close relatives (Case, 1978; Pregill, 1986; Meiri, 2007; Raia et al., 2010; Meiri et al., 2011; Senczuk et al., 2014; Slavenko et al., 2016; see also Arnold, 2000). Some conditions are quite different across these three islands but are not different in general from other Greek islands we visited. While house geckos are relatively rare on Kassos and Sifnos, they are abundant on Anafi (Itescu et al., unpublished). The only other gecko, and potential competitor of the house gecko, Kotschy's gecko (*Mediodactylus kotschyi*) occurs on all three islands (Anafi, Sifnos and Kassos), and on virtually all other Aegean Sea islands inhabited by *H. turcicus*. Thus character displacement is an unlikely cause of insular gigantism in *H. turcicus*. *Mediodactylus kotschyi* individuals, however, are not particularly large on any of these islands (and in fact are relatively small on Kassos, Itescu et al., 2016), thus these islands do not seem particularly favourable for the evolution of large size of geckos in general. The conditions often associated with the evolution of insular gigantism (Hasegawa, 1994; Meiri, 2007) vary across the three islands: Sifnos and Kassos are inhabited by snakes that are important gecko predators, whereas Anafi is snake-free (as its Greek name – άνευ όφεως – 'snake free', implies). All three islands harbour other gecko predators such as rats and domestic cats. Release from competition is also an unlikely cause, as the herpetofaunal composition on these three islands is similar to that of many Greek islands (Valakos et al., 2008) where geckos are of average size (Itescu et al., 2016). Neither island enjoys significant amounts of marine subsidies (i.e., food brought in by nesting sea birds), a factor associated with reptile gigantism in many places (Bonnet et al., 2002; Hasegawa, 2003; Keogh et al., 2005), including the Greek Islands (Pafilis et al., 2009).

We are not sure if *H. turcicus* is really native on these islands. On Kassos, for example, some resident lizards may have been introduced recently, based on conversations with locals, who started observing these species only in 2011 (Itescu et al., unpublished). Gigantism in reptiles may well be the result of founder effects, with colonisers being larger than average (Arnold, 2000; see also Cernansky et al., 2015), although in this case, the islands with giant individuals are quite distant from each other, and there are several "normal-sized" population on islands between them (e.g., Sikinos, Folegandros, Karpathos). Thus, evolution is a more likely explanation. Evolution can be extremely fast in island lizards (e.g., Campbell & Echternacht, 2003; Stuart et al., 2014), and so even if the geckos are not native on these islands, they may well have evolved there. We are nonetheless unable at the moment to explain what selective forces drive the evolution of gigantism in this particular population.

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Newt mortalities on an urban cycle path

[1]STEVEN J. R. ALLAIN & LIAM T. SMITH

Cambridgeshire and Peterborough Amphibian and Reptile Group, UK
[1]Corresponding author Email: steveallain@live.co.uk

ABSTRACT - There are many anthropogenic obstacles faced by amphibians in the urban environment. An example of one of these is roads between habitat features such as hibernation sites and breeding ponds; this leads to traffic mortalities. In urban settings, cycle paths may be as busy as roads and present similar dangers; we report this in the case of smooth newts (*Lissotriton vulgaris*) attempting to cross a cycle path in central Cambridge. We also explore a potential solution, a drift fence, which we wish to implement in the future to prevent further declines.

INTRODUCTION

Roads are a common obstacle encountered by amphibians as they move to their breeding ponds in the spring (Mazerolle, 2004). Amphibians may also be at risk on the return migration to their hibernation sites or when they are foraging terrestrially (Meek, 2012). Amphibian metamorphs may also be at risk when they disperse from their ponds. During this initial migration it is well documented that road traffic may cause high mortality rates in common toads (*Bufo bufo*; Cooke, 1995). Unfortunately this is a widespread problem that is not just limited to the United Kingdom; many amphibian species are at risk of being killed by cars as roads get busier and as they cut through potential habitats (Glista et al., 2008).

The road traffic associated with amphibian mortality includes bicycles although they are less well documented than mortalities from motorised vehicles. The combination of noise and vibration gives the amphibians a warning that cars may be present, but due to the speed at which cars are travelling amphibians often do not have time to escape the path of oncoming traffic. Work in the USA has shown that car-associated stimuli such as bright headlights tended to lead to amphibians remaining immobile, perhaps because it disrupts their visual system (Mazerolle et al., 2005). Bicycles pose a threat as they do not make as much noise as cars and create fewer vibrations than motorised vehicles, when travelling over paved surfaces. We present evidence perhaps for the first time that urban cycle paths can be a source of mortality for newts.

MATERIALS AND METHODS

On 21 February 2014 after a period of mild and wet weather, the authors followed up reports from members of the public, via the Cambridgeshire and Peterborough Amphibian and Reptile Group (CPARG) of dead newts along a busy cycle path in central Cambridge. On arrival at the area at 1:30 pm on the Chesterton side of Riverside Bridge (TL 4640 5954), we discovered 12 dead smooth newts (*Lissotriton vulgaris*) that had been killed by bicycles. Of these, 9 were males and 3 were female. We continued to monitor the site over the next 13 weeks (between 27 February and 20 May 2014) and made nine more site visits during times when the weather was favourable for amphibian surveys (a night time temperature above 5˚C, preferably with rainfall). The cycle path was monitored by torchlight when we visited the breeding pond, which is roughly 10 m away. We visited at night as this is when we suspected the newts were active and so aimed to prevent any further mortality. We made an effort to go on the same evening every week, but were limited by the weather. Night-time was also when the cycle path was least busy and so safer for us to examine any newt carcasses. The breeding pond was surveyed using standard protocols for night-time amphibian surveys (Griffiths et al., 1996) and surveying of the pond only commenced after we were made aware that newts were present in the area.

RESULTS & DISCUSSION

During the time that both the cycle path and pond were monitored, only five more newts were found to have been killed by bicycles (Fig .1). In total we observed 17 smooth newts that had been squashed by bicycles, most of which were male. The newts were spread over an area of roughly 5 m^2 and all but one facing in the direction of the breeding pond. Typically we visited the site when the air temperature was between 7-11˚C but on our last survey the air temperature was 16.4˚C. Our peak count for smooth newts within the breeding pond during 2014 was 36 newts, a mortality event like the one observed may pose a threat to a small and isolated population of amphibians. On the first visit to the site it was clear that not all of the newts had been killed at the same time, due to varied levels of decomposition. Some individuals had been killed earlier than others, and those that had been struck earlier appeared to be crushed into the pavement. This may have been due to continual running over from cyclists, foot traffic, or due to the decomposition process. Weather conditions and the traffic intensity will influence the rate at which amphibians are likely to be struck (Mazerolle, 2004; Meek, 2012).

The newt habitat is divided by the narrow cycle path

Figure 1. A squashed smooth newt (*L. vulgaris*) on the cycle path.

Figure 2. A view of the site from Riverside Bridge, the breeding pond is 10 m to the right and the hibernation site can be seen on the left. The adjoining footpath can also be seen on the left hand side.

with the aquatic breeding habitat and ideal terrestrial habitat on either side. The presumed hibernation site consists of a mound of earth covered in brambles and shrubbery. It is only when the newts try to cross from one side to the other during the spring and summer migration that they are at risk of being struck by a bicycle. Newts may be more vulnerable to such threats than anurans due to the fact that they are much slower moving (Gibbs & Shriver, 2005). In addition, their elongated bodies means that they are more likely to be struck when crossing roads and cycle paths as their lower profile makes them harder to see than a toad or frog. Newts are also less active than frogs or toads and so may spend more time on the road/path increasing the risk of them being squashed.

This negative impact was not observed in the population during 2015 or 2016, despite the risk still being present. There may have been no newt mortalities as the conditions may have been different to those in 2014. The bridge was built between the years of 2007-2008 and so is a relatively new. Observed foot traffic on the bridge is usually quite low in comparison to the cycle traffic. All of the dead newts were observed at a junction at the foot of the bridge (Fig. 2), where a footpath joins the main route. At this point cyclists may not be paying as much attention to the ground ahead of them, as they may be more focused on trying to avoid pedestrians especially around such a tight corner. It is likely that the 12 individuals we first discovered were killed during the initial migration between the presumed hibernation site and the breeding pond. The reason why we failed to find as many newts again over further site visits may be because the majority of smooth newts had completed their migration.

With a view to assessing the impact of cycle paths on amphibians in Cambridge and locations around the country it may be prudent to work towards identifying potentially problematic sites. To help minimise the risk of bicycle related mortality, site specific solutions need to be implemented. In the future we aim to question cyclists in the area to find out if they are aware of the presence of newts and whether or not they care about the risk facing them on the cycle path. Raising awareness of the newts presence may help reduce future impacts of cyclists in the area. A more permanent solution to the problem at Chesterton would be to put drift fencing along the cycle path to prevent the newts crossing, redirecting them to a safer crossing location. This alternate crossing is only 2 m away leading the newts under Riverside Bridge directly to their breeding pond. Drift fencing was not implemented in either 2015 or 2016 due to a lack of time, funds and permission. We will be hoping to implement this solution in time for the 2017 migration. An alternative solution would have been to create a tunnel connecting the hibernation site to the breeding pond. However, this would be impractical as the foundations of the bridge are too deep.

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Lamination as a method of preserving reptile sloughs

[1]STEVEN J. R. ALLAIN & MARK J. GOODMAN

Cambridgeshire and Peterborough Amphibian and Reptile Group, UK
[1]Corresponding author Email: steveallain@live.co.uk

The sloughed skins of snakes and lizards can sometimes be found in reptiles' habitats. In Europe they are more commonly found during the spring after snakes and lizards have emerged from hibernation than at other times of the year (Bauwens et al., 1989; Nilson, 1980). These can then be used to assist in identification of winter hibernacula locations. Unfortunately sloughs tend to dry out quickly in warm climates and break down readily in the environment. This means that searching for sloughs at a known reptile site may increase the likelihood of encountering freshly sloughed skins. If collected early enough, they can be stored dry for later reference or used as a non-invasive sampling method to extract DNA (Jones et al., 2008).

A number of reptiles are often used as educational tools in outreach aimed at children. During these events, the children may come into close contact with the reptiles and may even get to hold the animals. There is a risk here of the handled reptiles infecting the children with salmonellosis, although this risk is only small. The use of antibacterial hand gel when children handle such animals is vital. We propose a method in which the sloughs of reptiles can be preserved, meaning they can be used as an up-close educational tool during such outreach events. The method of lamination means the slough can be handled without damage and without distorting any detail. This doesn't remove the need for reptiles at such events but instead adds another level of interaction and interest.

An inspection of the slough should be performed to ascertain if there is any damage and if present to what degree. If there are multiple sloughs to choose from then selecting a different one for lamination may be the best choice to ensure the optimum detail is captured and as little wastage of the laminate sheets is achieved. This visual inspection also allows for the selection of potential cut points as well as a visualisation of the final lamination. To prepare the slough for lamination it is advised that it is lightly misted with a spray bottle. If the slough has been in dry storage then leave the skin for a short while after misting (time varies on size, length and ambient room temperature). Ensure that the slough is only lightly rehydrated and isn't brittle or saturated.

Once the slough has been sufficiently rehydrated and is ready to be laminated, it should be cut to size. If the dorsal and ventral surfaces are desired to be fully preserved, then the slough can be cut longitudinally. At this stage a suitable pouch size should be selected, for most snakes we recommend A4 pouches. The prepared sections should then be placed on a hard surface such as an ironing board, with a tea towel placed on top of them. This step is taken in case the slough has become warped as part of the storage process. To avoid burning the slough during the ironing, lightly mist the tea towel and then pass a hot household iron over the slough until the tea towel is sufficiently ironed. Care should be taken not to apply too much pressure and ironing should replicate the care one would take with a silk shirt. This not only protects the delicate skin beneath but also acts as an indicator. The process works to both flatten the skin ready for lamination and to fix it into position. The process of ironing the slough sections may stretch them slightly.

Figure 1. The laminated sloughs from a captive corn snake (*Pantherophis guttatus*).

Depending on the size and colour of trimmed sloughs, they can either be placed directly into an A4 lamination pouch (for larger and darker sloughs) or be glued onto a sheet of A4 paper. Being glued onto the a sheet of paper

prevents the sloughs from slipping during the lamination process and allows a greater level of clarity of any patterns that may be present on the slough. This is particularly helpful if a slough is light in colour and any patterns present are desired to be displayed. Multiple cut sloughs sections can be fitted within a single lamination pouch; printed labels can also be added before the final process of lamination. If preferred these can be added afterwards with a permanent marker pen. Once the pouch is full, run through a laminator as normal. There is an alternative method to the one we have described but it requires greater skill. It involves applying the slough to a Super Loxol backing sheet, this avoids use of adhesives or lamination (Kaleta, 1979).

The lamination of sloughs may also be a useful tool for a population based study where sloughs of individuals are sometimes found. This could work well for species such as the northern viper (*Vipera berus*) as individuals of the species have unique scale patterns (Sheldon & Bradley, 1989). This novel technique could over time add richness to a population study by way of having a visual database and associated DNA profile of an individual without having to encountering it. The proportion of sloughs compared with animals present is likely to be low and so should only be restricted to a minor role compared with visual surveys. Lamination will likely denature the DNA contained within the laminated sections of the slough and so part of the discarded or excess trim could be used for DNA extraction. This is an area which could be explored further in the future and would work well if partnered with a photographic census of a known population.

Due to the size limitations of standard laminate sheets only smaller skins can be fully laminated, for larger skins a selection of sections can be made to ensure that any identifying features such as dorsal head scales are preserved. Figure 1 shows three sections of which the left and right have been orientated so that the dorsal surfaces are facing upwards but the centre section is orientated so that the ventral surface faces upwards. Dorsal patterns on either the head or body are easier to capture than those of ventral markings, at least in our limited experimentation with the sloughs of captive corn snakes (*Pantherophis guttatus*).

Our preferred use for the laminated sloughs is as a tool to educate people about squamates and their life histories. These are often used in outreach events to add another level of intrigue although they are not a substitute for live animals. Another use for the lamination of sloughs could be as a training aid, helping to train individuals in the identification of varying scalation types across a number of species. Being able to recognise morphological features such as scale groups can be used in identification of visually similar species. We feel that this technique is of so much value that we will provide each classroom where we exhibit with at least one lamination for future reference. This we hope will serve to lessen the fear associated with snakes and improve the public image of reptiles.

The method of preservation works better on the sloughs from captive animals which tend to be in a single piece and are encountered more often than those of wild animals. Lamination may not work well for fragmented sloughs although this may to some extent be overcome by gluing the fragments to sheets of paper of the appropriate size. This method ensures that the sloughs can be handled and studied far past the lifetime of an unpreserved slough.

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Northern crested newt (*Triturus cristatus*) migration in a nature reserve: multiple incidents of breeding season displacements exceeding 1km

PHILLIP J. HAUBROCK[1,2,*] & JAN ALTRICHTER[3]

[1]Department of Biology, University of Florence, Via Romana 17, 50125 Florence, Italy
[2]NEMO, Nature and Environment Management Operators s.r.l., Piazza M. D'Azeglio 11, 50121 Florence, Italy
[3]University of Kassel, Department of Zoology. Heinrich-Plett Str. 40, 34340 Kassel, Germany
[*]Corresponding author Email: Phillip.haubrock@hotmail.de

ABSTRACT - The northern crested newt *Triturus cristatus* is a model organism for the study of amphibian migrations but is in decline and is prioritised for conservation efforts. We monitored 15 adults in the Dönche Nature Reserve. During the breeding season five of these individuals migrated from one pond to a group of subjacent ponds. Surprisingly, in four cases their displacements were 1,520 m and in one case 1,610 m.

INTRODUCTION

Although the northern crested newt *Triturus cristatus* is widely distributed across Europe and present in parts of western Asia, populations have been declining (Griffiths et al., 2010). In Germany, *T. cristatus* can be found nationwide. In the Dönche Nature Reserve (Kassel, Central Germany), suitable habitats are steadily decreasing with little prospect of amelioration. The species was present in around 400 ponds after WWII, now only five ponds remain of which only three or four ponds hold enough water throughout the breeding and larval development period to support *T. cristatus* (Haubrock & Altrichter, 2016). The largest of the ponds is at a higher elevation and lies 1,430 m to the north-west of the others, and hence the newts in these ponds appear to be too far apart to be included within the same metapopulation. In previous monitoring studies, potential movements between ponds during the breeding season have received little attention (e.g. Kupfer & Kneitz, 2000; Griffiths et al., 2010), although it is known that certain species of newt are capable of travelling in excess of 1 km when returning to their breeding pond (reviewed in Jehle & Sinsch, 2007); indeed displacements of a much as 1,290 m have been recorded in the case of northern crested newts (Kupfer, 1998). Herein we describe migration displacements during the breeding season of a population of *T. cristatus* based on mark-release-recapture.

MATERIALS AND METHODS

Newts were sampled from March until the end of June 2015, using weekly scoop-netting and bottle trapping (volume 5 L) in four ponds. Ponds 1-3 were old bomb craters, which are now water filled. Pond 4 is an artificial open air laboratory used as part of the "Forschen und Lernen durch Offenes Experimentieren" (FLOX) project (Wulff et al., 2015). It is associated with a set of much younger and smaller to medium sized ponds with artificial foliage. All bottle traps were secured to prevent sinking. To forestall the possibility of overheating or drowning of newts we examined all bottle traps every 24 - 48 hours. Captured newts were collected in a 0.5 m × 0.3 m × 0.15 m sized plastic container and were then placed in a transparent box for belly-pattern photography. To facilitate individual recognition, these belly patterns were analysed using the open source programs "extract/compare" (http://www.conservationresearch.org.uk; (Hiby, 2013) and "Wild-ID" (Bolger et al., 2012).

RESULTS

From all ponds there were a total of 33 records of *T. cristatus* and following belly pattern analysis it appears that these records were obtained from fifteen different individuals, eight of which were females. In the more distant Pond 4, *T. cristatus* were only observed and caught until April 14th. Newts were observed in the three lower ponds and travelled between them (Table 1). Recapture data (Fig. 1) indicated significant movements of two females and three males from Pond 4 towards the subjacent ponds achieving four displacements of at least around 1,520 m and one of 1,610 m, in a mean period (± standard deviation) of 55±19.4 days (Table 1).

DISCUSSION

The majority of *T. cristatus* adults and metamorphs would be expected to stay in the vicinity of the ponds after leaving the water in the summer and autumn (Arntzen & Teunis, 1993; Jehle & Arntzen, 2000), but they are also known to migrate between ponds during the breeding season (Jehle et al., 2011). Furthermore, Latham et al., (1996) reported that 13% of *T. cristatus* were captured within the breeding season while moving between a pond and terrestrial habitat and that immigration is affected by temperature and emigration by rainfall. Our observations

Table 1. Individual inter-pond migrations of *T. cristatus*.

Encounter	ID	Gender	Location	Date (2015)	Days	Distance [m]
1	1	M	Pond 4	09.04	54	1,520
2			Pond 3	02.06		
3	2	M	Pond 1	29.05	7	100
4			Pond 3	05.06		
5	3	M	Pond 1	26.05	10	100
6			Pond 3	05.06		
7	4	M	Pond 4	10.03	72	1,610
8			Pond 1	21.05		
9			Pond 3	27.05	6	100
12	6	M	Pond 4	24.03	35	1,520
13			Pond 3	28.04		
29	14	M	Pond 1	27.05	6	100
30			Pond 3	02.06		
31	15	M	Pond 2	05.06	4	60
32			Pond 3	09.06		
33			Pond 3	12.06	3	-
10	5	F	Pond 4	19.04	37	1,520
11			Pond 3	26.05		
14	7	F	Pond 4	10.03	77	1,520
15			Pond 3	26.05		
16	8	F	Pond 1	21.05	12	100
17			Pond 3	02.06		
18	9	F	Pond 1	28.04	35	100
19			Pond 3	02.06		
20	10	F	Pond 1	21.04	7	50
21			Pond 2	28.04		
22			Pond 3	05.0	38	60
23	11	F	Pond 3	05.05	21	100
24			Pond 1	26.05		
25	12	F	Pond 2	05.05	-	-
26	13	F	Pond 1	28.04	35	-
27			Pond 1	02.06		
28			Pond 3	09.06	7	100

of the early appearance of *T. cristatus* in Pond 4 (the open-air laboratory) may be explained by higher than average temperatures and lower precipitation at the beginning of 2015 (Imbery et al., 2015). During the breeding season, we recorded five long distance migrations from Pond 4 to the other ponds, which resulted in displacements of 1,520 m and 1,610 m. These were potentially directed movements for mating purposes. However, it is possible that the five specimens observed had previously migrated into the Dönche Reserve from the north-west adjoining Habichtswald nature reserve and were continuing their migration in 2015 because Pond 4 has been, or has recently become, unsuitable for breeding purposes. It is uncertain whether the stream (Krebsbach) that connects the ponds may have assisted in the unusually long migration.

Our study clearly highlights the extraordinary migratory abilities of *T. cristatus* in the Dönche nature reserve. This should be investigated in more detail, perhaps employing radio telemetry (e.g., Schabetsberger et al., 2004). The displacement of 1,610 m is a new maximum for the species, which has relevance for conservation biology.

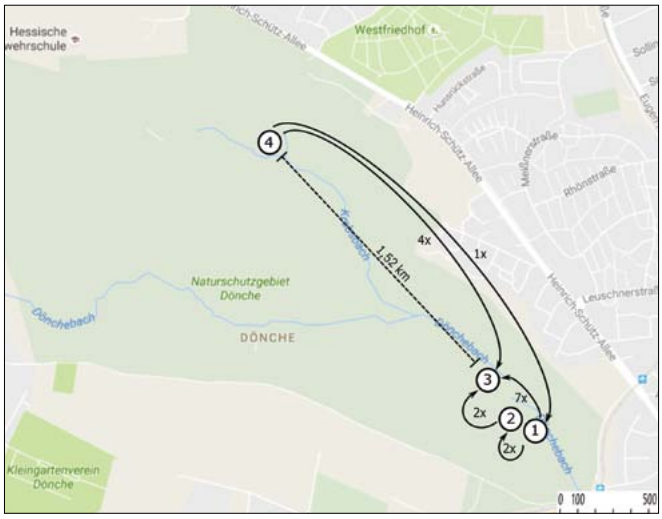

Figure 1. Recorded inter-pond movements of northern crested newts. Distance between subjacent and higher situated ponds is indicated by a dashed line. Numbers denote observed migrations.

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Larva of the tick *Ixodes ricinus* found attached in the oral cavity of a green lizard (*Lacerta viridis*)

DANIEL KOLESKA*, VERONIKA SVOBODOVA & TOMAS HUSAK

Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
*Corresponding author Email: koleska@af.czu.cz

The Green lizard (*Lacerta viridis*) is a member of the family Lacertidae and is widely distributed throughout the central and south-eastern part of Europe from southern Greece up to central Germany and Poland (Sillero et al., 2014). This species inhabits mostly lowland south-facing slopes and prefers xerothermic areas with rather sparse vegetation. The Castor bean tick (*Ixodes ricinus*) is a common parasite of reptiles or other smaller vertebrates distributed from northern Africa to Scandinavia and from Ireland to central Russia (Gern & Humair, 2002). This note reports an *I. ricinus* larva found attached to the mucous membrane inside the oral cavity of *L. viridis*. Such a case has not been previously reported.

On 13 June 2015 around 12:00h local time, one of us (DK) found an adult male of *L. viridis* near the Šobes vineyards, Znojmo, southern Czech Republic. The individual was found basking on the border of a sparse pinewood and a low rocky slope near a busy cycle path (48.81669°N, 15.97550°E; 300 m a. s. l.) in the Dyje river valley. After capturing the individual, it was closely inspected. During manipulation of the lizard it demonstrated some defensive behaviour including defecation, attempts to bite and opening the mouth as a threat. With the individual's mouth open we noticed a significant dark spot inside its oral cavity (Fig. 1A). This spot proved to be an *I. ricinus* larva firmly attached to the lizard's mucous membrane (Fig. 1B). After removing the *I. ricinus* larva it proved to be alive and moving. Together with this larva we observed several more on the lizard's body, mainly around its forelimbs. As far as our knowledge goes, observation of *I. ricinus* parasitizing inside an oral cavity of a lizard has not been previously reported.

The distribution of ticks on their host's body seems to be mainly determined by finding a suitable spot with minimal disturbance (Nelson et al., 1975). The ticks seem to choose spots with thin skin providing more firm attachment (Bauwens et al., 1983). Ticks are usually found on a lizard's body around forelimbs, arm-pits and sides of their heads. These spots also offer ectoparasites to be partly protected from being scratched off by their host or being brushed against vegetation. The mucous membrane of oral cavity seems a very unusual spot for ticks to attach to. We assume this was a rather rare case with very poor chances of the tick's successful survival.

However, all stages of ticks are able to survive in extreme environmental conditions. The oral cavity is a wet and low oxygenated place. Although ability of anaerobic respiration of ticks has not been reliably proven yet, there are reports of resistance to hypoxia and anoxia (Fielden et al., 2011). It was also reported, that some ticks are able to survive quite a long time e. g. under water or even in the automatic washing machine (Cançado et al., 2006; Carrol, 2003; Smith, 1973). Fielden et al. (2011) reported that the American dog tick (*Dermacentor variabilis*) is able to utilise dissolved oxygen in the water. Oxygen is obtained via a plastron formed by the complex spiracular plates.

Figure 1. *L. viridis* male with an *I. ricinus* larva attached to its mucous membrane. **A** – wider view with noticable dark stain inside lizards oral cavity. **B** – close up view with a detail of *I. ricinus* larva.

However, even with the plastron disabled or in water with very low oxygen content, *D. variabilis* can still survive for several days. Mihalca et al. (2012) report the European pond turtle (*Emys orbicularis*), a predominantly aquatic species, as a host of *I. ricinus*.

Lizards play an important role in the life cycle of *I. ricinus* (Dantas-Torres, 2015; Földvári et al., 2009). Their infections by ticks is currently being investigated because of the large number of tick-borne diseases potentially able to affect humans (e. g. Gryczyńska-Semiątkowska et al., 2007, Majláthová et al., 2013). Many tick-borne diseases have shown a significant increase in parts of Europe in the last two decades (Jones et al., 2008) and therefore any new observations might have value.

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Cryptobranchus alleganiensis bishopi (Ozark Hellbender): larval habitat and retreat behaviour

AMBER L. PITT[1*], JOSEPH J. TAVANO[2] & MAX A. NICKERSON[3]

[1]Environmental Science Program and Department of Biology, Trinity College, Hartford, Connecticut 06106, USA.
[2]Reptile and Amphibian Conservation Corps, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA.
[3]Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, USA.
[*]Corresponding author email: amber.pitt@trincoll.edu

The aquatic salamander *Cryptobranchus alleganiensis bishopi* (Ozark Hellbender) is a long-lived (est. 30-55 years), large-bodied (≤ 62 cm) species endemic to the Ozark region of southern Missouri and northern Arkansas, USA (Nickerson & Mays, 1973). This species has undergone dramatic population declines throughout its range (Trauth et al., 1992; Wheeler et al., 2003), resulting in its listing as an Endangered Species under the US Endangered Species Act in 2011 (U.S. Fish and Wildlife Service, 2011).

Little is known about larval *C. a. bishopi* habitat use and behaviour *in situ* because few have been found, including in pre-decline populations, but those that were observed occupied stream reaches typified by gravel rather than bedrock substrate (Nickerson & Mays, 1973; Nickerson et al., 2003). Some evidence suggests that larval *C. a. bishopi* utilise the interstitial spaces among the gravel and cobble as their primary habitat which has been posited as a measure to avoid predation and obtain macroinvertebrate prey (Nickerson et al., 2003).

On 19 July 2006 at 15:10 (US Central Time Zone) we observed two un-gilled larval *C. a. bishopi* (Total length1 = 13 cm; 14.1 g; Total length2 = 16.5 cm; 29.5 g) under the same rock during a snorkeling-based field survey in the North Fork of White River, Ozark County, MO (precise locality coordinates withheld due to collection concern and conservation status). As soon as we lifted the rock, the larvae began to quickly retreat into the interstitial spaces between the gravel and cobble. Before they fully retreated, we were able to carefully extract them by lifting a few (≤ 5) pieces of gravel and cobble which completely revealed the larvae. Neither larvae had any visible abnormalities, which have commonly been observed in adult *C. a. bishopi* in recent decades (Wheeler et al., 2002; Hiler et al., 2005). We carefully replaced the disturbed gravel, cobble, and cover rock in their original locations and orientations. Hellbenders were released at the bottom edge of the rock so that they could move back under the rock on their own. We observed them until they moved back underneath the rock to ensure that they were not predated during this transition. This observation demonstrates that when disturbed, larval *C. a. bishopi* will actively retreat into interstitial spaces, suggesting this behaviour and habitat use are adaptations for avoiding predation. However, direct observations of such behaviour in response to a predatory species have not been published. The use of interstitial spaces by larval *C. a. bishopi* may make them particularly vulnerable to siltation and sedimentation. Siltation and sedimentation have been hypothesised as factors contributing to the decline of *C. a. bishopi* due to the degradation and reduction of habitat for both *C. a. bishopi* and their macroinvertebrate prey (Briggler et al., 2007).

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Reproductive biology of *Sylvirana nigrovittata* (Blyth, 1856) (Anura, Ranidae) from Kedah, Peninsular Malaysia

SHAHRIZA SHAHRUDIN

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
Email: shahriza20@yahoo.com

The medium-sized ranid frog *Sylvirana nigrovittata* has a total length of 55-60 mm (Berry, 1975). This riparian evergreen forest dwelling species can be recognised by its rounded snout, distinct tympanum, finger and toe tips dilated into small disks, disk of toe tips larger than finger, upper arm with dark-centred glandular swelling and a very broad dark brown stripe on both lateral sides (Berry, 1975). It is widely distributed, being found across Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Thailand and Vietnam, usually between 200-600 m asl (IUCN, 2015). In Peninsular Malaysia the species is mostly found in primary rainforests (Berry, 1975) and is known from Temenggor Forest (Kiew et al., 1995), Bukit Larut and Gunung Bubu (Grismer et al, 2010), and Bukit Perangin (Ibrahim et al., 2012).

On 19 March 2016, between 20:00 and 20:30, an axillary amplexed pair of *S. nigrovittata* was observed in a small rock pool at Lata Bayu, Baling, Kedah, Peninsular Malaysia (5º43'N, 100º48'E; <200 m asl) (Fig. 1). The rock pool was 65 x 25 cm across/5-20 cm deep and approximately 1 m from a waterfall (Fig. 2). It contained clear stagnant water, a sandy-gravel bottom and was filled with dead leaves and twigs. The pool was directly exposed to sunlight and there had no vegetation around it. The air temperature and humidity at the site were 24ºC and 64% respectively. Another six males of the same species performed advertisement calls locally, and were also detected around the area, confirming the area as a breeding resource.

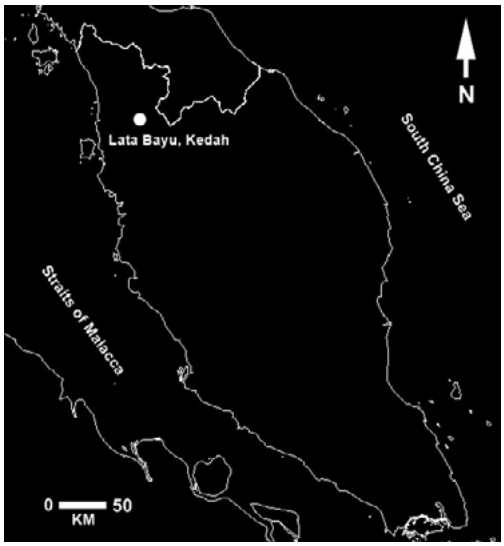

Figure 1. Map of Lata Bayu, Kedah, Peninsular Malaysia

Figure 2. Breeding pool of *S. nigrovittata*

Between 23:00-23:30, the female frog was observed depositing its eggs in the pool, whilst the male remained perched on a rock nearby (Fig. 3). Both specimens were captured and measured its snout-vent length (male=44 mm, female=56 mm), head width (male=14 mm, female=15 mm) and mass (male=6 g, female=10 g) using digital calliper and electronic balance. After measurement, both specimens were released back to their natural habitat. The clutch size comprised 553 eggs with diameter between 0.9 and 1.1 mm (*mean*=0.99 ± 0.057 mm, n=10) (Fig. 4). The eggs were rounded in shape, pigmented, black and white in colour, and enclosed by viscous jelly. The clutch was clumped in shape and deposited on the water surface in sunlight.

The egg clutch was collected and raised in a laboratory setting until hatching. Eggs and larvae were reared in a glass aquarium (60 cm x 30 cm x 30 cm), consisting of tap water, dead leaves and an aerator to supply oxygen. On 22 March 2016, around 10:00-11:00 (after approximately 59-60 hours), the eggs hatched into small tadpoles (Gosner's stage 19) that had a total length of about 3 mm. Only 455 (82.28%), out of 553 eggs were hatched and developed into tadpoles. Seven days later, the total length of the tadpoles were 5-6 mm (*mean*=5.4 ± 0.52 mm, n=10) (Gosner's stage 25). The tadpoles remained dark brown in colour, oval in

Figure 3. A calling male of *S. nigrovittata*

Figure 4. Egg clutch of *S. nigrovittata*

shape with a light tapering tail. The larval development phase followed Gosner (1960). All the tadpoles were released back to their natural habitat after measurements have been made.

Currently, 107 amphibian species inhabit the forests of Peninsular Malaysia but information and knowledge on their reproductive biology is poorly known. Previous data for Malaysia on breeding ecology of frogs has been documented for *Fejervarya limnocharis* and *Fejervarya cancrivora* (Ibrahim et al., 1999), *Chalcorana labialis* (Shahriza et al., 2010: 2016) and *Ingerophrynus parvus* (Shahriza et al., 2012: 2015). *Sylvirana nigrovittata* is a common frog but one currently considered in decline across its range (IUCN, 2015). The observations herein therefore add to the understanding of frog reproductive biology from Peninsular Malaysia.

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Mimophis mahfalensis (common big-eyed snake) predation on *Furcifer major* (giant carpet chameleon) in Isalo, Madagascar

GONÇALO M. ROSA[1,2,3*], JOÃO CASTELO BRANCO[3], HENRIQUE COUTO[3], PAOLO EUSEBIO BERGÒ4 & FRANCO ANDREONE[4]

[1]Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury CT2 7NR, Kent, UK
[2]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
[3]Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2, 1749-016 Lisbon, Portugal
[4]Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, 10123 Turin, Italy
[*]corresponding author Email: goncalo.m.rosa@gmail.com

Snakes are carnivorous and many are specialist feeders on a particular type of prey. Some of the prey may include other reptiles such as lizards and even other snakes (Bauchot, 1994; Mercurio et al., 2006). In Madagascar there are about 100 species of non-marine colubrids in which the Lamprophiidae family represents over 80 of the described species (Cadle, 2003; Laduke et al., 2010; Crottini et al., 2012). *Mimophis mahfalensis* (Grandidier, 1867), is an endemic colubrid with a widespread distribution in Madagascar (except on the east coast) (Glaw & Vences, 2007). The diet of this snake includes frogs, other snakes and lizards (Glaw & Vences, 2007; Jenkins et al., 2009).

During our ongoing research on the herpetofauna of the Isalo Massif, central-southern Madagascar, an adult individual of *M. mahfalensis* was observed preying on an adult male of *Furcifer major* (Brygoo, 1971) on 14 February 2011. The episode took place in Malaso, 22°35'29.28"S; 45°21'26.82"E (Fianarantsoa Province, Antsohy Fivondronona, Ranohira Firaisana).

When the team approached the animals (at 13:00), the snake was already wrapped tightly around the chameleon, which was still alive. For few minutes the prey was moving but struggling in failed attempts (Fig. 1A). After the lizard apparently abandoned attempts at resistance and showed no signs of life, the snake adjusted its prey in order to start swallowing from the head (Fig. 1B). Twenty minutes later the snake began swallowing the lizard head, first briefly pausing when reaching the front limbs (Fig. 1C). The rest of the swallowing process took about 40 mins (Fig. 1D), which made the total observation last over an hour since the animals were first spotted.

F. major is a common chameleon endemic to Madagascar (Raxworthy et al., 2003; Florio et al., 2012). As part of *F. lateralis* complex, it has also been reported as a prey for other snake species (e.g. *Ithycyphus ourci*; Domergue, 1986; Crottini et al., 2010). Although a not recorded previously as a prey species for *M. mahfalensis*, it is known to prey on other chameleons (e.g. *F. labordi*; Andriamandimbiarisoa, 2007). This opisthoglyphous snake (Rosa et al., 2014), uses a combination of mild envenomation and constriction to immobilise and kill its prey. As commented by Domergue (1989), *M. mahfalensis* seems to have a preference for saurians. Given how common both species are, particularly in the Isalo region, predation on *F. major* by *M. mahfalensis* is likely a frequent event, especially when *F. major* moves through open areas.

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Figure 1. Predation of the chameleon *F. major* by a snake, *M. mahfalensis* in Isalo Massif, central-southern Madagascar: **A**. snake seizing the chameleon; **B-C**. snake starting to swallow its prey by the head; **D**. snake unwrapped the chameleon and swallows in gentle motions. Photos by P. Eesebio Bergò.

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An "upside-down" juvenile slow-worm (*Anguis fragilis*): could this be a thermophilic behaviour?

ELLIOTT LEWIS HAILS[1*] & COLIN THOMAS STRINE[2]

[1]EAD Ecology, Exeter, U K.
[2]Suranee University of Technology, Nakhon Ratchasima, Thailand.
[*]Corresponding author Email: elhails01@gmail.com

During a routine reptile survey on 4 July 2016 at 12:45 hrs, we found two adult and five juvenile slow-worms *Anguis fragilis* motionless beneath an artificial refuge (roofing felt, 0.5 m^2). One of the juveniles was in a partially "upside-down" position. The posterior ventral and subcaudal scales were facing upwards, as shown in Fig. 1. The juvenile remained in this position for approximately 10 seconds before slowly retreating into adjacent vegetative cover. The observation was made within the district of Torquay, Devon, England (alt. 102 m) although the exact location has been withheld because of confidentiality issues. The habitat was classified as semi-improved neutral grassland with sections of ruderal vegetation, hard standing and semi-natural broadleaved woodland in the wider area (JNCC, 2010). The refuge had been approached slowly and quietly in an attempt to minimise disturbance to any reptiles in the area.

It is possible that the individual was flipped over in the motion of lifting the refuge. The roofing felt that is commonly used in reptile surveys can become adhesive in hot conditions. If the adhesive strength of this was strong enough to invert an individual, it seems plausible that this would be strong enough to restrain an individual, something that would present a problem to current UK practices. As the individual remained motionless for an extended period, it would appear to be undisturbed, so this is considered to be an unlikely explanation.

Slow-worms, like most reptiles, are ectothermic, requiring an external heat source to meet their temperature requirements. However slow-worms are most commonly found under refugia, rarely basking in exposed areas (Beebee and Griffiths, 2000). Under refugia they maintain body contact with the warm surface as a means of thermoregulation, making the use of artificial refugia (typically corrugated iron and roofing felt) a useful tool for surveying as they absorb and retain heat quickly (Reading 1996, Platenberg, 1999, Hubble & Hurst, 2006).

This thermoregulatory function of refugia provides a potential explanation for the inverted posture described here. A study of thermoregulation in melanistic *Vipera berus* found that the monitored individuals were able to heat faster than normally pigmented individuals (Forsman, 1995). The juvenile we observed had black ventral and subcaudal scales with a copper coloured dorsum, so possibly it might have been able to increase its temperature faster or maintain a high body temperature more effectively if these were positioned towards the external heat source.

Figure 1. An inverted juvenile and adult *A. fragilis*.

This hypothesis is sufficiently intriguing to warrant further investigation.

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Regarding Michaels (2016), published in Issue 137, the author regrets that the name of a correspondent was spelled incorrectly. 'H. Jansens' should in fact be spelled 'H. Janssen'. The authors apologises for any confusion or offense caused.

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