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# THE HERPETOLOGICAL BULLETIN

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**Front Cover:** A juvenile midwife toad from the Cambridge population shortly before it was captured and measured.  
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# A new species of blind snake, *Xerotyphlops*, from Iran

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**ABSTRACT** - A new species of blind snake is described from Lorestan Province, western Iran. This is a cryptic species close to the *Xerotyphlops vermicularis* complex. It is readily distinguished by hemipenal characters. When everted the right hemipenis is coiled and the left is curved, in *X. vermicularis* both hemipenes are straight.

## INTRODUCTION

Three genera and five species of Typhlopidae, are distributed in Iran: *Indotyphlops braminus* (Daudin, 1803), *Myriopholis blanfordi* (Boulenger, 1890), *M. macrorhyncha* (Jan, 1860), *Xerotyphlops vermicularis* (Merrem, 1820), and *X. wilsoni* (Wall, 1908) (Uetz and Hallermann, 2016). The genus *Xerotyphlops* Hedges, Marion, Lipp, Marin & Vidal, 2014 is easily distinguishable from other related genera of the subfamily *Asiatyphlopinae* by having segmented testes and 7-13 subcaudals, as well as other traits described by Pyron and Wallach (2014). *Xerotyphlops* comprises four species (*X. etheridgei*, *X. socotranus*, *X. vermicularis*, and *X. wilsoni*). In the present study a new cryptic species of *Xerotyphlops* is described and compared to other typhlopids snakes of Iran.

## MATERIAL AND METHODS

Six specimens of a new *Xerotyphlops* were collected during field trips in March and May 2016 to the western slope of the central Zagros Mountains in western Iran (Fig. 1). All six specimens are included in the type series. They are now all deposited in the herpetological collections of the Paris Natural History Museum (MNHN-RA; France).

The specimens were compared with other members of *Xerotyphlops* using the original species descriptions and other publications containing morphological accounts of these snakes (Wall, 1908; Wallach, 2009; Afroosheh et al., 2012; Hedges et al., 2014; Pyron and Wallach, 2014). Characters were selected to facilitate comparisons with data from Pyron and Wallach (2014). Measurements were taken using a dial caliper with 0.01 mm precision.

### Data collected included the following:

LSRab: longitudinal scale rows at anterior body; LSRmb: longitudinal scale rows at mid-body; LSRpb: longitudinal scale rows at posterior body; TSR: transverse scale rows at mid-body; SRR: scale row reduction (longitudinal or transverse); SC: subcaudals; LOA: total length (mm); W: mid-body diameter; TL: tail length; TW: mid-tail diameter; SIP: supralabial imbrication pattern; INS: inferior nasal



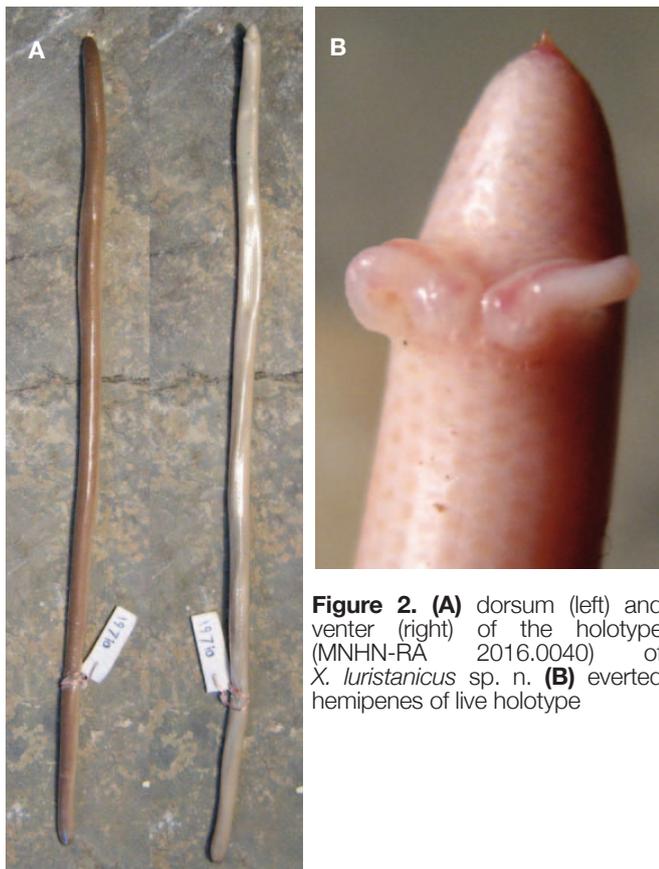
**Figure 1.** Distribution map of *X. luristanicus* sp. n. in western Iran (Badavar region, Nourabad, Lorestan Province)

suture contact with supralabials (1, 2), preocular (PO) or rostral (R); RW: mid-rostral width; HW: interocular head width; PO: postoculars; HP: everted hemipenes (S: straight; C: coiled); HPC: everted hemipenis coiled; TL/LOA: tail length/total length; TL/ TW: tail length/tail diameter; L/W: total length/mid-body diameter; RW/ HW: mid-rostral width/ interocular head width. For skull anatomy three specimens were examined (FTHM019700-02), the skull abbreviations were: P: parietal; F: frontal; N: nasal; SO: Supraoccipital; EO: Exoccipital; PO: Prootic; PM: Premaxilla; SM: Septomaxilla.

### *Xerotyphlops luristanicus* sp.n.

#### Holotype (Fig. 2)

Adult male, MNHN-RA 2016.0040 (former FTHM 19710), collected by Farhang Torki on 29 March 2016, 1,750-2,100 m a.s.l., on the western slope of the central Zagros Mountains, Badavar region, Nourabad, Lorestan Province, western Iran, 34°07' N, 47°53' E.



**Figure 2.** (A) dorsum (left) and venter (right) of the holotype (MNHN-RA 2016.0040) of *X. luristanicus* sp. n. (B) everted hemipenes of live holotype

### Paratypes

Five specimens: MNHN-RA 2016.0041-45 (respectively former FTHM 19711-15); 2 adult males: MNHN-RA 2016.0042 and 2016.0045 (respectively former FTHM 19712, 19715); 3 adult females: MNHN-RA 2016.0041, 2016.0043-44 (respectively former FTHM 19711, 13-14), collected by Farhang Torki on 18 May 2016; same data as for holotype.

### Diagnosis

A stout-bodied blind snake, small body size (maximum: 219 mm), moderate body form, short tail, rostral moderate. *X. luristanicus* sp. n. can easily be distinguished from all other *Xerotyphlops* species by the combination of the following characters: right side hemipenis in a single coil, left side curved, not straight; segmented testis; 22-24 longitudinal scales rows; scale reduction present or not; 355-398 transverse scale rows at mid-body; 10-12 subcaudals; supralabial imbrication pattern T-III; parietal is apparently fused as there is no suture in the middle of this bone on the external side, on the internal side a groove is observed in this place; exoccipital is completely separated from supraoccipital; the dorsal body has strong uniform pigmentation throughout while in contrast all ventral scales lack pigmentation.

### Description of holotype

Measurements (in mm): total length: 216; tail length: 5.01; mid-body diameter: 4.90; mid-tail diameter: 3.38; transverse scale rows at mid-body: 355; scale rows round the body: at proximal: 22, at mid-body 22, at distal 22.

Body more or less cylindrical; small tail; upper head flattened and smaller than anterior body (95%), but not

distinct from neck; eye diameter 0.31 mm, eyes dorsolateral, not visible in ventral view, clearly visible in dorsal view, only located on upper ocular scale. Rostral long, oval and surrounded by nasals (left + right), posteriorly in contact with prefrontal, width 1.2 mm, one third the head breadth, tip broadly round. Four supralabials, first is smaller than 2nd, and 2nd smaller than 3rd, and 4th is much larger than 3rd; three infralabials (IL), 1st IL larger than mental, one scale (larger than mental) between first IL, 3rd IL larger than 2nd IL. Ocular acute contacts 3rd and 4th supralabials; preocular in contact with 3rd supralabials. Prefrontal in contact with rostral and between nasals, prefrontal smaller than supraoculars (approximately 0.5x), width and length of prefrontal equal; frontal larger than prefrontal; parietal much wider than frontal; interparietal wider than frontal; parietal slightly wider than interparietal. Nostril anterolateral, oblique crescent-like slit, the nostril opening approximately same size as the eye, much nearer the tip of snout than eye, nasals not in contact and slightly distinct from rostral and prefrontal; anterior nasal in contact with 1st and 2nd supralabials; posterior nasal in contact with 2nd supralabial; inferior nasal suture in contact with 2nd supralabial. Twenty two scales around mid-body, 6 scales on dorsum, 14 on ventral and 2 lateral, dorsal scales strongly pigmented, in contrast, ventral scales are without any pigmentation, dorsolateral scales semi-pigmented; all ventral and dorsal body scales imbricate, dorsal scales uniform, ventral scales are uniform, dorsal scales smaller than ventral scales. Subcaudals mostly homogenous, one or two scales proximal and distal to subcaudals are smaller than mid-part; dorsal scales of subcaudal homogenous, last scales near spine are smaller. The testes are elongated, right is much longer and is composed of nine testicular units, it is placed more anteriorly than the left, which is composed of 10 units; right hemipenis coiled (one coil when everted), everted left hemipenis curved (not striated and not completely coiled).

Scale pigmentation of dorsal body (including head, dorsum, caudal, ventral of subcaudal) is similar being dark brownish; venter (including head, body, cloacal and subcaudals) is whitish; spine color is same as dorsal body.

### Variation and Dimorphism

All specimens have similar colour pattern. All specimens have supralabial imbrication pattern T-III, inferior nasal suture in contact with 2nd supralabials, and 2 postoculars. All male specimens have hemipenes similar to holotype (right is coiled and left is curved). See Table 1 for variation in measurements, scale counts, and various ratios.

All male specimens of *X. luristanicus* sp. n. have 22 longitudinal scales rows without scale reduction from anterior to posterior of body, this is true for one female (MNHN-RA 2016.0044) and is in contrast to two other females (Table 1). The mean number of transverse scales rows for females is greater than that for males (384 vs. 366). Males have longer tails than females (4.9 vs. 3.9) while females have wider tails (males: 3.29 vs. females: 3.45), the TL/TW ratio for males is greater than for females (1.5 vs. 1.1).

### Comparison with other species

*Xerotyphlops* includes four species and can be diagnosed by several combinations of characters described by Pyron and

**Table 1.** Morphological characters for holotype and paratype specimens of *X. luristanicus* sp. n. (for abbreviations see Material and Methods)

MNHN - RA code	Sex	LSRab	LSRmb	LSRpb	TSR	SC	LOA	W	TL	TW	RW	HW
2016.0040	M	22	22	22	355	12	216	4.9	5.01	3.38	1.20	3.58
2016.0041	F	24	24	23	398	12	219	4.26	3.07	3.38	1.10	3.16
<b>2016.0042</b>	M	22	22	22	366	11	186	4.06	4.89	2.99	1.22	3.04
2016.0043	F	24	23	22	388	11	203	4.89	3.88	3.29	1.32	3.34
2016.0044	F	22	22	22	368	12	198	4.74	4.80	3.68	1.26	3.13
2016.0045	M	22	22	22	377	12	198	4.25	4.81	3.51	1.14	2.97

Wallach (2014). *X. luristanicus* sp. n. has all the combined characters; it is clearly a new species of *Xerotyphlops*. Comparisons of *X. luristanicus* sp. n. with the published descriptions of other species (Wall, 1908; Wallach, 2009; Afroosheh et al., 2012; Hedges et al., 2014; Pyron and Wallach, 2014) are as follows.

Easily distinguishable from *X. etheridgei* by everted hemipenes (coiled vs. straight), scale reduction present (yes and no vs. only yes), TSR (355-398 vs. 424), TL/LOA (1.4-2.43 vs. 1.1), L/W (41-46 vs. 55).

Easily distinguished from *X. socotranus* by low longitudinal scale rows (22-24 vs. 26-30), SIP (T-III vs. T-V), scale reduction present (yes and no vs. only yes), TL/LOA (1.4-2.43 vs. 3.1).

Easily differentiated from *X. wilsoni* by ocular in contact with labials (vs. separated from labials by the subocular), small body size (total length: 186-219 vs. 338-345) as well as slender body size (L/W: 41-46 vs. 38), scale reduction present (yes and no vs. only yes).

Distinguished from *X. vermicularis* by coiled everted hemipenis (vs. straight).

Other slight differences between *X. luristanicus* sp. n. and *X. vermicularis* are as follows: subcaudals (11-12 vs. 7-13); TL/TW is different between studies (see Table 2 and 3) as follows: mean of TL/TW is greater for *X. luristanicus* sp. n. than *X. vermicularis* (1.31 vs. 1.18) and range of *X. luristanicus* sp. n. is close to *X. vermicularis* (Afroosheh et al., 2012), in contrast *X. luristanicus* sp. n. has greater variation in TL/TW than *X. vermicularis* (0.91-1.63 vs. 1.3-1.5) (Pyron and Wallach, 2014); mean of TL/LOA in *X. luristanicus* sp. n. is greater than *X. vermicularis* (0.02 vs. 0.01) (Afroosheh et al., 2012), or is slightly different (1.4-2.43 vs. 1.8-2.5) (Pyron and Wallach, 2014). More comparisons between *X. luristanicus* sp. n. and other *Xerotyphlops* as well *X. vermicularis* are shown in Tables 2 and 3.

There are several morphological differences between *X. luristanicus* sp. n. and *Indotyphlops braminus* (Daudin, 1803), which also inhabits Iran. In particular, *X. luristanicus* sp. n. has segmented testes (vs. unsegmented); the inferior nasal suture is in contact with 2nd supralabial (vs. preocular); LSR (22-24 vs. 20); SRR (both vs. neither); postocular (2 vs. 1); and, coloration of submental, tip of the tail and cloacal region in *X. luristanicus* sp. n. is white not yellowish (Wallach, 2009; Afroosheh et al., 2010; Pyron and Wallach, 2014).

Some differences between the skull bones of *X. luristanicus* sp. n., *X. vermicularis* and *I. braminus* were identified. In general, nasal, supraoccipital, and occipital



**Figure 3.** Habitat of *X. luristanicus* sp. n. at type locality, Badavar, Nourabad, Lorestan, Iran

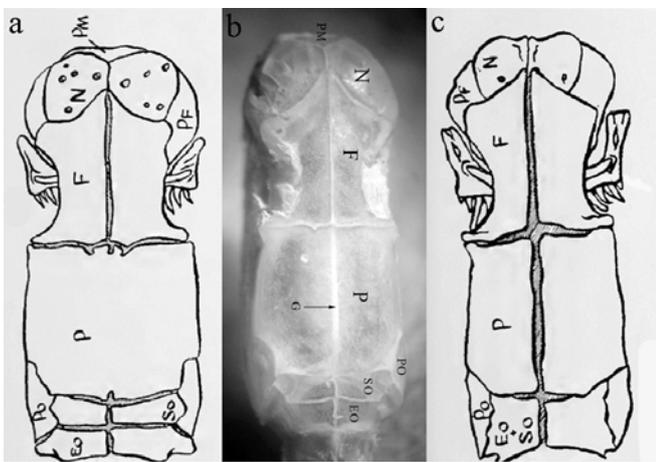
**Table 2.** Comparison between *X. luristanicus* sp. n. with *X. vermicularis* inhabitant Iran (data from Afroosheh et al., 2012); (for abbreviations see Materials and Methods)

Characters	<i>X. vermicularis</i>			<i>X. luristanicus</i> sp. n.		
	Min	Max	Mean	Min	Max	Mean
LSRab	22	24	23.2	22	24	22.6
<b>LSRmb</b>	21	24	22.8	22	24	22.5
LSRpb	20	24	21.7	22	23	22.1
TSR	340	438	389	355	398	375
SC	7	12	9.01	11	12	11.6
LOA	90	277	178	186	219	203
W	1.76	7.22	3.69	4.06	4.9	4.51
<b>TL</b>	0.60	5.75	3.01	3.07	5.01	4.41
TW	0.83	5.21	2.67	2.99	3.68	3.37
RW	0.78	1.79	1.14	1.1	1.32	1.2
HW	1.73	4.13	2.73	2.97	3.58	3.2
TL/LOA	0.01	0.03	0.01	0.01	0.02	0.02
LOA/W	32.7	85.1	49.4	41.5	51.4	45.2
TL/TW	0.5	2.15	1.18	0.91	1.63	1.31
RW/HW	0.27	0.57	0.42	0.33	0.4	0.37

are paired. The parietal is apparently fused as there is no suture in the middle of this bone on the external side. On the internal side a groove is observed in this place. This character is in contrast to what seen in the parietal of *X. vermicularis*, where it is single or quite unique and without groove, and in *I. braminus* it is paired or partially divided (Mookerjee and Das, 1932). According to fossil evidence the character of paired parietals is usually considered to be more primitive than the single fused homologue (List, 1966).

**Table 3.** Comparison of important characters among all species of *Xerotyphlops* (Pyron and Wallach, 2014) with *X. luristanicus* sp. n., (for abbreviations see Materials and Methods)

	<i>X. etheridgei</i>	<i>X. socotranus</i>	<i>X. wilsoni</i>	<i>X. vermicularis</i>	<i>X. luristanicus</i>
LSR	24	26-30	24	20-24	22-24
SRR	yes	yes	yes	both	both
TSR	424	370-435	-	346-410	355-398
SC	10	-	-	7-13	11-12
TL/LOA	1.1	3.1	-	1.8-2.5	1.4-2.43
LOA	220	200-255	338-345	92-405	186-219
TL/ TW	1	1	-	1.3-1.5	0.91-1.63
SIP	III	V	-	III	III
LOA/W	55	37-50	38	34-56	41-51
INS	2	2	2	2	2
PO	2	2-3	-	2	2
RW/ HW	0.41	0.30-0.35	-	0.27-0.41	0.33-0.40
HP	S	-	-	S	C (left)
HPC	-	-	-	-	1 (right)

**Figure 4.** Skull comparison of (a) *X. vermicularis* CNHM 28572 - Chicago Natural History Museum; specimens from Benyaminiya, Israel; (b) *X. luristanicus* sp. n. (c) *I. braminus* JCL 1022 - James C. List, private collection, specimens taken from Dadanduwa, Ceylon (for abbreviations see Material and Methods)

In *X. luristanicus* sp. n. this character shows an intermediate state since there is a groove in the mid-line of interior surface of the parietal. Moreover, in *I. braminus* the exoccipital is fused with supraoccipital (List, 1966), but in *X. vermicularis* and *X. luristanicus* sp. n. they are separate and paired. Also, in *I. braminus* the exoccipitals meet with each other behind the former (List, 1966), this contrasts with what is observed in *X. luristanicus* sp. n. and *X. vermicularis*.

According to List (1966) the type locality of *X. vermicularis* is near the Mediterranean Sea (Benyaminiya, Israel) which is close to Clade A of Kornilios et al. (2012) (which includes Syria and Jordan populations). Clade A shows a strong genetic divergence from other clades of *X. vermicularis*, and colonisation for this population occurred about 9.8 Mya. There appear to be no significant morphological differences between Clade A and other clades, but the current study indicates some skull differences (especially in the parietal) between the Benyaminiya and Lorestan populations (Fig. 4).

### Distribution

*X. luristanicus* sp. n. is at present known only from the type locality, Badavar region, Nourabad, Lorestan Province, western Zagros Mountains, western Iran.

### Etymology

“*luristanicus*” refers to Lorestan province (habitat of new species), “Laki Blind Snake” would be more appropriate as a common name for the new species.

### Habitat and ecology

The type locality is the valley of the Badavar River which is surrounded by mountains (Fig. 3). *X. luristanicus* sp. n. was found beneath stones from both the base and top of Chghasalman Mountain (1,750-2,100m a.s.l.). The new species is syntopic with the following reptiles: *Natrix tessellata*, *Platyceps najadum*, *Laudakia nupta*, *Ophisops elegans*, and *Trachylepis aurata*. Important threats to the new species (as well as other animals and vegetation) are as follows: (i) waste disposal, the area is a trash dump for Nourabad city and urban rubbish is deposited near the Badavar River; (ii) agrichemicals, much of the type locality is used by farmers and gardeners; (iii) burning of postharvest residues around mid-summer; and (iv) recreation, the area is used by city dwellers mainly at weekends.

## DISCUSSION

The present study shows that the external morphology of *X. luristanicus* sp. n. is similar to that of *X. vermicularis* (e.g., Wall, 1908; Wallach, 2009; Afroosheh et al., 2012, 2013; Pyron and Wallach, 2014), although there is a distinct difference in hemipenial morphology. The genetically distinct populations and wide distribution of *X. vermicularis* among completely different ecological and climatically areas from northern Africa, southern Europe towards the Middle East, indicate that *X. vermicularis* is a complex taxon and the current study suggests that *X. luristanicus* sp. n. is a cryptic species, close to the *X. vermicularis*-complex.

A genetic study of *X. vermicularis* inhabiting Iran shows the species split into two clades (Kornilios et al., 2012): clade E, that includes a population from Kermanshah (near

type locality of *X. luristanicus* sp. n.), and clade I in other areas including northeastern, southern and southwestern Iran. Based on geological maps, the type locality of *X. luristanicus* sp. n. is close to a population of *X. vermicularis* from Ravansar and Kermanshah (e.g., Wrobel-Daveau et al., 2010; Verges et al., 2011). Therefore, it is suggested that *X. luristanicus* sp. n. is close to clade E of Kornilios et al. (2012). Although statistical tests of taxonomical characters do not show differences between Iranian populations (including clade I and E), phylogenetic data indicate differences between the two clades; clade E diverged from clade I in 3.3 Mya (Kornilios et al., 2012). The western Zagros Mountains are considered as “hot spots” for reptile diversity. Owing to the geological history of these mountains there is an unusual number of narrowly endemic species in this region, many of which have been described only within the last two decades.

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# An evaluation of the diet of *Cyclura* iguanas in the Dominican Republic

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**ABSTRACT** - Understanding the diet of endangered species can benefit conservation efforts that involve habitat preservation and restoration. Caribbean rock iguanas, *Cyclura* spp., are among the most endangered lizards in the world, yet little is known about the diets of many of these species. In particular, the diets of the Rhinoceros rock iguana, *Cyclura cornuta*, and Ricord's rock iguana, *C. ricordii*, from the Dominican Republic, are poorly understood. Here we present diet information from scat analyses of these two threatened endemic species. We report on a variety of new dietary items and identify seasonal core plant species (*Consolea moniliformis*, *Stenocereus hystrix*, *Ximeniopsis horridus*, *Capparis flexuosa* and *Acacia* sp.). *Cyclura* from the Dominican Republic are generalist herbivores, although animal matter is consumed at times and preference for particular vegetation exists. Variation in diet likely exists between the two species and is discussed. The information presented here advances our understanding of the ecology of these two species and will aid in management decisions and activities.

## INTRODUCTION

Within reptiles, iguanas of the subfamily Iguaninae are known for their unique herbivorous feeding strategy (Iverson, 1982). While most reptiles have adopted a more omnivorous strategy, many iguana species are known to specialise on fruits, seeds, and leaves, making them “truly herbivorous” (Alberts, 2004; Iverson, 1982). Indeed these herbivorous lizards have a distinct enlarged colon that is likely related to this ecologically important life strategy (Iverson, 1982). Herbivory plays a key role in plant growth and reproduction, aiding in seed dispersal and germination. Thus, this feeding strategy is essential to maintaining and perpetuating healthy ecosystems (Hartley et al., 2000).

Understanding the diet and feeding behaviour of herbivorous lizards, such as iguanas, will not only improve our understanding of their ecological role in a given environment, but can also provide useful information for conservation planning. Given that iguanas are some of the most endangered lizards in the world (ITWG, 2016), dietary information is vital to management efforts focused on the restoration of current and future habitats, as well as translocation strategies, as these data will ensure that the proper food sources can be made available. Restoration and translocation are both common approaches explored in iguana conservation programs (Knapp & Hudson, 2004).

To date, detailed information regarding the diet of many iguana species, such as those endemic to Hispaniola, is unavailable. Hispaniola is unique in that it is the only Caribbean island where two native species of *Cyclura* co-occur: *Cyclura cornuta* (Bonaterre) (Rhinoceros iguana) and *C. ricordii* (Duméril & Bibron) (Ricord's iguana). The broadly sympatric distribution of *C. cornuta* and *C. ricordii* likely arose when Hispaniola was formed through the joining of North and South Paleoislands (Banbury & Ramos, 2005). *C. cornuta* occurs throughout Hispaniola; however, many populations seem to be dwindling (Powell

et al., 2000; SAP pers. obs.). *C. ricordii* is restricted to four populations: three in southwestern Dominican Republic (Los Olivares, Lago Enriquillo and Isla Cabritos) and one in southeastern Haiti (Alberts, 2000). *C. cornuta* is categorised as Vulnerable under IUCN Red List criteria and *C. ricordii* is listed as Critically Endangered (Ottenwalder, 1996 a, b). The introduction of exotic mammals, illegal pet trade, human consumption, and most prominently, habitat alteration and degradation are the main threats to these iguanas (Ottenwalder 1996 a, b).

*Cyclura* spp. are thought to be generally herbivorous; however, precise feeding strategies may differ depending on food availability and preference. Previous studies of *Cyclura* on Hispaniola have produced limited observations of diet (reviewed by Iverson, 1979). Herein we evaluate the diet of *C. cornuta* and *C. ricordii* through an in-depth scat analysis, in an effort to characterise the diet and address the aforementioned factors affecting feeding behaviour. Specifically we aimed to: (1) qualitatively and quantitatively describe the diet of *Cyclura* in the study area and (2) determine if preference for certain plant species occurs by comparing the resources used to those available during the study period.

## FIELD-SITE DESCRIPTION

We conducted our study in the southwest portion of the Dominican Republic, in the province of Pedernales, where *C. cornuta* and *C. ricordii* are found in both sympatry and allopatry. Our three focal study sites are within, or border, Jaragua National Park (17° 47' 22" N and 71° 29' 56" W). Limestone terraces, with deep soil depressions (fondos) interspersed, characterise this region (Rupp et al., 2008). Iguanas use the fondos year round but they concentrate their activity there during the nesting season (*C. ricordii* from mid-March to early June and *C. cornuta* from mid-September to mid-October; Rupp et al., 2008), and thus

our capture success was relatively high during those time periods. We focused our efforts on Sites A (47 ha) and B (10 ha) with 1.5 km distance apart and opportunistically visited a third site, Site C, but were unable to conduct the same level of research due to time constraints. Site A was a known location for *C. ricordii*, but may have supported *C. cornuta* around the periphery, whereas Site B was a well-known sympatric location. Site C only supported *C. cornuta*. Both Sites A and B were impacted by livestock grazing, as well as feral dogs, cats, and mongoose. Plant species such as Cayuco (*Stenocereus hystrix*), Alpagata (*Consolea moniliformis*), Bayahonda (*Prosopis juliflora*), and Guasabara (*Cylindropuntia caribea*) were common in the fondo areas of Site A and B. In the limestone terraces surrounding the fondos of Sites A and B there was a greater diversity of plants and in general open canopy vegetation (see Arias et al., 2004). Site C was characterised by a high abundance of Acacia trees and heavily impacted by a nearby highway.

## MATERIALS AND METHODS

### Data collection

We obtained dietary data from scats that we collected during the summer of 2015 (June, July and August). We walked two loops of approximately 1.5 – 2 km each within Sites A and B, opportunistically collecting scat samples from the ground throughout the study period. We also collected limited scat samples along the paths leading to Sites A and B. On two separate occasions in July we visited Site C and opportunistically collected scat samples over the course of one hour. We verified that all scat samples were from iguanas by checking for the presence of urea, the absence of hair, and noting the level of digestion. We collected scats that were dry but deposited recently (1-2 days). We noted the site, date, and GPS coordinates whenever possible. In many instances it was not possible to definitively determine which species of iguana the scat belonged to while using this opportunistic collecting method. This is most relevant to the sympatric Site B, but should also be considered for Site A as the level of allopatry is not entirely understood. However, no *C. cornuta* were captured at Site A over the four year course (2012-2015) of study by SAP.

To supplement scat samples from individuals of unknown origin, we simultaneously carried out trapping at Sites A and B for the last 25 days of our field season in order to obtain scats from known individuals. We used Tomahawk cage-traps baited with mango and sardines, and we hid the traps within the vegetation. We checked and re-baited traps daily. Upon capture of an iguana, we collected scat samples from the traps or maintained iguanas in a cloth bag to allow scat deposition. For all scats collected we classified and counted each leaf, seed, and fruit present, as well as other elements that appeared, such as vertebrate parts, shells, arthropods and human-made materials. We took into account both full-bodied individuals and arthropod parts when counting arthropod individuals, but carefully accounting for possible oversampling of different arthropod parts. We refer to these total counts as the “abundance of a given item” both within and across all scats.

During scat collection, we simultaneously collected and classified the leaves, fruits, and seeds from the majority of plant species in Sites A and B to aid in the identification of plant material from scats. We assessed the

plant availability in Sites A and B through an evaluation of 20 randomly selected vegetation plots (10 in each site) of 10 m<sup>2</sup> each, within the fondos and a buffer of 100 m<sup>2</sup>. Our design included both typical fondo habitat and the rocky limestone area surrounding the fondo in order to encompass the likely foraging grounds for these iguanas. Within the plots, we identified all plant species and recorded their abundance. We did not collect grass samples because we were unable to identify them confidently to the species level and because grasses were never observed within the scat samples.

### Data analysis

For each fully intact scat collected, we recorded total mass to the nearest 0.1 g with an electric balance before deconstructing the scat for examination. The use of scat examination as a method to determine the diet of iguanas has been commonly used and is a widely accepted noninvasive method (e.g., Beovides-Casas & Mancina, 2006; Hines, 2016; Perera, 1985). In our study system, this method was preferable over stomach flushing and post mortem examination as *C. cornuta* and *C. ricordii* are threatened species. After sorting materials from an individual scat sample, we determined the ratio of the mass of identifiable items to total mass of the scat. We ranked the elements that were present in the scats according to their frequency across scat samples as we felt this most accurately reflected foraging (or used) events. For comparison, we ranked the plant species found in the vegetation quadrats by abundance. Based on frequency of occurrence in scats (used values) and abundance in nature (available values), we determined selection preference for the plants following Manly’s selection ratios for design I, using a conservative approach (Manly et al., 2004). We chose the conservative approach, as the available dietary items were assessed from a sample set of plots and not from surveying the entire area of study.

We determined the core plants in the diet during the study season by considering the abundance and frequency of occurrence of each taxon in the scats. We tested for differences in abundance of the core plant species and arthropods between Sites A and Site B using t-tests and Mann-Whitney U tests. We used R package adehabitatHS for the Manly’s Selection Ratio analysis (Calenge, 2006), and the statistical program SPSS 20 IBM for all other analyses with an alpha level of 0.05.

## RESULTS

We opportunistically collected 158 scat samples across the three study locations (69 from Site A, 69 from Site B and 20 from Site C) and 10 additional scats by the trapping method (5 from *C. cornuta* and 5 from *C. ricordii*), to qualitatively describe the overall diet of the *Cyclura* in this region (Table 1). No *C. cornuta* were captured at Site A. To avoid pseudoreplication that would result from the inclusion of multiple scats from the same individual over time, we used only a single scat from a given location within 5 meters for all quantitative analyses, which resulted in a reduce sample set (see below for exact numbers for each analysis). Samples from Site C were also excluded from quantitative analyses due to a lack of location information and thus possible pseudo-replication.

From the 168 scats collected, we recovered 21 plant taxa, as well as arthropods, vertebrates, shells, and

**Table 1.** Dietary elements resulting from 168 *C. cornuta* and *C. ricordii* scats from study sites A, B, and C in the province of Pedernales, southwest Dominican Republic. We report the: abundance of various plant items in the scat, broken down into leaves, seeds, and fruits, RFO (ranked frequency of occurrence across scats [actual frequency]), vegetation abundance rank (VAR) of the 21 plant taxa (total number of individuals), and species specific consumption. All calculations were based on 93 samples (83 opportunistically collected and 10 from the traps) collected in Sites A and B. Superscripts indicate sources identified in the footnote.

Diet elements	Abundance	% Leaves	% Seeds	% Fruits	RFO (frequency)	VAR (total number of individuals)	Known consumption by species
<i>Consolea moniliformis</i>	1675	0	93.1	6.9	1 (51)	2 (646)	<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Stenocereus hystrix</i>	436	0	96.6	3.4	2 (38)	5 (323)	<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Capparis flexuosa</i> <sup>+,1,4,5,7</sup>	179	60.5	26.5	13	3 (31)	18 (53)	<i>C. cornuta</i>
<i>Ximeniopsis horridus</i>	171	10.2	89.8	0	4 (30)	33 (10)	<i>C. cornuta</i> ; <i>C. ricordii</i>
Arthropods	55				5 (28)		<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Cordia salvifolia</i> •, <sup>1,5,6</sup>	70	4.2	68.1	27.8	6 (21)	16 (68)	<i>C. cornuta</i>
<i>Acacia</i> sp. <sup>+,1,5,6,7</sup>	156	2.3	78.1	19.6	7 (16)	26 (22)	<i>C. cornuta</i>
<i>Croton discolor</i> <sup>+,1,3,5,6</sup>	27	44.5	0	54.5	8 (15)	7 (248)	<i>C. cornuta</i>
<i>Capparis ferruginea</i> <sup>+,1,4,5</sup>	25	24	68	8	9 (13)	25 (24)	<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Guaiacum</i> sp. <sup>+,1,3,7</sup>	23	14.3	85.7	0	10 (11)	20 (50)	<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Prosopis juliflora</i>	76	7.9	92.1	0	11 (10)	9 (165)	<i>C. ricordii</i>
<i>Cameraria linearifolia</i>	22	100	0	0	12 (6)	28 (19)	<i>C. ricordii</i>
Vertebrates	7				12 (6)		<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Eugenia</i> sp. <sup>+,1,3,5,6,7</sup>	5	100	0	0	13 (5)	31 (12)	<i>C. cornuta</i> ; <i>C. ricordii</i>
<i>Bursera simaruba</i> <sup>+,3</sup>	8	0	0	100	14 (4)		
<i>Lantana</i> sp. <sup>+,1,4,5,6</sup>	3	0	0	100	15 (3)	1 (648)	<i>C. cornuta</i> ; <i>C. ricordii</i>
Shells	8				16 (2)		
<i>Opuntia</i> sp. <sup>+,1,4,5,6,7</sup>	3	0	0	100	17 (1)	19 (52)	
<i>Harrisia nashii</i> •, <sup>1,4</sup>	3	0	100	0	17 (1)	32 (11)	<i>C. cornuta</i>
<i>Colubrina elliptica</i>	1	0	0	100	17 (1)	24 (29)	
Man-made material	1				17 (1)		
<i>Phyllostylon rhamnoides</i> *						8 (209)	
<i>Melochia tomentosa</i> *						15 (74)	
<i>Hippomane horrida</i> <sup>+,1,5,6</sup>						34 (8)	
<i>Hippomane mancinella</i> •, <sup>1,5,6</sup>							
<i>Cissus trifoliata</i> <sup>+,4,5</sup>						10 (162)	
Iguana skin*							
<i>Ziziphus rignoni</i> <sup>?</sup>							

\*Additional species found in scat samples only used in qualitative assessments

+Species already reported in other *Cyclura* sp.

• Congeners already reported in other *Cyclura* sp.

<sup>1</sup>Iverson, 1979; <sup>2</sup>Hartley et al., 2000; <sup>3</sup>Auffenberg, 1982; <sup>4</sup>Carey, 1975; <sup>5</sup>Lemm et al., 2010; <sup>6</sup>Lemm & Alberts, 2012; <sup>7</sup>Hines, 2016

man-made materials (Table 1). On average 29.77 % (0.15% - 98.8%) of the items in the scats were identifiable. Of the 21 plant taxa identified, we considered *Consolea moniliformis*, *Stenocereus hystrix*, *Capparis flexuosa*, *Ximeniopsis horridus* and *Acacia* sp. as the core diet of the iguanas (Table 1) because the abundances of other plants were discernably less (Table 1). Overall seeds were the most commonly found element from the plants, which is to be expected as they are likely the most difficult to digest (Table 1).

Manly's selection ratios demonstrated that *Cyclura* in this area have a significant preference for four plant taxa (*Capparis* sp., *Ximeniopsis horridus*, *Acacia* sp. and *Cordia salvifolia*) as the occurrence in the scats was significantly greater than expected given the availability of those plants in the environment (Table 2). Six plant taxa (*Lantana* sp., *Opuntia* sp., *Phyllostylon rhamnoides*, *Melochia tomentosa*, *Hippomane horrida*, *Cissus trifoliata*) were shown to be avoided by *Cyclura* using this analysis. In fact, the latter four plant species were never observed in *Cyclura* scats (Table 2) despite they are commonly present in the study sites. We collected and classified 56 plant species

during our floral surveys, 32 of which were reproductive (flowering, fruiting or both; Table 3).

We used 79 samples (42 from site A and 37 from site B) in regression analyses of location and richness or abundance. Scats collected from the trapping method were excluded due to their deconstructed state. Plant species richness was not dependent on location ( $F=0.41$ ;  $P=0.839$ ;  $d.f.=78$ ). Abundance of *Capparis flexuosa* was significantly greater in scats from Site B ( $t$  value=2.790;  $P=0.007$ ;  $df=77$ ), whereas *Ximeniopsis horridus* was more abundant in scats from Site A ( $t$ -value=-4.647;  $P=0.001$ ;  $df=77$ ). These patterns were not simply a reflection of plant abundance across sites as neither *Capparis flexuosa* (Mann-Whitney  $U=37.5$ ;  $P=0.328$ ) nor *Ximeniopsis horridus* (Mann-Whitney  $U=48$ ;  $P=0.829$ ) showed significant differences in vegetation abundance between sites.

Arthropod abundance was significantly increased in the scats from Site A compared to Site B ( $t$ -value=-3.054;  $P=0.003$ ;  $df=77$ ). The identifiable arthropods were mainly beetles (11) and ticks (5), and to a lesser extent flies and ants. We also observed hatchling iguana parts, an adult iguana jaw, and a bird bone in the scat samples.

## DISCUSSION

Understanding the dietary requirement of a species allows for a better understanding of the role that species plays in its environment. In addition, this information aids in conservation and management decisions focused on habitat restoration and translocation, as these data will ensure that the appropriate dietary items are provided in the new or restored habitat. In light of the increasing threats to these iguanas and on-going plans to restore their habitat, dietary information for *Cyclura* in the Dominican Republic is necessary in order to make informed management decisions.

Of the 21 plant taxa discovered in the diet of *Cyclura* across Sites A and B, several (or their congeners) have been previously reported in the diets of other *Cyclura* species (Table 1). Plant endemism can often explain the pattern of differing congeneric plant species consumed by *Cyclura* on different islands, as closely related congeneric plant species likely serve similar ecological roles. However, many plants species prominently identified in this study (*C. moniliformis*, *X. horridus*, *S. hystrix*, *P. juliflora*, *P. rhamnoides*, *C. elliptica* and *M. tomentosa*) have not been previously recorded from other *Cyclura* (Hines, 2016), though they occur sympatrically with other *Cyclura* species.

Dietary species richness varies greatly across Rock iguanas species. *C. lewisi* is reported to consume 105 different plant species (Burton, 2011), 54 plant species are reported for *C. carinata* (Iverson, 1979), and 54 plant species for *C. cyclura* (Hines, 2016). Other *Cyclura* species have fewer dietary species reported, such as *C. collei* (13 species) and *C. riley* (8 species); however, these studies were not exhaustive (Carey, 1975; Cyril, 2001; Hayes et al., 2004; Iverson, 1979; Vogel, 2000). With plant 21 species reported herein, the dietary richness of *Cyclura* in the Dominican Republic is comparatively low. However, we are likely underrepresenting the overall diet of these species since we did not examine all seasons or the full geographic range of the two species. Seasonal differences in diet have been noted for other species of *Cyclura* (Auffenberg, 1982; Hines, 2016; Iverson, 1979), and were also observed during the short time frame of this study. For example, it was not until the end of the field season that *Lantana* spp. and *Harrisia nashii* began fruiting, and only then, were first present in the scats. In addition, differences in digestibility between species could be a factor in observed dietary richness. Hines (2016) was able to identify 94% of the dietary items in *C. cyclura*. However, we were only able to identify on average 30% of the fecal material, the remainder of which was an unidentifiable powdery substance.

Despite the dietary richness recorded, we observed a seasonal core diet of five floral species in our study: *Consolea moniliformis*, *Stenocereus hystrix*, *Capparis flexuosa*, *Ximeniopsis horridus* and *Acacia* sp. The presence of a core diet has been shown previously in *Cyclura* (Auffenberg, 1982; Hines, 2016; Wiewandt, 1977). *C. moniliformis* and *S. hystrix* were common in both the vegetative surveys and the scat sampling, demonstrating generalised foraging by iguanas on these species (Table 2). However, iguanas demonstrated preferential foraging for *X. horridus* and *C. flexuosa*, as these plant species were selected more than expected given their availability in nature (Table 2). Iguanas also showed a significant

preference for *Acacia* sp. (Table 2); however, given the high abundance in habitat and relatively low frequency of *Acacia* sp. in the scats, this pattern may be indicative of individual preference rather than species level preference (Table 1). *Cordia salvifolia* and *Capparis ferruginea* were also significantly preferred by *Cyclura* but these were not included in the core diet due to their relatively low abundance in the scats (Tables 1 and 2); however, they should be considered in management planning.

All of the aforementioned preferred plant species were reproductive at the time of this study (Table 3). As previously mentioned *Lantana* spp. did not begin to fruit until the end of the study, which may account for the observed “avoidance” of this species. Likewise, *Hippomane horrida* and *Cissus trifoliata* were not reproductive during the time of the study, which likely accounts for the lack of observed consumption of these species. However, *Phyllostylon rhamnoides* and *Melochia tomentosa* were not preferably consumed by *Cyclura* even though they were reproductive. These two plants are not known to be toxic; however, the specific characteristics of their fruits may have made them less attractive food items. The fruit of *Melochia tomentosa* is extremely small and the fruit of *Phyllostylon rhamnoides* is not fleshy as it is a wind-dispersed plant. Although *Opuntia* sp. have been noted in the diets of various *Cyclura* sp. (Table 1; see also Hines, 2016) and these cacti were reproductive at the time of the study, we only observed consumption by one individual iguana.

We could not elucidate species-specific diet patterns for iguanas given our opportunistic sampling strategy, the small trapping sample size, and the undetermined range boundaries of *C. cornuta*. However, *C. cornuta* was not present in Site A based on four years of trapping effort in the area (2012-2015). Therefore, we feel that a cautious mention of potential species differences is warranted. *Ximeniopsis horridus* was more abundant in the scats found in Site A although it was equally available across sites, which may indicate that *C. ricordii* has a distinct preference for *X. horridus*, more so than *C. cornuta*. Likewise, arthropods were more abundant in the scats found in Site A than Site B, which may indicate that *C. ricordii* consumes more insect material than *C. cornuta*. Given the proximity and similarity of Site A and B, it is unlikely that this is simply a reflection of differences in arthropod availability. The presence of arthropods in the diet has been commonly reported for other *Cyclura* species (Burton, 2011; Cyril, 2001; Hines, 2016) and specifically in *C. cornuta* and *C. ricordii* at the National Zoo in Santo Domingo (Perera, 1985). Although arthropod parts (beetles, ticks, flies) were present in 30% of the scats, other animal parts (shells, iguana parts, bird parts) were also present to a lesser extent.

Reptile parts (including iguana) have been reported previously in the diets of iguanas (Hines, 2016; Iverson, 1979; Lemm & Alberts, 2012; Murphy, 1969). We encountered shed skin as well as hatchling scales and claws of *C. ricordii*, and lower jaw of a large adult *C. cornuta*. *Cyclura* is known to be mainly herbivorous (Iverson, 1979) and some authors suggest that arthropod consumption is accidental (Auffenberg, 1982; Perera, 1985; Wiewandt, 1977); however, given the abundance of arthropods and the presence of other vertebrate parts in the scats assessed herein we feel the consumption of animal matter is intentional as reported previously by Hines

**Table 2.** Dietary preference resulting from Manly Selection ratios design I within a conservative approach in Pedernales, southwest Dominican Republic. We report: Used plants (% frequency of occurrence in scats); available plants (% occurrence of plants in nature); Wi preference values; and p-values. All calculations were based on 93 samples (83 opportunistically collected and 10 from the traps) collected in Sites A and B. Bold p-values reflect those that are significant. Species highlighted in bold are preferred, while those underlined are avoided.

Species	Used plants (% freq. scats)	Available plants (% nature)	Wi	P-value
<i>Consolea moniliformis</i>	0.202	0.228	0.887	0.325
<i>Stenocereus hystrix</i>	0.151	0.114	1.320	0.126
<b><i>Capparis flexuosa</i></b>	0.123	0.019	6.568	<b>0.000</b>
<b><i>Ximenopsis horridus</i></b>	0.119	0.004	33.765	<b>0.007</b>
<b><i>Acacia</i> sp.</b>	0.063	0.008	8.173	<b>0.006</b>
<i>Prosopis juliflora</i>	0.040	0.058	0.684	0.147
<b><i>Cordia salvifolia</i></b>	0.083	0.024	3.474	<b>0.003</b>
<i>Croton discolor</i>	0.059	0.088	0.679	0.066
<b><i>Capparis ferruginea</i></b>	0.048	0.008	5.619	<b>0.018</b>
<i>Guaiacum</i> sp.	0.044	0.018	2.467	0.069
<i>Cameraria linearifolia</i>	0.024	0.007	3.576	0.119
<i>Eugenia</i> sp.	0.020	0.004	4.704	0.135
<u><i>Lantana</i> sp.</u>	0.012	0.229	0.052	<b>0.000</b>
<u><i>Opuntia</i> sp.</u>	0.004	0.018	0.221	<b>0.000</b>
<i>Harrisia hashii</i>	0.004	0.004	1.045	0.966
<i>Collubrina elliptica</i>	0.004	0.010	0.397	0.129
<u><i>Phyllostylon rhamnoides</i></u>	0.000	0.074	0.000	<b>0.000</b>
<u><i>Melochia tomentosa</i></u>	0.000	0.026	0.000	<b>0.000</b>
<u><i>Hippomane horrida</i></u>	0.000	0.003	0.000	<b>0.000</b>
<u><i>Cissus trifoliata</i></u>	0.000	0.057	0.000	<b>0.000</b>

(2016). Furthermore, it is likely that we underestimated the consumption of insects by assessing only scat rather than gut contents (Iverson, 1979). For example, Wiewandt (1977) reported the presence of *Pseudosphinx tetrio* larvae (a moth that is host specific to *Plumeria obtusa*) in the diet of *Cyclura* on nearby Mona Island. These larvae were very abundant on the *Plumeria obtusa* in our study sites; however, we did not discover them in our scat samples, likely because their soft bodies were fully digested.

We characterised the diet of *Cyclura* in the SW of DR during the most vital time of the year, nesting and hatching season. However, understanding how resources are being partitioned between these sympatric iguana species needs further investigation. In addition, future research should include all seasons and account for differences in seed, leave, and fruit size, as well as nutritional value, in order to obtain a better understanding of the preferences that these iguanas have for certain plant taxa and for their parts. Furthermore, addressing a more rigorous assessment of food availability would benefit further studies. Managers should consider the plant species that shape the core diet and those given preference as vital to maintain in the range of these iguana species and when contemplating reintroduction sites. Likewise, these plant species should be considered of upmost importance in on-going and future habitat restoration projects aimed at the conservation of these iguanas.

This study presents the first step in understanding the role that these Rock iguanas play in the tropical dry forest ecosystem of the Dominican Republic. Iguanas are known to be important seed dispersers in the areas in which they live (Benítez-Malvido et al., 2003; Hartley, 2000), aiding in maintenance and perpetuation of native plant communities. Two core dietary species, *Capparis* sp. and *Consolea moniliformis*, are known to play a key role in this dry forest ecosystem (García-Fuentes et al., 2015), and

thus these iguanas may be important seed dispersers for these species. Targeting management efforts towards these iguanas as flagships for conservation will aid in preserving the dynamics of this unique ecosystem.

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**Table 3.** Plant species identified from Sites A and B in the province of Pedernales, southwest Dominican Republic (DR). DR Red List status includes: VU (Vulnerable), EN (Endangered), CE (Critically Endangered). Vegetation abundance was ranked (VAR) from the most abundant to the least by considering the total number of individuals (Total Num. Ind.) observed. Species in bold represent the core diet.

Family	Species	Common name	DR Red List Status	Reproductive stage	VAR (Total Num. Ind.)
Apocynaceae	<i>Cameraria linearifolia</i>		Endemic	Not recorded	28 (19)
Apocynaceae	<i>Pentalinon luteum</i>	Ahogavaca	Native	Flower	20 (50)
Apocynaceae	<i>Plumeria obtusa</i>	Aleli	Native	Flower, Fruit	22 (33)
Asparagaceae	<i>Agave antillarum</i>	Magüey	Endemic (EN)	Flower	
Bignoniaceae	<i>Jacaranda ekmanii</i>	Abey	Endemic (CE)	Fruit	
Boraginaceae	<i>Cordia salvifolia</i>		Endemic	Flower, Fruit	16 (68)
Boraginaceae	<i>Heliotropium angiospermum</i>	Alacrancillo	Native	Not recorded	5 (323)
Burseraceae	<i>Bursera simaruba</i>	Almacigo	Native	Fruit	
Cactaceae	<b><i>Consolea moniliformis</i></b>	Alpargata	Native	Flower, Fruit	2 (646)
Cactaceae	<i>Cyindropuntia Caribaea</i>	Guazabara	Native	Fruit	6 (251)
Cactaceae	<i>Dendrocereus undulosus</i>	Cagüey	Endemic (EN)	Not recorded	
Cactaceae	<i>Harrisia divaricata</i>			Fruit	21 (34)
Cactaceae	<i>Harrisia nashii</i>		Endemic	Flower, Fruit	32 (11)
Cactaceae	<i>Mammillaria prolifera</i>	Bombilito	Native (VU)	Not recorded	11 (129)
Cactaceae	<i>Melocactus intortus</i>	Melon espinoso	Endemic (EN)	Flower, Fruit	14 (76)
Cactaceae	<i>Opuntia dillenii</i>	Tuna brava	Native	Flower, Fruit	19 (52)
Cactaceae	<i>Opuntia</i> sp.			Flower	19 (52)
Cactaceae	<i>Pilosocereus polygonus</i>		Native	Fruit	28 (19)
Cactaceae	<b><i>Stenocereus hystrix</i></b>	Cayuco	Endemic	Flower, Fruit	5 (323)
Caesalpiniaceae	<i>Caesalpinia ciliata</i>		Native	Fruit	
Caesalpiniaceae	<i>Caesalpinia sphaerosperma</i>		Endemic (VU)	Fruit	39 (1)
Caesalpiniaceae	<i>Senna atomaria</i>	Palo de chivo	Native	Flower	11 (129)
Caesalpiniaceae	<i>Senna uniflora</i>			Not recorded	38 (2)
Capparaceae	<i>Capparis cyphallanophora</i>		Native	Fruit	39 (1)
Capparaceae	<i>Capparis ferruginea</i>	Olivo	Native	Flower, Fruit	25 (24)
Capparaceae	<b><i>Capparis flexuosa</i></b>	Mostazo	Native	Fruit	18 (53)
Commeliaceae	<i>Commelina erecta</i>		Native	Not recorded	17 (67)
Euphorbiaceae	<i>Croton discolor</i>		Native	Flower	7 (248)
Euphorbiaceae	<i>Croton polytomus</i>		Endemic	Flower	3 (638)
Euphorbiaceae	<i>Hippomane horrida</i>		Endemic	Not recorded	34 (8)
Euphorbiaceae	<i>Jatropha gossypifolia</i>	Tuatua	Introduced	Flower	23 (31)
Fabaceae	<i>Galactia</i> sp.			Not recorded	13 (78)
Flacourtiaceae	<i>Samyda dodencandra</i>		Native	Flower	37 (3)
Leguminosae	<b><i>Acacia macracantha</i></b>		Native	Flower	26 (22)
Leguminosae	<b><i>Acacia skleroxylla</i></b>	Candelón	Endemic	Fruit	37 (3)
Leguminosae	<i>Coursetia caribaea</i>	Roblecillo		Flower, Fruit	36 (5)
Leguminosae	<i>Prosopis juliflora</i>	Bayahonda	Native	Flower, Fruit	9 (165)
Malvaceae	<i>Abutilon umbellatum</i>		Native	Flower	30 (16)
Malvaceae	<i>Corchorus hirsutus</i>	Tremolina	Native	Flower	12 (95)
Malvaceae	<i>Hibiscus brasiliensis</i>			Flower	
Meliaceae	<i>Trichilia</i> sp.	Guau		Not recorded	33 (10)
Mimosaceae	<i>Calliandra pedicellata</i>		Native	Not recorded	36 (5)
Myrtaceae	<i>Eugenia pitrensis</i>		Native (VU)	Not recorded	39 (1)
Myrtaceae	<i>Eugenia</i> sp.		Native	Fruit	31 (12)
Olcaceae	<b><i>Ximeniopsis horridus</i></b>	Me voy contigo	Endemic	Fruit	33 (10)
Portulacaceae	<i>Portulaca rubricalis</i>	Verdolaguilla	Native	Not recorded	27 (21)
Rhamnaceae	<i>Colubrina elliptica</i>		Native	Fruit	24 (29)
Rubiaceae	<i>Scolosanthus triacanthus</i>		Native	Not recorded	35 (6)
Sapindaceae	<i>Thouinia domingensis</i>		Endemic	Fruit	35 (6)
Sterculiaceae	<i>Melochia tomentosa</i>	Malva cimarrona	Native	Flower, Fruit	15 (74)
Turneraceae	<i>Turnera diffusa</i>		Native	Flower	4 (423)
Ulmaceae	<i>Phyllostylon rhamnoides</i>		Native	Fruit	8 (209)
Veberaceae	<i>Lantana</i> sp.*		Endemic	Flower	1 (648)
Vitaceae	<i>Cissis trifoliata</i>		Native	Not recorded	10 (162)
Zygophyllaceae	<i>Guaiacum officinale</i>	Guayacan	Native (CE)	Fruit	20 (50)
Zygophyllaceae	<i>Guaiacum sanctum</i>	Vera, Guacayancillo	Native (VU)	Flower	29 (17)

\*Two species of *Lantana* were found: *L. ciferriana* (Endemic) and *L. reticulata* (Native), but we were unable to differentiate them in the vegetation plots.

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# *Leptodactylus latrans* tadpoles predated the eggs and tadpoles of sympatric anurans

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**ABSTRACT** - The Criolla frog, *Leptodactylus latrans*, is one of the most common and widely distributed anuran species in the Neotropics. Tadpoles of this species were previously reported to be omnivorous, feeding on algae and animal remains but observations in the wild suggested that they predate small tadpoles of *Physalaemus* sp. These observations were confirmed by a laboratory test where *L. latrans* tadpoles predated the larvae and eggs of *Physalaemus biligonigerus* and larvae of *Rhinella fernandezae*. There was evidence that the tadpole of *R. fernandezae* was relatively unpalatable.

## INTRODUCTION

Most anuran larvae are primarily herbivores or detritivores; however, some species are carnivores (Alford, 1999) and may be cannibalistic (Duellman & Trueb, 1994), a behaviour associated with a high density of tadpoles in water bodies (e.g. *Scaphiopus* spp, *Bombina variegata*, *Epidalea calamita* and *Hyla arborea*) (see Heusser, 1971; Pomeroy, 1981). Some species (e.g. *Anomaloglossus beebei*, *Aparasphenodon arapapa*, *Oophaga pumilio*) (see Pramuk & Hiler, 1999; Bourne et al., 2001; Lourenço-de-Moraes et al., 2013) that breed in the small water bodies retained by plants (*phytotelmata*) show a special case of cannibalism. Low food availability in this situation induces females to lay unfertilised eggs that are then consumed by their tadpoles. The tadpoles of other species breeding in temporary ponds obtain a high-protein diet by being carnivorous. This accelerates metamorphosis and so decreases the risk of death by desiccation (Heusser, 1970; Crump, 1992; Petranka & Thomas, 1995).

For leptodactylid tadpoles, carnivory is unusual but is known in two species; *Leptodactylus labyrinthicus* (Spix, 1824) and *L. pentadactylus* (Laurenti, 1768) (Laurenti, 1768; Silva & Giaretta, 2008; Piraini et al., 2010).

*L. latrans* (Steffen, 1815) is one of the most widely distributed leptodactylids in South America (Maneyro & Carreira, 2012; Pimenta et al., 2014) and its tadpole is among the largest within the Pampa biome. Previous studies have shown that these tadpoles are omnivorous, feeding on algae and animal remains (Lajmanovich, 1994). However, our casual observations in the field indicated that *L. latrans* tadpoles were preying upon smaller tadpoles of *Physalaemus* sp. The current study describes a laboratory investigation of the potential predatory behaviour of *L. latrans* tadpoles on the eggs and smaller tadpoles of sympatric anuran species.

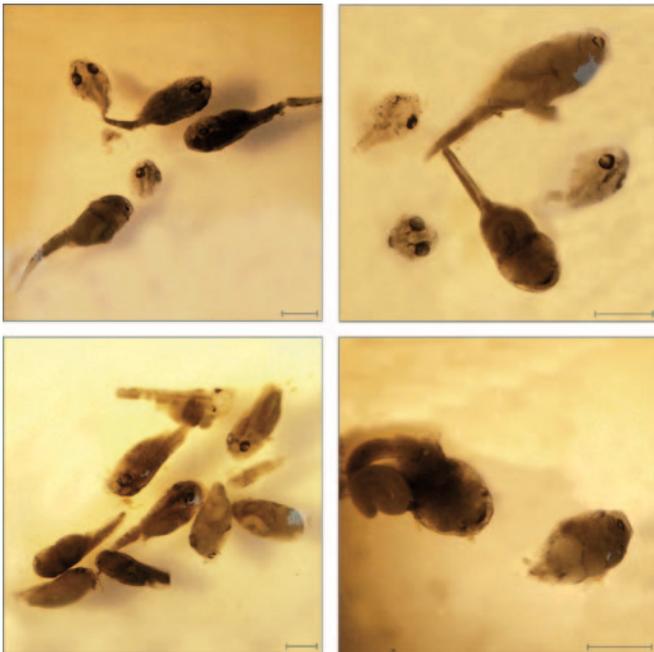
## MATERIALS AND METHODS

To perform the experiment, we used nine *L. latrans*

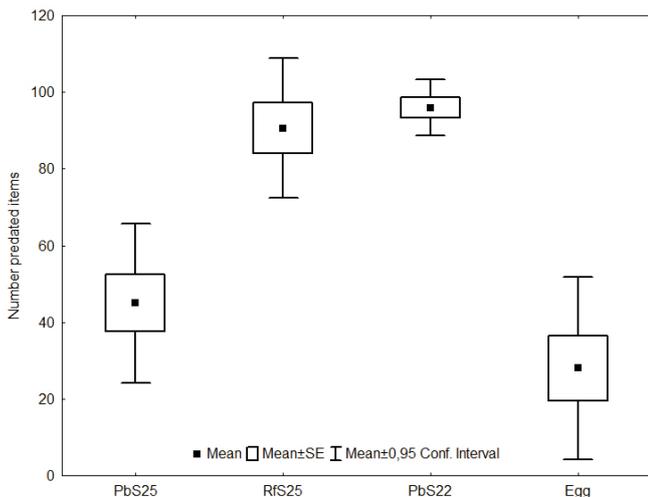
tadpoles, which were split into two size groups. The first group, hereafter named 'smaller' tadpoles, ranged from 40.1 to 46.2 mm total length (stage S 36, Gosner, 1960) and the second group, hereafter named as 'larger' tadpoles, ranged from 69.8 to 71 mm total length (S 40). These were offered fertilised eggs of *Physalaemus biligonigerus* (Cope, 1861) and tadpoles of *P. biligonigerus* in two development stages (mean = 5.79 mm total length, S 22; and mean = 5.90 mm total length, S 25); and tadpoles of *Rhinella fernandezae* (Gallardo, 1957) (mean = 5.67 mm total length, S 25). These were chosen because they are syntopic with *L. latrans* tadpoles in the coastal plain of Rio Grande do Sul, southern Brazil. All individuals were collected in temporary ponds at the university campus of Universidade Federal do Rio Grande (FURG) (33.075694°, 52.168390°, 7 m above sea level, Datum WGS 84). A collection permit was authorised by Instituto Chico Mendes de Conservação da Biodiversidade (Licence Number 43658-1).

Eggs and tadpoles were collected in the wild and no tadpole was submitted to starvation conditions. Photoperiod in the laboratory simulated natural conditions, that is 12h/12h. Each tadpole of *L. latrans* was placed in a transparent container filled with 0.55 L of rain water (21-23°C) and then simultaneously offered 15 eggs of *P. biligonigerus*; 15 tadpoles of *P. biligonigerus* (S 22), 8 tadpoles of *P. biligonigerus* (S 25) and 15 tadpoles of *R. fernandezae* (S 25). Feeding behaviour of *L. latrans* tadpoles was observed during the first 30 minutes but the total number of tadpoles and eggs consumed was counted only after 12h. The number of tadpoles and eggs were counted, including those that were rejected, that is, those *P. biligonigerus* and *R. fernandezae* tadpoles that were partially consumed (see Fig. 1).

The influence of *L. latrans* tadpole size (smaller vs larger than 50 mm) on the number of prey items consumed was analysed using the Mann-Whitney *U*-test. To detect significant differences between the numbers of consumed items consumed by larger tadpoles, we used the Friedman nonparametric ANOVA for dependent samples. Values were considered statistically significant at  $p \leq 0.05$ . Results



**Figure 1.** Examples of *R. fernandezae* tadpoles found dead, partially eaten or rejected by tadpoles of *L. latrans* during the experiment. Scale bars represents 1 mm.



**Figure 2.** Mean, standard error and confidence intervals for the number of prey items consumed by larger *L. latrans* tadpoles in the experiment. Pb = *P. biligonigerus*; Rf = *R. fernandezae*; S = Gosner developmental stage.

were expressed in mean percentage ( $\pm$  standard deviation). Statistical analyses were performed using Statistica 7.0 software.

## RESULTS

Smaller tadpoles consumed fewer prey than larger individuals ( $U = 28.5$ ,  $p < 0.001$ , see Table 1). Therefore, to compare feeding preferences we used only the larger group ( $>50$  mm) and detected a significant difference in consumption between different items (Friedman test,  $\chi^2 = 12.62$ ,  $df = 3$ ,  $n = 5$ ,  $p = 0.006$ ). That is, larger tadpoles of *L. latrans* consumed all items offered to some degree but with

**Table 1.** The size of *L. latrans* tadpoles (cm) and the number of tadpoles and eggs of other species consumed by each. Values in parentheses are partially consumed prey (considered rejected).

Size (cm) of <i>L. latrans</i> (S 36-40)	<i>P. biligonigerus</i>		<i>R. fernandezae</i>
	Eggs	Tadpole (S 22)	Tadpole (S 25)
No. prey items presented/replicated	15	15	8
			Tadpole (S 25)
<b>Larger tadpoles</b>			
7.17	1	15	5
7.10	6	15	2
7.10	8	14	3
7.06	2	13	3
6.98	4	15	5
<b>Smaller tadpoles</b>			
4.62	1	2	0
4.48	4	4 (1)	2
4.10	4	0	3
4.01	8	1	0

higher mean values for *P. biligonigerus* (S 22) ( $96\% \pm 5.96$ ) and *R. fernandezae* (S 25) ( $90.7\% \pm 14.6$ ) and lower mean values for *P. biligonigerus* (S 25) ( $45\% \pm 16.8$ ) and eggs of *P. biligonigerus* ( $28\% \pm 19.1$ ) (Fig. 2). Out of 135 *R. fernandezae* tadpoles 19 feeding attempts were made but rejected, while only one *P. biligonigerus* tadpole was rejected, out of 207 (Fig. 1 and Table 1).

## DISCUSSION

These results support our initial hypothesis that *L. latrans* tadpoles will attack and consume larvae and also the eggs of other anurans. The lower predation capacity of smaller *L. latrans* tadpoles ( $<50$  mm total length) may be attributed to limitations of smaller size. In many kinds of carnivorous and cannibalistic tadpoles, such behaviour is facultative and usually occurs under crowded conditions or when the food supply is limited (Crump, 1992; Duellman & Trueb, 1994). Although we observed predatory behaviour in *L. latrans* tadpoles it is apparent that their mouthparts have no specific morphological adaptations for carnivory, unlike other carnivorous species of the *L. pentadactylus* group (Heyer et al., 1975). The findings in relation to *R. fernandezae* tadpoles, suggest they may be unpalatable. *Rhinella* tadpoles, and related species are known to produce alciaphilic mucous that has an unpleasant taste, a possible anti-predator adaptation (Gunzburger & Travis, 2005). Similarly, it has been reported that *L. pentadactylus* tadpoles avoided *Rhinella marina* tadpoles after initially seizing and killing them (Heyer et al., 1975).

It is important to emphasise that this is the first evidence of carnivory for a species from the *L. latrans* group. Similar behaviour was previously recorded for *L. labyrinthicus* and *L. pentadactylus*, both belong to the *L. pentadactylus* group, which are known for preying upon both con- and heterospecific tadpoles (Heyer et al., 1975; Cardoso & Sazima, 1977; Wells, 1979; Silva & Giarretta, 2008; De Sousa et al., 2014). The ability to forage actively for animal protein may be an essential component of a tadpole's development

(Heyer et al., 1975) leading to enhanced growth (Heusser, 1970; Crump, 1992; Petranka & Thomas, 1995). Also, carnivory could increase tadpole survivorship, particularly in temporary ponds with limited food resources (Blair, 1976; Pomeroy, 1981; Crump, 1983). Our results along with field observations indicate that these tadpoles have the capacity to prey upon smaller tadpoles and anuran eggs, a behaviour that may influence survivorship rates of tadpoles of other anurans and small organisms that are syntopic.

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# Notes on reproduction and development of *Pleurodeles nebulosus* (Caudata: Salamandridae) in captivity

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**ABSTRACT** - The reproduction of captive *Pleurodeles nebulosus* was followed during five breeding seasons. The breeding season lasted from the onset of autumn to early spring. Most eggs were laid in October, although oviposition occurred throughout the breeding season. Each season a female would produce one or more clutches of 19-224 eggs. Diameter of the ova was 1.63-1.65 mm. Eggs hatched after 8-12 days, and at hatching the larvae were in Harrison stages 37-38. It took 73-108 days for the larvae to reach metamorphosis and the metamorphs measured 25.6-32.1 mm in standard length. A modest correlation was found between standard length at metamorphosis and larval development time. Juveniles, which were kept aquatic, reached maturity after 9-10 months, whereas juveniles, which were reared on land, did not reach maturity until approx. 20-22 months after metamorphosis.

## INTRODUCTION

The ribbed newts of the genus *Pleurodeles* consist of three species. While *P. waltl* is found both on the Iberian Peninsula and in northern Morocco, the distribution of the two other species is limited to North Africa. *P. nebulosus* is distributed across northern Tunisia and Algeria, except on the Edough Peninsula and surrounding lowland areas of north-eastern Algeria, where it is replaced by *P. poireti* (Carranza & Wade, 2004).

*P. waltl* is a well-studied species (e.g., Schleich et al., 1996, Salvador & García-París, 1999, García-París et al., 2004), whereas both *P. nebulosus* and *P. poireti* remain poorly known. Although Samraoui et al. (2012) provided some information on the natural history and conservation of *P. poireti*, almost all research into these two smaller species has focused on *P. nebulosus*, and most studies made on *P. poireti* before the work of Carranza & Wade (2004) actually refer to *P. nebulosus*. In this study data obtained from captive newts during five consecutive breeding seasons from 2012 to 2016 is compared with the available data from the field and other captive breedings.

## MATERIALS AND METHODS

The newts used in this study were the offspring (F2 and F3) of individuals collected in the vicinity of Tabarka, Tunisia. Eight newts were used in the study. They were kept in groups consisting of two females and two males in each tank. The tanks were standard aquaria measuring 60 x 30 x 35 cm. At a height of about 17 cm from the bottom a glass shelf covering a third of the bottom area was glued in place with silicone. This constituted the land area of the tank and allowed the water part to cover the entire bottom area of the tank. The shelf was covered with a 2 cm thick sheet of dark grey foam rubber, which was always kept slightly moist.

As hiding places for the newts pieces of cork bark were placed on top of the foam. A piece of driftwood allowed the newts to easily reach the land area when leaving the water. The water level was kept at a height of 15-16 cm. To allow for easy cleaning no substrate was used in the water part of the tank and aside from the piece of driftwood no other decorations were used. Various aquatic plants filled the water part, such as aquatic mosses of the genera *Taxiphyllum* and *Vesicularia*, hornwort (*Ceratophyllum demersum*) and Brazilian waterweed (*Egeria densa*). Over the summer filamentous green algae would occasionally appear in the tank. These were not removed as the newts would often use them for egg-laying. The water in each tank was filtered by a large air-driven sponge filter, which was cleaned about once a month. The tanks were lighted with fluorescent light year round for 12 hours a day. As they were placed near a window, the tanks also received some ambient light. Water temperature ranged from 20-26 °C in summer to 15-20 °C in winter. When terrestrial the newts were primarily fed with buffalo worms (*Alphitobius diaperinus*) and earthworms, whereas in the water the main foods were defrosted bloodworms (*Chironomidae*) and live *Tubifex* sp.

As the adult newts would often consume their own eggs, they were removed from the tanks as soon as they appeared and were placed in small water filled plastic boxes. To prevent infection, a small amount of methylene blue was added to the water with the eggs. Upon hatching the larvae were moved to densely planted aquaria ranging in size from 25 to 63 litres. The larvae were fed once a day with live brine shrimp naupliae (*Artemia* sp.) and banana worms (*Panagrellus nepenthicola*). As the larvae got bigger the brine shrimp and banana worms were gradually replaced by *Tubifex*, chironomid larvae and water fleas (*Daphnia* spp.).

Eggs and newly hatched larvae were measured to the nearest 0.01 mm by photographing them next to a stage

micrometer, whereas metamorphosed juveniles were measured to the nearest 0.1 mm using dial callipers. To restrain movement of the juveniles they were placed in small plastic bags while measurements were made.

## RESULTS

The breeding season began when the newts entered the water in early autumn. The newts would leave the water regularly and stay on land for up to a week at a time. From late spring the newts stayed on land where they would remain until the following autumn, signalling the end of the breeding season. To indicate the onset of the mating period the males developed brown nuptial pads on the underside of the humeral and their cloacas swelled slightly. After a short time in the water both sexes also developed low tail fins, just barely discernible in the females. Amplexus was observed within a day after the newts entered the water and would continue sporadically during the entire breeding season.

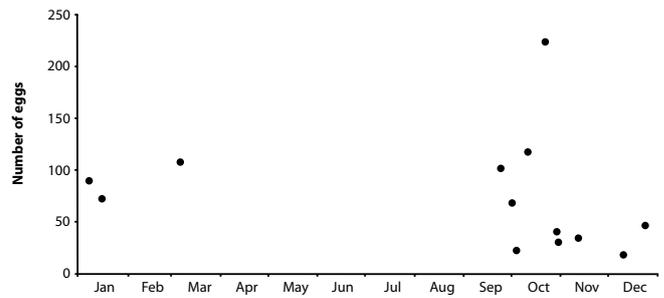
### Oviposition and eggs

Oviposition occurred from September to March (Fig. 1), and appeared to be triggered by a water change. The earliest recorded clutch was deposited on 23 September 2015, the latest on 4 March 2016. The most prolific month was October, in which five of the 13 recorded clutches were deposited. Eggs were deposited several times during the breeding season. The number of eggs in each clutch was highly variable and ranged from 19 to 224 eggs; averaging  $75.4 \pm 55.862$  eggs (mean  $\pm$  1 SD; N = 13). Eggs were deposited on aquatic plants, mosses, algae, driftwood and even on the filter sponge, either singly or in clumps of 2-32 eggs. Average number of eggs per clutch in each clutch ranged from  $2.9 \pm 1.552$  to  $11.8 \pm 7.786$  eggs (mean  $\pm$  1 SD; N = 8 and 10, respectively). A total of 120-355 eggs were produced in each tank during a breeding season. Assuming they each produced an equal number of eggs, each female laid 60-178 eggs during a breeding season. Considering the largest observed clutch size, this is obviously an underestimation. The maximum number of eggs produced by a female must therefore be at least 224 eggs.

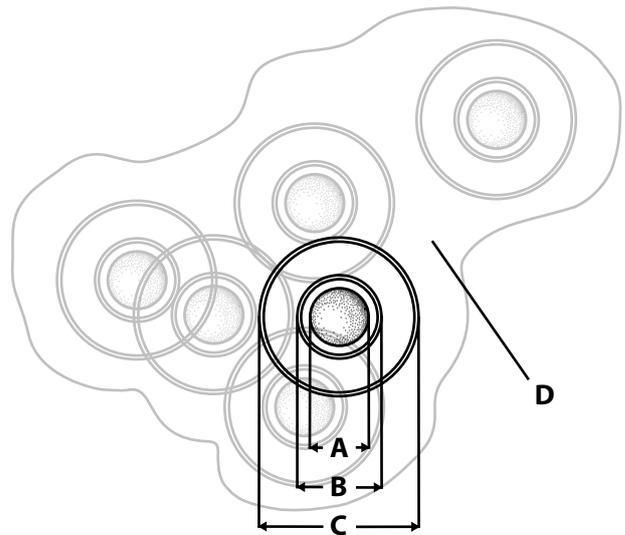
In the freshly laid eggs (Fig. 2) the diameter of the ovum ranged from 1.63 to 1.65 mm and averaged  $1.64 \pm 0.007$  mm, whereas the diameter of the perivitelline chamber measured 2.19-2.39 mm with an average of  $2.283 \pm 0.059$  mm. The largest outer capsule was slightly oval and its largest diameter ranged from 4.74 to 6.25 mm with an average of  $5.41 \pm 0.490$  mm (mean  $\pm$  1 SD; N = 10).

### Egg development and hatching

At temperatures of 16-23 °C the eggs hatched after 8-12 days. Hatching did appear to be somewhat dependent on temperature, so that eggs kept at 21-23 °C would hatch after 8 days, whereas eggs kept at 16-20 °C took up to 12 days to hatch. At time of hatching the larvae (Fig. 3) had a total length of 7.90-8.94 mm, averaging  $8.365 \pm 0.352428$  (mean  $\pm$  1 SD, N = 10). They were whitish-yellow with darker pigment arranged into two distinct stripes on each side of the body. The fins were clear to slightly milky. The



**Figure 1.** Time of oviposition in *P. nebulosus*. Oviposition occurred from September to March but peaked in October. Each dot (●) represents a clutch of eggs.



**Figure 2.** Schematic drawing of a clump of eggs showing the measurements taken: **A.** diameter of the ovum; **B.** diameter of the perivitelline chamber; **C.** diameter of the largest outer capsule; **D.** viscous jelly



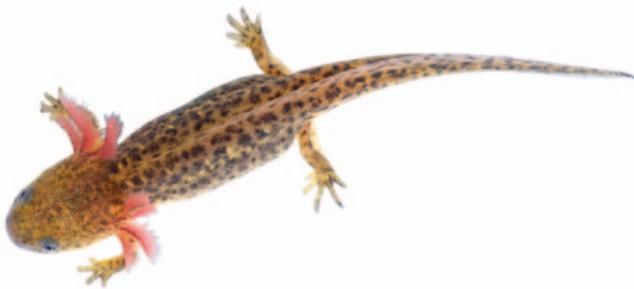
**Figure 3.** Newly hatched larva. Note the large balancers, branching of the gill filaments and beginning pigmentation.

size difference among the larvae was also reflected in their stage of development. All the larvae had three distinct gills on each side of the head. The gill filaments varied in length and would in some larvae reach the base of the forelimb; in others they would not. Branching was just beginning. In the smallest larvae only the tiniest sprout could be discerned on largest of the gill filaments, whereas sprouts

were clearly visible on all gill filaments in the largest larvae. The balancer was slightly shorter than the gill filaments in the smallest larvae, but just as long and very slightly club-shaped in the larger larvae. Forelimb buds were visible in all larvae, although more distinctly bud-shaped in the largest larvae. This corresponds to Harrison stages 37-38 (Harrison, 1969).

### Larval development and metamorphosis

At a temperature of 17-20 °C it took 73-108 days for the larvae (Fig. 4) to reach metamorphosis, with an average of  $86.90 \pm 10.621$  days (mean  $\pm$  1 SD; N = 17). The standard length (as measured from the tip of the snout to the posterior end of the vent) at metamorphosis was 25.6-32.1 mm with an average of  $28.89 \pm 1.75$  mm (mean  $\pm$  1 SD; N = 17). Although the smallest individual took the shortest time to reach metamorphosis and the largest took the longest, there was only a modest correlation between standard length at metamorphosis and larval development time (Spearman Rank Correlation Coefficient ( $r_s$ ) = 0.472; N = 17). Tail length was far more variable than standard length and the tail length to total length ratio ranged from 0.421-0.501, averaging  $0.463 \pm 0.021$  (mean  $\pm$  1 SD; N = 17). This was probably due to the larvae biting each other's tail tips off, which would often happen during feeding if the larvae were kept at high densities. No deaths resulted from this, though, and the tails healed fast.



**Figure 4.** Large larva at an age of 85 days. Total length is 62 mm.



**Figure 5.** Juvenile with a total length of 61 mm, one month after metamorphosis

After metamorphosis the juveniles (Fig. 5) were either reared in small terraria on moist foam rubber with pieces of cork bark for cover or in densely vegetated aquaria with relatively low water levels (up to 20 cm). Growth was much faster in the aquaria, where most of the juveniles would reach maturity just 9-10 month after metamorphosis. Most juveniles reared on land were not mature until 12 months later, i.e., at a postmetamorphic age of approximately 20-22 months. Mortality was, possibly due to drowning, much higher when the juveniles were raised in water than when raised on land. However, this was not quantified.

## DISCUSSION

Pasmans & Bogaerts (2001) observed that in captivity *P. nebulosus* would mate in the autumn, while Ben Hassine et al. (2012) found that mating begins with falling temperatures in October and lasts until the beginning of March. This is consistent with my observations, although mating was observed to begin slightly earlier, which can probably be attributed to the earlier onset of autumn at the more northern latitudes at which the newts were kept.

Almost nothing is known about oviposition in the wild. Blanc (1935) witnessed egg-laying in January and in February Pasmans et al. (2002) found larvae, ranging in size from 1 to 3 cm, in a ditch, which led them to speculate that oviposition occurred in December and January. At least in captivity, oviposition took place during the entire breeding season but with a peak in October, suggesting that *P. nebulosus* is primarily an autumnal breeder. Out of 36 newts found by Pasmans et al. (2002) in February only one was observed in the water, which supports the hypothesis that the peak mating season of *P. nebulosus* under natural conditions probably lies in autumn and early winter. The annual egg production of 60-224 eggs per female is more or less in accordance with the 100-200 eggs noted by Ben Hassine et al. (2012).

Descriptions of the eggs of *P. nebulosus* are almost nonexistent. Ben Hassine et al. (2012) did report an average diameter of the ovum of just 1.2 mm, which is, however, much lower than the 1.64 mm observed in this study. At 20 °C Ben Hassine et al. (2012) found that it took 10 days for the eggs to hatch, whereas at 15-16 °C hatching was prolonged to 20 days. This is similar to my findings and corroborates the observed effect of temperature on egg development.

Under natural conditions larvae have been found from the beginning of December to the first half of April (Pasmans et al., 2002, Sicilia et al., 2009) and according to Ben Hassine et al. (2012) it takes the larvae 2-3 months from hatching to metamorphosis. This is slightly less, than what I have observed, but the time it takes the larvae to reach metamorphosis seems to be highly variable. Although there was only a modest correlation between size and development time, availability of prey/food could be a determining factor. As the larvae were kept at relatively high densities, some were probably better at obtaining food than others and as a result grew faster, eventually reaching metamorphosis earlier than their siblings.

## REFERENCES

- Due to lack of data it is not possible to compare the reproductive biology of *P. nebulosus* to that of *P. poireti*. In comparison to the well-studied *P. waltl*, however, the similarities seem to outweigh the differences. In the lowlands of North Africa *P. waltl* breeds, similar to *P. nebulosus*, from October to April (Schleich et al., 1996). Despite its often much larger size (at least on the Iberian Peninsula, females of *P. waltl* may reach total lengths of up to 286 mm (González de la Vega, 1988) compared to the maximum total length for female *P. nebulosus* of 170.4 mm reported by Ben Hassine et al. (2012)) the eggs of *P. waltl* are only slightly larger than those of *P. nebulosus*. According to González de la Vega (1988) the ova of the eggs of *P. waltl* have diameters of 1.7-2.0 mm, just slightly larger than the ovum diameter of 1.63-1.65 mm in *P. nebulosus*. The reproductive advantage due to the larger size of *P. waltl* over the smaller *P. nebulosus* seems, therefore, to be limited to a much larger egg production with large females producing up to 1303 eggs annually (González de la Vega, 1988), perhaps even up to 2000 eggs (Schleich et al., 1996). This is a substantial egg production when compared to the annual production of 60-224 eggs, which I observed in *P. nebulosus*.
- Larvae of *P. waltl* have been reported to hatch after 2-26 days (Gallien & Durocher, 1957; González de la Vega, 1988; Schleich et al., 1996), which is a much wider time span that I was able to observe in *P. nebulosus*. Despite similarities in ovum size larvae of *P. waltl* appear to hatch at a larger size than *P. nebulosus*. Reported sizes for newly hatched *P. waltl* range from 6.5-8 mm (González de la Vega, 1988) to 10-14 mm (Gallien & Durocher, 1957; Schleich et al., 1996).
- It takes the larvae of *P. waltl* 3-5 months to reach metamorphosis (González de la Vega, 1988; Schleich et al., 1996). While this is similar to *P. nebulosus*, *P. waltl* seems to transform at a larger size. According to Schleich et al. (1996) newly metamorphosed *P. waltl* reach snout-vent lengths of 55-80 mm, although other authors report somewhat smaller sizes (e.g., González de la Vega, 1988). Growth in *P. waltl* after metamorphosis is fast and well-fed juveniles may reach sexual maturity within 6 months (Schleich et al., 1996), which is much faster than even my fastest developing *P. nebulosus*.
- The knowledge of the reproductive biology of *P. nebulosus* obtained in this and other studies has been fairly predictable and hasn't revealed any surprises. However, much of the available data was, as in the present study, obtained from captive animals and may be biased by the conditions of captivity. To wholly understand the natural history of *P. nebulosus* more detailed studies on wild populations are needed.
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# First report of the co-existence of the three endemic *Phelsuma* species of Mayotte Island (Indian Ocean) in anthropogenic habitats

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**ABSTRACT** - We report the first syntopic observation of all introduced and endemic *Phelsuma* species of Mayotte Island from a restricted area dominated by plantations and degraded forest. However, we suggest that more in-depth studies of the interactions between *Phelsuma* species in anthropogenic habitats are urgently needed to guide efforts towards the conservation of the endemic species.

## INTRODUCTION

The island of Mayotte is part of the volcanic Comoros archipelago located in the Northern Mozambique Channel in the Western Indian Ocean region. As in many highly diverse tropical regions, 90 to 95% of the island's original habitats are degraded or destroyed (UICN, 2013), but still support a high diversity and abundance of reptiles (Hawllitschek et al., 2011). Five species of day geckos of the genus *Phelsuma* occur sympatrically on the island. The three endemics *P. robertmertensi*, *P. nigristriata*, and *P. pasteuri* mostly occur in natural habitats and more specifically in elevations above 200 m a.s.l. for *P. nigristriata*, while the two introduced species *P. dubia* and *P. laticauda* are very abundant in lowland plantations and degraded forests (Hawllitschek et al., 2011). Substantially modified areas were previously shown to be valuable habitats for some species of *Phelsuma* on other islands (see Thorpe & Crawford, 1979; Randrianantoandro et al., 2012; Theisinger & Ratianarivo, 2015). Possibly because of their divergence in ecological preferences, up to two endemic species of *Phelsuma* were already reported to occur in syntopy in Mayotte, i.e., in one observation site, along with the two introduced species (see Hawllitschek & Glaw, 2014; Wang et al., 2016). Here, we present the first data on the syntopic occurrence of all five *Phelsuma* species of Mayotte in anthropogenic habitats.

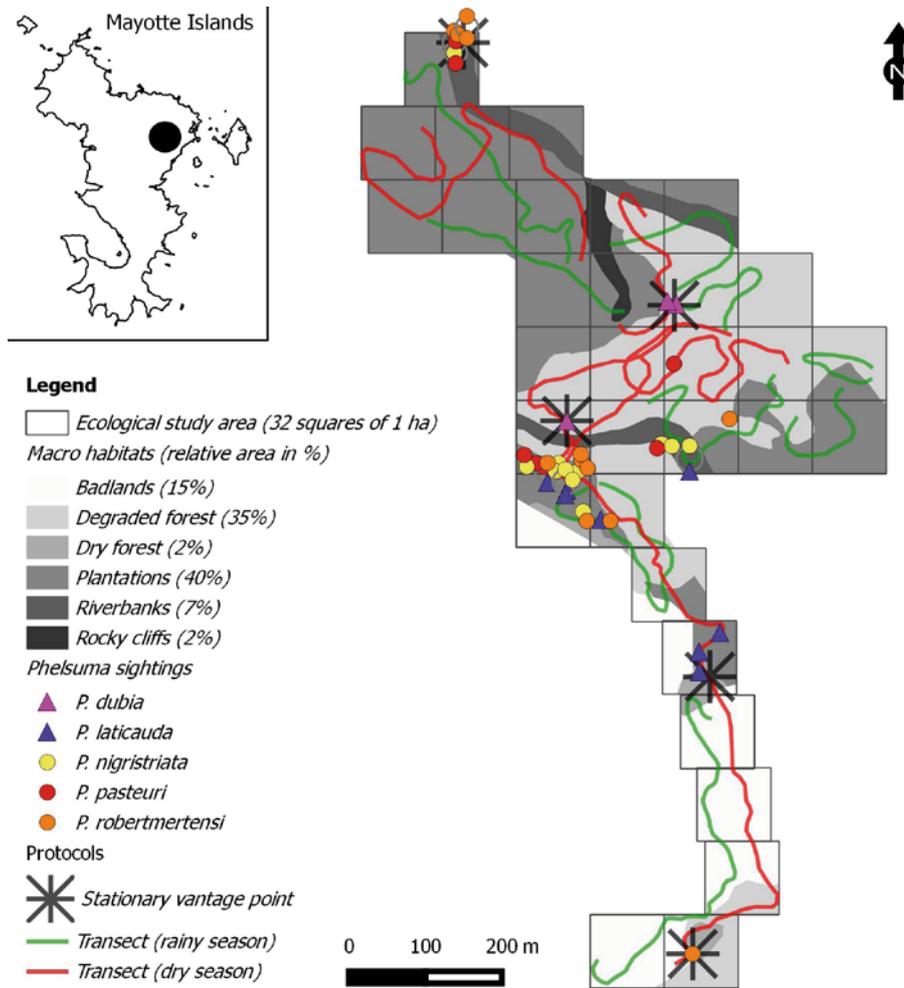
## METHOD

In 2015, we conducted a complete ecological assessment (unpublished data) for a designated project located in Grande Terre (12°46'S, 45°11'E; 150-250 m a.s.l.; 0.32 km<sup>2</sup>). We specifically searched for *Phelsuma* species by walking nine transect lines (Eberhardt, 1978) during the rainy season (March) for a total of 3,237 meters and a duration of 5.4 hours, and five additional transect lines during the dry season (September) for a total of 3,421 meters and a duration of 5.7 hours (Fig. 1). During the second campaign, five stationary vantage points were conducted in areas and types of vegetation where no geckos were found before. They

consisted in scanning the surrounding area for 60 minutes with binoculars (8X42) within a radius of 30 meters (canopy to ground, 360°), by allowing the observer to slightly move and improve his visibility through tree crowns (especially for *Arecaceae*). All surveys were conducted during the optimum period survey advised by Hawllitschek & Glaw (2014) from 7 to 11 am and 3 to 6 pm. Habitat classes were categorised according to Hawllitschek et al. (2011). Complete descriptions and identification keys for *Phelsuma* species were provided by Hawllitschek & Glaw (2014). We apply a strict definition of syntopy based on the potential overlap of home ranges of adult *Phelsuma* species. The only published data on home range extent is from Ikeuchi et al. (2005), where home ranges of male *P. kochi* (from Madagascar) extended over up to 516 m<sup>2</sup>, with a maximum distance of 45 meters between two perches used by the same individual. This relates to a hypothetical perfect circle with a diameter of 45 m, specifically for males that potentially overlap with as many female home ranges as possible. We therefore consider any occurrence of *Phelsuma* spp. within twice this distance, i.e., 90.0 m or less, as potentially overlapping home ranges and therefore as syntopic. Within this distance, we also consider observations in different habitat classes as syntopic because in the boundaries between habitat classes in the study area, with the exception of the river, are 'soft' and only marked by thresholds in the abundance of plant species. Therefore, they should not be expected to form barriers to the movement of *Phelsuma* spp.

## RESULTS AND DISCUSSION

While Mayotte's natural habitats have become extremely scarce, our surveys yielded the first data on the co-existence of all five *Phelsuma* species of Mayotte in a degraded area close to urban settings and mainly composed of plantations and degraded forests (Table. 1, Fig. 1). A total of 40 observations of *Phelsuma* geckos were made. The two most commonly observed species were the endemics *P. robertmertensi* (30% of total counts) and *P. nigristriata* (27.5%), while the least commonly observed was the



**Figure 1.** Location map of the study site with habitat types and gecko observations

introduced *P. dubia* (7.5%). Endemic geckos represented 72.5% of total observations. The highest numbers of observations were made in plantations (dominated by banana and coconut trees) along with the maximum species richness (4 taxa); the two most abundant taxa recorded in this habitat were *P. laticauda* and *P. nigristriata* (both representing 30% of the counts in this habitat class). Riverbanks are the second most occupied habitat, with 32.5% of total counts, mainly represented by endemics (30%) (Table. 1). All five species were observed within a minimum distance of 71 m, therefore considered syntopic because their home ranges can be expected to overlap and the species may compete for

resources.

We have the view that four major factors may explain species richness in the study area: i) the high diversity and availability of macro- and microhabitats (Arecaceae, Musaceae, Pandanaceae, indigenous wooden trees in canopy); ii) the singular biogeographical context of the study site as a buffer zone between the natural area of Majimbini known to host endemic *Phelsuma* populations (Brückmann, 2010; Hawlitschek et al., 2011), and the nearest human settlements of Mamoudzou, largely colonised by the introduced *P. laticauda* (Hawlitschek et al., 2011; S.A., pers. obs.); iii) the fact that human transportation of plant material

**Table 1.** Macro habitat types and *Phelsuma* gecko species counts and percentage per type. Σ: total counts; %: percentage of counts; D.O.: duration of observation; PD: *P. dubia*; PL: *P. laticauda*; PN: *P. nigristriata*; PP: *P. pasteuri*; PR: *P. robertmertensi*.

Vegetation types	Total			PD		PL		PN		PP		PR	
	Σ	%	D.O. (h)	Σ	%	Σ	%	Σ	%	Σ	%	Σ	%
Badlands	0	0.0%	1.43	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Degraded forest	8	20.0%	6.65	3	7.5%	0	0.0%	0	0.0%	2	5.0%	3	7.5%
Dry forest	3	7.5%	2.30	0	0.0%	1	2.5%	0	0.0%	0	0.0%	2	5.0%
Plantations	16	40.0%	3.94	0	0.0%	6	15.0%	6	15.0%	2	5.0%	2	5.0%
Riverbanks	13	32.5%	1.67	0	0.0%	1	2.5%	5	12.5%	2	5.0%	5	12.5%
Rocky cliffs	0	0.0%	0.10	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
<b>TOTAL</b>	<b>40</b>	<b>100.0%</b>	<b>16.09</b>	<b>3</b>	<b>7.5%</b>	<b>8</b>	<b>20.0%</b>	<b>11</b>	<b>27.5%</b>	<b>6</b>	<b>15.0%</b>	<b>12</b>	<b>30.0%</b>

(e.g. banana plants) from one remote forested area to another on foot is a common practice throughout Mayotte and iv), the presence of rivers crossing the study site and providing riparian habitats that can be seen as refuges excluded from farming and human influences. While anthropogenic ecosystems will probably cover a larger fraction of Mayotte in the near future, the co-existence of closely related endemic and introduced day geckos in these areas may lead to niche overlap, microhabitat partitioning, habitat shift or even species exclusion (Schoener, 1968). Knowledge about these mechanisms in the species from Mayotte is limited to anecdotal reports, but quantitative studies are urgently needed considering the fast and permanent changes in land use and the potentially high pressure from introduced species on this island. Some studies on microhabitat use and intra-generic competition were conducted on the endemic *Phelsuma* species of Mauritius and the Seychelles (Harmon et al., 2007, Cole & Harris 2011, Noble et al., 2011, Buckland et al., 2014, Hagey et al., 2015). Therefore, more in-depth studies of the interactions between the five *Phelsuma* species from Mayotte are urgently needed to guide efforts towards their conservation.

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## Deimatic behaviour exhibited by the green and black poison frog (*Dendrobates auratus*) after exposure from a cover object

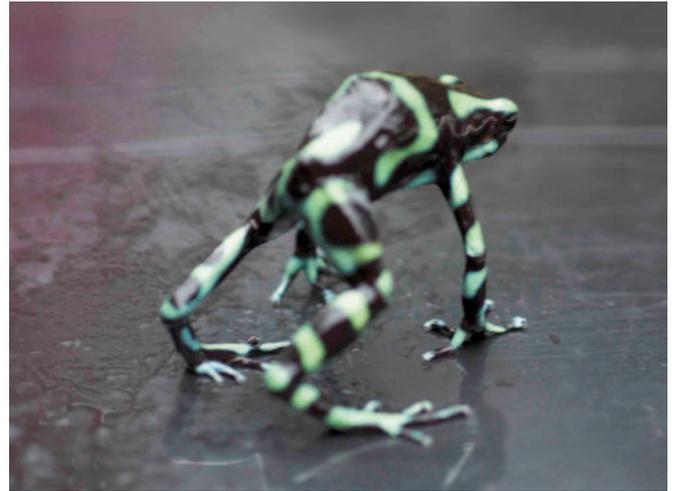
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Anurans employ a variety of morphological and behavioural strategies to avoid or prevent predation. Deimatic behaviours are characterised as sudden postures or displays that are intended to deter predators from attacking, and are common among aposematic organisms (Skelhorn et al., 2015; Umbers et al., 2017). For example, the unken reflex in certain salamanders involves lifting of the appendages, body arching, and exposing conspicuously coloured throat and ventral regions (Toledo et al., 2011). Body raising is another deimatic behaviour in which anurans stretch out their appendages vertically or laterally, lifting their bodies off the ground and enhancing conspicuous colouration (Toledo et al., 2011). This defensive behaviour has been described in certain members of Hylidae, Leptodactylidae, Leiuperidae, and Bufonidae (Toledo et al., 2011), observed in the field in *Ameerega (Epipedobates) flavipicta* (Dendrobatidae) (Toledo et al., 2004), and was recently reported in lab-raised *Dendrobates auratus* (Dendrobatidae) when individuals were exposed from under a cover object or after experiencing simulated predation (Blanchette & Saporito, 2016). Herein, we report deimatic vertical body raising behaviour in a natural population of *D. auratus* from the Firestone Center for Restoration Ecology (FCRE) in the Pacific lowlands of Costa Rica (9.2749°N, -83.8589°W).

Between 13 June 2016 and 25 June 2016, and as a part of a larger field-based study on antipredator behaviour, 20 adult *D. auratus* were captured and housed in small plastic containers with leaf-litter. The goal of the study was to understand how *D. auratus* respond to different simulated predators (humans and birds) as measured by their escape behaviours. As part of the larger study, behavioural assays were conducted on a black plastic experimental arena (30.5 × 30.5 cm) that was flat, level, and flush against the ground in a forest clearing. To begin each assay, an individual *D. auratus* was placed in the center of the arena under a darkened plastic cover object (10 × 10 × 7.5 cm) for a five-minute acclimatisation period. Frogs were handled minimally between initial capture and their use in behavioural trials. Following acclimatisation, the cover object was lifted and removed from the arena by a researcher that was ca. 2.5 m away and behind a blind. Frogs were then allowed 10 seconds to adjust to their surroundings before one of the two simulated predators approached from a starting distance of 9 m away. The human approached by walking at a speed of approximately 1.8 m/s and stopped when *D. auratus* began moving. If the frog did not move, the human stopped once it reached the arena. The model bird was constructed



**Figure 1.** Adult *D. auratus* exhibiting body raising by vertical stretching of the legs

using a 3D printer, and was made of white hard plastic and painted cream with gray tipped wings to represent a general bird form (body length: 28 cm; wingspan: 33 cm; body depth: 6.4 cm). The bird was fitted to glide silently on a nylon line at approximately 1.8 m/s, after being released by a researcher. The bird began at a height of 2 m, was at a height of 50 cm when directly overhead the frog, and came to rest approximately 6.5 m behind the frog. Over the course of the experiment, each individual frog was exposed to both simulated predators once, with approximately 24 hours in between each behavioural trial. In total, 10 out of 20 individual *D. auratus* exhibited body raising each time they were exposed to the outside environment from under the cover object (Fig. 1). The body raising behaviour was exhibited by individuals in response to the removal of the cover object, independent of the direction they were facing or the type of simulated predator. All individuals exhibited body raising for the entire 10 second adjustment period, and slowly relaxed their bodies to the ground before the predator began its approach. The larger research project included the study of two additional natural populations of *D. auratus* in the Atlantic lowlands of Costa Rica; however, only individuals from FCRE exhibited body raising.

Body raising was previously reported in captive, lab-raised *D. auratus* upon exposure from under a cover object in a lab setting (Blanchette and Saporito, 2016), which is identical to the present report of this behaviour in the field. In both instances, the cover object may have been

considered a safe location for individuals, whereas quickly lifting the object was perceived as dangerous, prompting immediate body raising as a defensive behaviour. On the basis of our observations, body raising in *D. auratus* appears to provide increased exposure of their chemically defended dorsum to a potential predator, while also enhancing their aposematic signal. The absence of body raising in some populations of *D. auratus* suggests that this behaviour may be an adaptive response to differences in predation pressure; however, the nature of this behaviour in *D. auratus* (and other dendrobatids) will certainly require further study. Individuals that exhibit this behaviour may gain a fitness advantage by startling a predator and deterring attack, preventing the loss of an acquired resource (e.g., mate, territory, food) or energy expended in escape. To the best of our knowledge, this is the first report of deimatic body raising in a natural population of *D. auratus*, and supports the previous finding of this defensive behaviour in captive lab-raised *D. auratus*.

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# Comparison of methods for controlling *Saprolegnia*-like infection in the egg sacks of Asiatic salamanders (*Hynobius*)

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**ABSTRACT** - Asiatic salamanders, genus *Hynobius*, deposit paired egg sacks rather than individual or clumped uncovered spawn. This oviposition mode means that clutches are vulnerable to water mould infections of non-viable ova. I trialled five methods of arresting mould infections in the egg sacks of *Hynobius dunni*, in pairwise comparisons with control sacks. Chemical treatment using methylene blue was unsuccessful in controlling infections. Mechanically removing infected eggs or parts of egg sacks was more successful. Open sacks were successfully resealed using nylon fishing line, and individual eggs were successfully incubated outside of the sack. These methods may be particularly useful to captive breeders and experimenters working with these salamanders in laboratories and the field.

## INTRODUCTION

Salamanders of the family Hynobiidae, and especially the genus *Hynobius*, are notable in their reproductive biology for exhibiting external fertilisation and for depositing paired egg sacks (Fig. 1), rather than individual ova. The egg sacks consist of a tough external membrane containing numerous ova within individual jelly envelopes. At eclosion, larvae break through the individual jelly envelopes and swim down the sack, leaving via an opening at the tip.

Male *Hynobius* exhibit scramble competition and release sperm over the egg sacks shortly after oviposition (Hasumi, 1994; Park & Park, 2000). It is very rare for all ova to be fertilised (Hasumi, 2001) and it is common for infertile ova to become opportunistically infected with ubiquitous *Saprolegnia*-like water moulds, which rapidly spread to and kill healthy embryos (Wallays, 2002). Additionally, clumps of dead, water mould infected embryos and ova can block the exit of healthy larvae when they are ready to hatch, leading to death (pers. obs.). In this way entire or partial clutches may be lost.

In captivity, a number of *Hynobius* species are routinely reproduced, largely in private hands (Rafaelli, 2013). *Hynobius dunni* Tago 1981, from lowland central Japan, has long been established in captivity in Europe. A single genealogical line initially propagated by Henk Wallays in the 1990s is still in existence (Wallays, 2002; Rafaelli, 2013) and likely represents the vast majority of *H. dunni* in captivity today. I have repeatedly reproduced this species and line in captivity, and trialled several techniques to reduce the number of embryos lost to *Saprolegnia*-like infection; the results are presented here.

## METHODS

Trials took place in the springs of 2015, 2016 and 2017; egg sacks were deposited between mid-March and mid-April depending on prevailing weather conditions. Techniques trialled were methylene blue (a chemical available

over-the-counter to treat fungal infections in a variety of aquatic organisms) baths and injections, removal of infected ova via syringe, and removal of parts of egg sacks with and without resealing of the sack. I also trialled incubation of eggs removed from the external sack membrane, with and without methylene blue baths. The different techniques are detailed in Fig. 1.

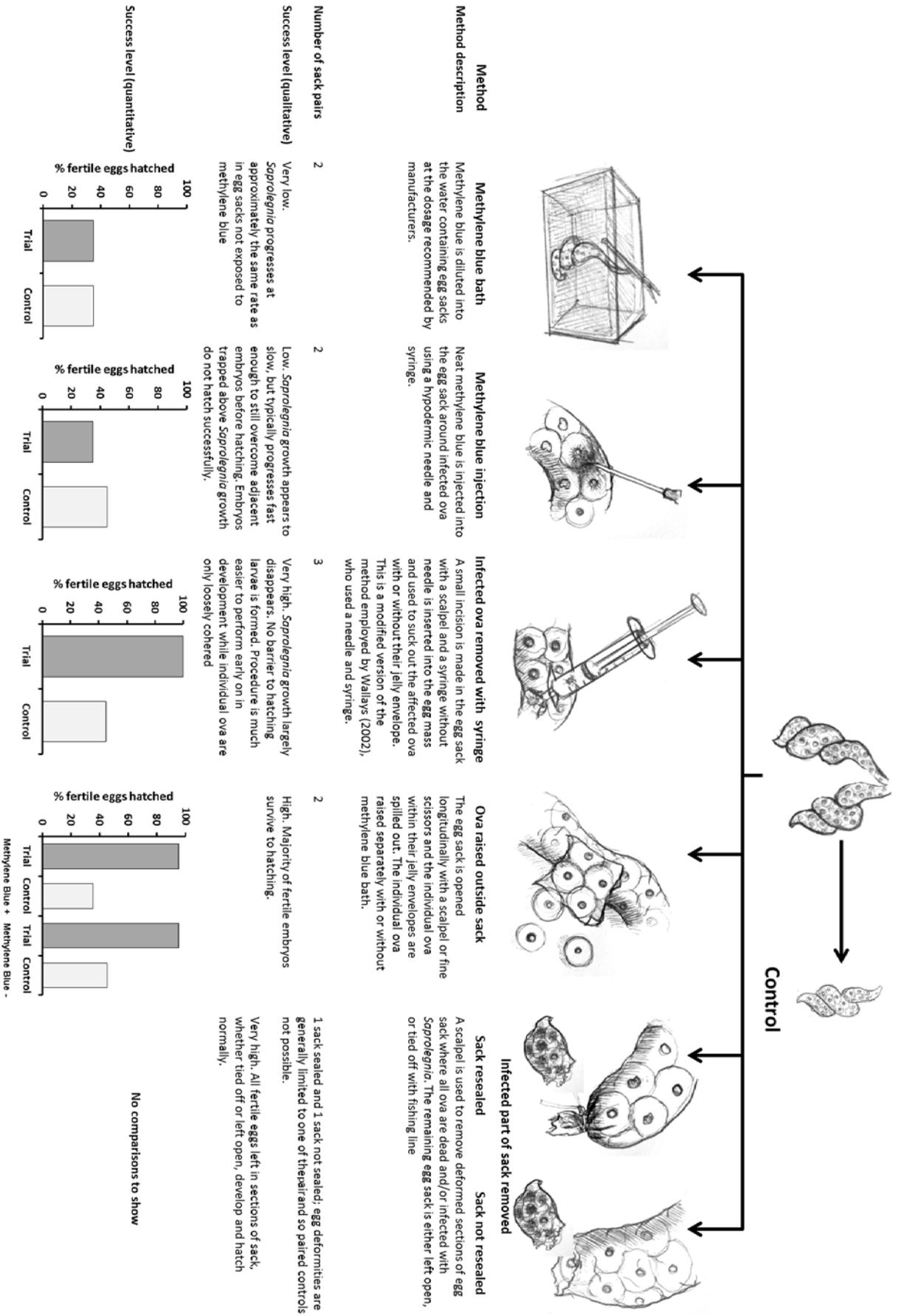
After deposition, pairs of egg sacks were used as control and trial sacks, with one sack in each pair exposed to each treatment, and the other allowed to develop naturally with no interventions, unless otherwise stated in Fig. 1. All egg sacks were incubated in substrate-less glass aquaria in the absence of adult salamanders with water (alkalinity c. 180mg/l at tank setup) at 10-15°C, taken from the breeding aquarium.

## RESULTS AND DISCUSSION

The results of different trialled methods are reported in Fig. 1. I report median percentages of the total number of eggs that were fertile and that hatched successfully, rounded to the nearest 5%, across the indicated number of trialled egg sacks. Sample sizes (usually 2, no more than 3 replicates) were not large enough for statistical analysis.

Results indicate that treatment of sacks with methylene blue, either through addition to the incubation medium or injection directly into the sack itself is not very effective at controlling *Saprolegnia*-like infections. Methylene blue at similar concentrations is used with success to treat fungal and fungal-like infections, including saprolegniasis, in post-hatching amphibians (Maruska, 1994; Wright & Whitaker, 2001; Crawshaw, 1992; Raphael, 1993; Smith, 2007), but is apparently of little use against such infections in *Hynobius* egg sacks.

The egg sacks and the ova contained therein proved to be relatively robust against interference and tolerated partial or complete opening of the sack, removal of non-viable ova and even incubation once separated from the sack for the duration of development. Methods involving such manipulation of the sacks were much more successful in



**Figure 1.** Methods, qualitative and quantitative results of techniques trialled to address water mould infection of non-viable eggs in the egg sacks of *H. dumii*. Each paired egg clutch was split between control and trial conditions; graphs show median percentage of fertile eggs that survived to hatching across the number of trials indicated by number of sack pairs.

controlling saprolegniasis and hatch rates were very close to total fertility rates; once non-viable ova were removed as a food source for water moulds, the pathogen was apparently unable to infect developing embryos. There was no observed difference in proportion of fertile ova that hatched between eggs kept in methylene blue solution (made up from aquarium water) and those in standard aquarium water after being separated from the egg sack. Re-closing an opened egg sack with nylon fishing line was trialled with success (Fig. 1), but this does not appear to be necessary to ensure further development and needed to be removed in order to allow larvae to escape at eclosion.

The data presented here are not well replicated, but the combination of the use of a split clutch design and the magnitude of apparent positive effects suggests that they are likely to be realistic.

These results are for the egg sacks of *H. dunni* only and data are not available for other *Hynobius*. However, it seems likely that such techniques could be transferred to other species as egg sack structure is very similar. These techniques are likely to be limited to use in captivity only, as the tough outer membranes of the egg sacks of *Hynobius* are important in protecting ova from mechanical damage and from predation. Opening the egg sack, especially early in development, will also allow individual ova to fall out. The use of nylon fishing line to reseal egg sacks may allow egg sacks to retain protective function and so extend some of the methods trialled here to be used in wild sites, but repeat visits to free larvae trapped in the sack at hatching would be necessary. Given the threatened status of some *Hynobius* (including *H. dunni*; Kaneko & Matsui, 2004), these data may have relevance conservation projects, both for application in the field to improve recruitment, and in captivity to maximise the output of captive breeding programmes.

The tolerance for egg sacks being cut open and the possibility of incubating eggs successfully to hatching outside of the egg sacks may be useful beyond controlling the spread of *Saprolegnia*-like infection. Several *Hynobius* species are used as model systems in laboratories for, inter alia, developmental, evolutionary and ecological work (e.g. Michimae & Wakahara, 2002; Nishihara, 1996; Moriya, 1982; 1983; Wakahara, 1994). The production of egg sacks makes it difficult to use hynobiids for studies requiring clutches to be split into groups before hatching or for embryos to be clearly visualised or manipulated. Therefore, opening egg sacks early in development may allow for a greater variety of experimental work to be performed on this group.

## ACKNOWLEDGEMENTS

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# Using call playbacks to investigate a population of non-native midwife toads *Alytes obstetricans* (Laurenti, 1768) in Cambridge, UK

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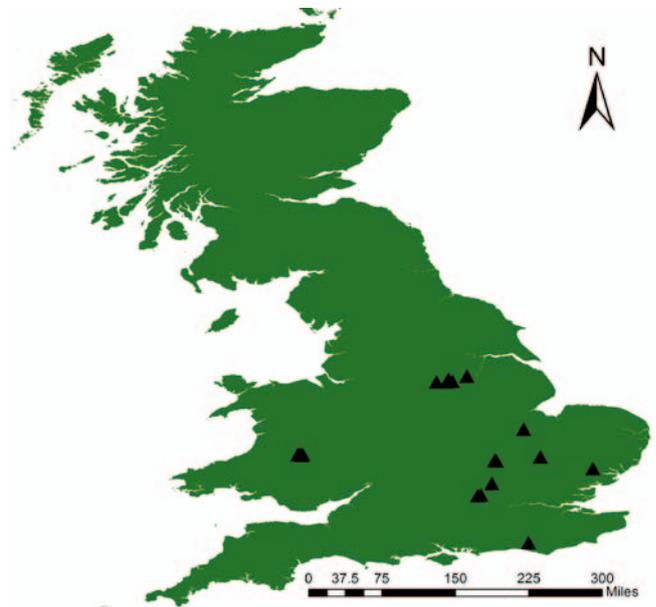
**ABSTRACT** - We report on a lesser known midwife toad (*Alytes obstetricans*) population within central Cambridge, England, UK with an initial investigation of the population's extent. In order to achieve this we used call playbacks to locate and count individual toads which responded with a return call.

## INTRODUCTION

The common midwife toad (*Alytes obstetricans*) is a widespread anuran found throughout most of western Europe (Gasc et al., 1997). This species, although not native to Britain, can be found in isolated populations across England (Fig. 1) due to the presence of escaped pets and intended releases (Muir-Howie, 2007). *A. obstetricans* are nocturnal and do not congregate around breeding ponds like the native UK amphibian species (Beebee & Griffiths, 2000). There is a need to monitor the impacts of non-native species such that intervention to prevent them from causing damage or harm to local species and ecosystems can be enacted where necessary (Manchester & Bullock, 2000); for such a secretive species an unusual method was developed to aid in this. A population of *A. obstetricans* is known from central Cambridge, England (Baker, 2007), and the aim of this study was to determine the range of toads in this area using recorded call playback and toad response to estimate the number of calling males. This is an established method in other demographic studies in a variety of species (e.g. Lor & Malecki, 2002; Sung et al., 2005) but relatively novel in terms of amphibians. This method was combined with active searches in order to locate females, juveniles and non-calling males. Our method was combined with the aim of trying to establish the full extent of the Cambridgeshire population, the expected hypothesis was that the toads occurred within all of the gardens of the houses of our survey area.

## METHODS

Communication with local residents identified an area in which *A. obstetricans* was most likely present. To gain access to the properties for surveying, we performed an informative mail drop after reports from a successful outreach and social media campaign. This established the presence of *A. obstetricans* in a smaller area than previously hypothesised. The Dragon Finder App from Froglife, UK, was used to play a pre-recorded mating call of male midwife toads. Both an



**Figure 1.** A map of the known UK distribution of midwife toads

Apple iPhone 5 and iPhone 6S were used to play the call using the app in order to elicit a response from males within our search area, which we hoped would illicit a response. Surveys were undertaken during 2015 and 2016 in the summer and autumn months. When a toad was discovered, dorsal photographs of each individual were taken. Toads could then be individually recognised by the manual comparison of these photos.

## RESULTS & DISCUSSION

During the 2015 season we made six visits to the gardens of three houses between the 26th July 2015 and 6th October 2015; this is not the peak breeding season. In 2016 we returned four more times between 14th June and 22nd August. Using the MAGIC Map Application and its measuring tool, we estimated the overall extent of the population to be 2200 m<sup>2</sup>.



**Figure 2.** An individual of *A. obstetricans* from the Cambridge population

On July the 26th of July 2015 we observed one male carrying a string of approximately 16 eggs; tadpoles have never been observed by us at any of the sites. In 2016, we used the same methodology and over the course of four visits we were able to locate a total of seven toads. We were able to confirm that the toads (Fig. 2) were present in the rear gardens of two rows of parallel Victorian houses in the heart of the city. No tadpoles of *A. obstetricans* were found. The larvae have been known to overwinter in the UK but we found no evidence of this during our surveys in the winter months. The gardens were also home to the common frog (*Rana temporaria*), common toad (*Bufo bufo*) and smooth newt (*Lissotriton vulgaris*).

As in other populations of *A. obstetricans* in the UK, the Cambridge population is small, localised and present in a highly urbanised environment (Beebee & Griffiths, 2000). The local distribution is more restricted than previously thought, with the toads occurring within only one third of the area we originally expected them to occupy; therefore we accept the null hypothesis. This expected range was based on the communication with local residents; if residents had the toads in their garden then they may have expected their neighbours did too. The restricted range of *A. obstetricans* in Cambridge may be directly linked to the urban gardens being enclosed rendering longer distance movement less unlikely since as well as walls and houses they are also surrounded by four relatively busy roads which must act as barriers to movement. Some dispersal has occurred since their initial introduction but only within a restricted area.

However, *A. obstetricans* could be heard calling from gardens close by, with up to a dozen males heard during one visit. Further study is needed as there were clearly more males present than we could identify. Due to their size, colouration and habits the toads were difficult to locate and success in being able to locate male toads is almost solely due to the Dragon Finder App from Froglife. Also lack of eye-shine and the way their call is reflected from surrounding surfaces such as walls and fences increases detection difficulties. Unfortunately the pre-recorded calls from the app were taken at an undisclosed location so we cannot infer how this regional call may affect our method for such a widely distributed species as *A. obstetricans*.

No visits were made in the spring since local residents reported not having heard the toads calling, despite a peak breeding activity between April and June (Beebee & Griffiths, 2000). It is unlikely that the toads bred earlier in the year and that their tadpoles have metamorphosed before our surveys. As the toads make a conspicuous call to attract mates to breed, the lack of calling would also indicate a lack of breeding. This also seems unlikely as metamorphs haven't been found during manual searches and males have been seen to be carrying eggs throughout both years of the study so far. None of the garden ponds have fish present which has made the absence of larvae even more puzzling.

Across the site visits that we made, male *A. obstetricans* were not seen to have been travelling any more than 20 m with the same individuals found in similar and not too distant locations on each visit. Individuals were identified using the dorsal photos taken during encounters and compared with one another. This is most likely due to the fact that the species has a low dispersal rate (Tobler et al., 2013).

*A. obstetricans* is clearly coexisting with native species and there is no evidence of negative effects on native amphibians, perhaps expected as they frequently occur sympatrically in continental Europe. The only potential threat is as a disease vector of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) which is responsible for amphibian declines globally (Skerratt et al., 2007). *A. obstetricans* has a low tolerance to the fungus (Bosch et al., 2001) although it has been shown that *A. obstetricans* colonies can persist if there is low chytrid fungus prevalence (Tobler et al., 2012).

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## Breeding biology of *Microhyla heymonsi* Vogt, 1911 (Anura, Microhylidae) from Kedah, Peninsular Malaysia

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Currently eight species of microhylids, including *Microhyla heymonsi* are present in Peninsular Malaysia (Amphibia my, 2009). *M. heymonsi* is a small-sized frog, with snout-vent length of males and females reaching at 16-21 and 22-26 mm, respectively (Amphibia my, 2009). This commensal species is widely distributed in Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Singapore, Taiwan, China, Thailand and Vietnam, and can be found from sea level up to 1,400 m asl (IUCN, 2015).

In Peninsular Malaysia, it can be encountered in clearings and disturbed forests (Berry, 1975; Ibrahim et al., 2008). Ephemeral pools (Ibrahim et al., 2008), temporary rain puddles, paddy fields, ditches, marshes and slow flowing streams (IUCN, 2015) are utilized as breeding sites.

In December 2015, between 2100 and 2300, two amplexed pairs of *M. heymonsi* were detected and captured at Sungai Sedim Recreational Forest, Kedah, Peninsular Malaysia (5° 25'N, 100° 46'E; < 150 m asl). Both amplexed pairs were collected at the edge of ephemeral puddle, approximately 2 m length, 1 m width and 2-15 cm depth, and located at the open areas, approximately 6 m from the main river. The puddle was directly exposed to the sunlight, and these usually dry up within two weeks, if there is no rain. Other frog species, including *Polypedates leucomystax* and *Fejervarya limnocharis* were also sighted around this area. Both amplexed pairs were captured after heavy downpours. The air temperature and relative humidity of the sampling site were 23° C and 82%, respectively.

In the laboratory, each amplexed pair was reared in a

medium-sized aquarium (60 x 30 x 30 cm), consisting of tap water, dead leaves and drift woods. Both pairs displayed axillary amplexus, where males clasped the females at axillary area. The amplexus activity persisted until the females deposited eggs, with both females depositing eggs in the aquariums within 10 hours after capture. The eggs were rounded in shape, pigmented, dark-brown in colour, coated with viscous jelly and floating on the surface of the water. The egg clutches were composed of 386 and 492 eggs. Five eggs from each clutch were randomly selected and measured its diameter by using a microscope, accompanied with ocular micrometer. The mean  $\pm$  SD (min-max, N) of egg diameter was  $0.9 \pm 0.11$  (0.8-1.1, 10) mm. The snout-vent length (SVL), head width (HW) and mass (W) of male (SVL=21 and 18 mm; HW=7 and 7 mm; W=2 and 1 g) and female (SVL=24 and 27 mm; HW=7 and 8 mm; W=2 and 3 g) specimens were measured by using digital calliper and electronic balance. SVL was measured from the tip of the snout to cloacal area, HW is the widest area of the head, and W is the weight of the specimens. All specimens were released back to their natural habitats.

Each egg clutch was raised in the same aquarium (60 x 30 x 30 cm) until hatching. The aquarium consisted of tap water, dead leaves and an aerator to supply oxygen. Between 48 and 60 hours from oviposition the eggs from both clutches hatched. Only 342 (89%) out of 386, and 428 (87%) out of 492 eggs were hatched and became free swimming tadpoles. At this phase, the size of the tadpole is approximately 2-3 mm (Gosner's stage 19). After a week, the mean  $\pm$  SD



**Figure 1.** An adult male of *M. heymonsi* from Kedah, Peninsular Malaysia



**Figure 2.** Axillary amplexus performed by *M. heymonsi*



**Figure 3.** Free swimming tadpoles of *M. heymonsi*

(min-max, N) total length of the tadpole was  $4.5 \pm 0.71$  (4-6, 10) mm (Gosner's stage 25). The tadpole is dark-brown to black in colour, slender in shape, tapering tail, dorso-lateral eyes, and having a white spot on its head. The tadpoles are filter feeders, frequently swimming on the surface of the water. Previous studies on reproductive biology of a range of frogs from Peninsular Malaysia and Singapore have been carried out, including Berry (1964), Ibrahim et al. (1999), Sheridan et al. (2009) and Shahriza et al. (2010, 2012, 2015, 2016). This work adds to this knowledge and increases our understanding on frog breeding biology, especially from tropical region. Photos of adult male, amplexed pair and tadpoles of *M. heymonsi* are shown in Figs. 1-3.

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# Notes on the breeding ecology of monocled cobras (*Naja kaouthia*) from areas adjacent to Sundarban, India

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## INTRODUCTION

Monocled cobras (*Naja kaouthia*) are the commonest venomous snakes in Sundarban and adjacent areas such as Canning (West Bengal, India). Though these snakes are a major cause of venomous snakebite and an important component of the ecosystem in this area, their ecological and behavioural aspects have received limited attention. As in other *Naja* spp., *N. kaouthia* are egg layers but periods for egg deposition vary. For example, Kopczynski (1993) reported that captive monocled cobras, imported from Thailand, deposit eggs between January and March whilst Das (2002) and Whitaker & Captain (2004) indicated January – March as the egg laying season. The incubation period of *N. kaouthia* is around 50 days (Kopczynski, 1993; Das, 2002) indicating hatchlings should appear from the end of February to May. However, Ahmed et al. (2009) reported that neonate cobras have been observed in Assam (India) in August which suggests *N. kaouthia* may have different breeding seasons in different regions. In this paper we present observational data on the breeding season of *N. kaouthia* from areas (Canning [88.76 E, 22.32 N] and Taldi [80.61 E, 22.35 N], Fig. 1) adjacent to the Sundarban mangrove forest in south West Bengal state, India.

## METHODS

A local organisation – Canning JSS – conducts snake rescue operations in these areas and rescued snakes are handed over to forest department officials of the Sundarban Tiger Reserve (STR). Between 1 September, 2016 to 31 April, 2017 we accompanied the rescuers of Canning JSS when they received calls for snake rescue. Whenever juvenile *N. kaouthia* were found, their snout to vent length (SVL) and tail length (TL) were measured and photographs were taken. To reduce stress to the snakes and also to minimise the risk of the rescuers, scale count and sex determination were not carried out.

## RESULTS & DISCUSSION

Neonates were rescued from October to early December (Fig. 2). In several instances, multiple snakes were found at the same site. The first six individuals were rescued on 3 October, 2016 from a hole in an abandoned house at Matla G.P. area, Canning (22.3133 N, 88.7344 E) and had a mean SVL of 30.07 cm and mean TL of 5.5 cm, the smallest

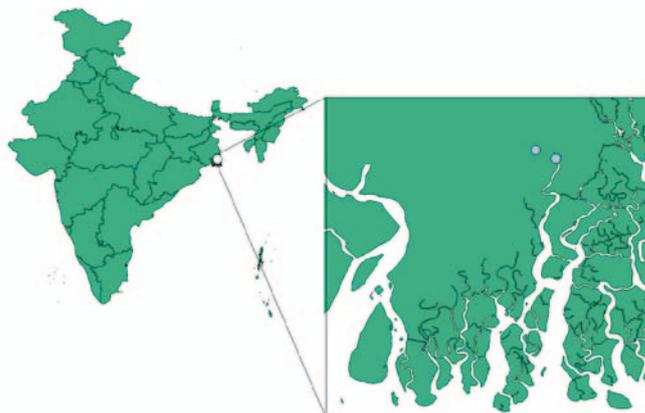


Figure 1. Map showing the locations of rescue

having a SVL of 28 cm and a TL of 5 cm. The highest number of juveniles (N = 19) were rescued in November. The last five snakes were found on 1 - 2 December. The data are tabulated in Table 1. It was reported that baby cobras first emerge in late September and females observed guarding eggs from late July to August (Joydeb Sardar pers. comm.). No juveniles were reported between December to early February, presumably due to this being the hibernation period. The first individual to be found after winter was rescued on 14 March, 2017 with a SVL of 41 cm and TL of 7 cm. These data therefore suggest that *N. kaouthia* show a late rainy - early winter reproductive season in this area.



Figure 2. Juvenile *N. kaouthia*

**Table 1.** Table summarising the measurement data of rescued juvenile monocled cobras (*N. kaouthia*); SD = Standard Deviation, Min. = Minimum SVL, Max. = Maximum SVL

Month (Year)	Number	SVL (mean $\pm$ SD)	Min. - Max.	TL (mean $\pm$ SD)
October (2016)	10	30.8 $\pm$ 1.95	28 - 35	5.67 $\pm$ 0.47
November (2016)	19	31.89 $\pm$ 1.99	29 - 36	6.08 $\pm$ 0.67
December (2016)	5	33.4 $\pm$ 2.77	29 - 36	6.1 $\pm$ 0.89
March (2017)	1	41 $\pm$ 0	-	7.0 $\pm$ 0

*N. kaouthia* in Sundarban, a low-lying coastal area, are said to be semi-aquatic (Whitaker & Captain, 2004). In this region agricultural areas (mainly paddy fields, a favourite habitat of *N. kaouthia*) remain wet from July to December. Being a coastal area, here winter is brief but cold (around 10° C) with lowest temperatures in January. These climatic factors likely play important roles in the breeding season of *N. kaouthia* in Sundarban and adjacent areas.

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## Genus *Malpolon*: New distribution area in Algeria

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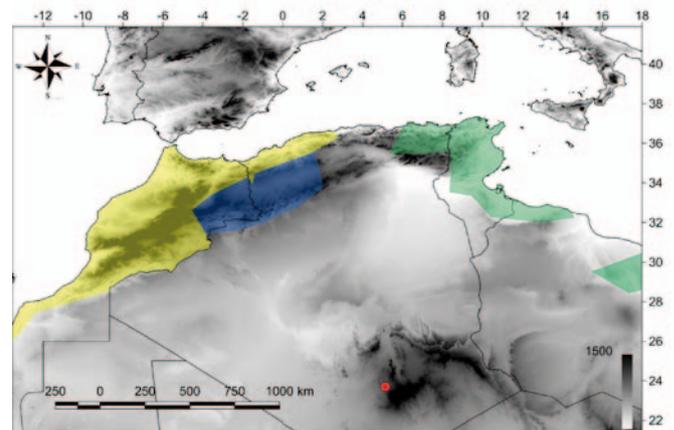
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The snakes of the genus *Malpolon* are distributed in the circum-Mediterranean region, and comprise two species *M. monspessulanus* (Hermann, 1804) (with two recognised subspecies in northwestern Africa, *M. monspessulanus monspessulanus* and *M. monspessulanus saharatlanticus*) and *M. insignitus* (Geoffroy de St-Hilaire, 1827) (Carranza et al., 2006). Both species appear in northwestern Africa, but *M. insignitus* occupies the eastern part of region, in Algeria, Libya and Tunisia and possibly also occurs in eastern Morocco (Geniez, 2015). In Algeria *M. insignitus* has been recorded in the mesic regions of the northeast of the country, between the wilaya of Constantine and the Tunisian border, its presence being progressively scarcer towards the arid regions of the south, reaching its limit in the region of Aurès (approximately at 35.30°N, 6.48°E; Sindaco et al., 2013).

In June 2015 female of a *Malpolon* species (Fig. 1) was found by the first author in activity among some riparian bushes. The site was located in the vicinity of the town of In Amguel (Tamanrasset, southern Algeria), at the coordinates 23.6930278° N, 5.1436389° E (WGS84 datum), elevation = 966 m (Fig. 2). The females of the two *Malpolon* species are difficult to differentiate, although those of *M. insignitus* show a pattern of 2–3 longitudinal discontinuous stripes on the flanks (Fig. 1), which in *M. monspessulanus* are not usually aligned (except in the southern form *M. monspessulanus saharatlanticus*; Geniez 2015). Nevertheless genetic studies of this population would be required to determine the species. The area of In Amguel is situated in the BWh (tropical desert) climate belt (Köppen-Geiger classification), with an average annual precipitation of 28 mm and annual average temperatures of 23.3°C. The habitat is formed by scattered patches of riparian trees (date palm trees) and bushes in a wadi (oued Tekouiat), close to a small town and cultivated lands. This new record significantly extends the southerly distribution of *Malpolon* in Algeria (approximately 1300 km) and is, together with those located in the Western Sahara, the southernmost of the genus. This observation adds another reptile species of Mediterranean origin found in the Hoggar Mountains. Species found previously include the skink *Chalcides ocellatus* and the snake *Macroprotodon* sp. (Sindaco & Jeremcenko, 2008; Sindaco et al., 2013). These reptile populations may be relics of a more humid period in the central Sahara (Neolithic subpluvial, 7000–3000 years ago; Hays, 1972), persisting in this hyperarid region favoured by the milder conditions offered by the Hoggar mountains (Cuesta et al., 2010).



**Figure 1.** Female of the *Malpolon* sp. (dorsal, ventral and head detail), preserved specimen from Amguel, southern Algeria. Photographed by B. Bakhouché.



**Figure 2.** Map of north-west Africa showing the known distribution of *M. insignitus* based on Sindaco et al., 2013 (green area), possible distribution of *M. insignitus* without genetic evidence (Carranza et al., 2006; blue area), distribution of *M. monspessulanus* (yellow area), and the new location (red dot).

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## The first record of a Slow-worm (*Anguis fragilis*) from the UK with blue ventral scales

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During a reptile translocation on 20 July 2016 at 09:30 hrs, a juvenile slow-worm *Anguis fragilis* was observed beneath an artificial refuge (roofing felt, 0.5 x 0.5 m). This individual was estimated at around 9 cm in length and had the typical dorsal colouration for *A. fragilis* given the size. It was not until capture that the blue ventral and subcaudal scales were observed. Juveniles typically have a colour pattern resembling an adult female with silver, copper, gold or bronze dorsal scales (Beebee & Griffiths, 2000). Juveniles also possess an enlarged black spot, known as a parietal spot, joining a black vertebral stripe that runs towards the end of the tail (Beebee & Griffiths, 2000; Platenburg, 1999). This is illustrated in Fig.1, which shows the anterior dorsal and posterior ventral of the lizard. The scales of the entire ventral surface of the lizard were a uniform pale blue colour.

The lizard was located within the district of Taunton, Somerset, England (alt. 25 m) but co-ordinates have been withheld due to confidentiality issues. The habitat was classified as semi-improved neutral grassland with sections of ruderal vegetation, intact hedgerows and fencing (JNCC, 2010). The wider area consisted of residential housing, arable and pastoral fields.

Blue colouration is well documented in *A. fragilis* with numerous studies focusing on the prevalence of dorsal blue spots in various populations (e.g. Capula et al., 1997; Platenburg, 1999; Sos, 2011). These blue spots can change in an individual over time, an aspect that was particularly thoroughly studied by Simms (1970). Completely blue ventral scales appear to be most unusual, however. In relation to UK, Beebee & Griffiths (2000) wrote "Both sexes have... a slate-grey, bluish or black belly...". Jablonski & Meduna (2010) described a male *A. colchica* with blue ventral scales, and Kaczmarek et al. (2016) described blue colouration of the ventral scales in two female *A. fragilis* from Poland.

It is possible that these observations are just a rare colour variation within populations but as Kaczmarek et al. (2016) hypothesised, high levels of sex hormones may be the cause. This hypothesis requires further study. Overall, further observations on blue ventral coloration in slow-worms would be welcome, since the true prevalence of this colour variation is unknown.

### ACKNOWLEDGEMENTS

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**Figure 1.** A juvenile slow worm showing typical dorsal colouration with blue ventral and subcaudal scales

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# Active male-male competition for mate access in the giant parrot snake *Leptophis ahaetulla* (Squamata: Colubridae), in the southwest Amazon, Brazil

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The genus *Leptophis* comprises 11 species occurring through the Neotropics and Mexico (Oliver, 1948). The largest species, *Leptophis ahaetulla* Linnaeus, 1758, is a medium-sized colubrid that reaches a snout-vent length of 958 mm in males and 835 mm in females (Albuquerque, 2008). The species is widely distributed, with records from Mexico to Argentina (Albuquerque, 2008; Oliver, 1948), though not from the Cerrado biome (Bernarde et al., 2012). Certain aspects of *L. ahaetulla* biology have been documented: it has a wide habitat tolerance, having been reported in both primary forests and disturbed environments (Martins & Oliveira, 1998), is semi-arboreal in habit and diurnal in activity patterns (Albuquerque et al., 2007; Martins and Oliveira, 1998). These snakes are oviparous and few aspects on their reproductive biology have been reported, including eggs and clutch size (Albuquerque, 2008; Linardi, 2016; Rand, 1969), communal nests and neonate biometry (Linardi, 2016). This paper presents the first report of male-male competition in *L. ahaetulla*.

On 20 October 2015 at 16:10 hrs, a pair of *L. ahaetulla* were observed in copulation, with a second male attempting to dislodge the first. Observations were made in a protected forest fragment at the Rondon II Hydroelectric Power Plant (11°58'40"S, 60°41'58"W, 296 m.a.s.l.), in Pimenta Bueno municipality, Rondônia State, the southwest Amazon, Brazil. The animals, two similar sized males (estimated 800 mm in total length) and a smaller female (around 700 mm), were first encountered at about 1.30 m above the ground in the branches of a small tree (*Casearia* sp., Salicaceae) and engaged in reproductive activity when detected (Fig. 1). As the second male approached, the male that was copulating began to move higher into the branches, dragging the female with him. The second male responded by following the pair and attempting to wind his body around the body of the first male. This continued until the animals reached the end of a tree branch some 4.5 m above the ground within the canopy. At this point, the second male bit the first one in the neck. Finally, the three snakes fell to the ground and the mating pair separated and all three exited in different directions across the forest floor. The entire sequence of observations lasted around 15 minutes.

We did not observe directly the initial pre-copula behaviour of the pair; but likely these performances were



**Figure 1.** Male-male competition between two males of the snake *L. ahaetulla* (climbing together the branches at the right hand), while one male was already mating with a female (located at the left side)

similar to those reported by Cruz-Lizano et al. (2013), where the male approached the female, aligned their bodies and began to roll over the female's body with caudocephalic waves. In contrast to the observations of Cruz-Lizano et al. who reported copulation in late January, during the driest season in their region, our observation occurred when the regional climate was changing from the dry to wet season. If the 89 day incubation period reported by these authors also occurred here, this would indicate hatching in the middle of January in our study area, a timing also observed by Linardi (2016) for the species in São Paulo State, south-eastern Brazil. Our report showed that access for mating can generate active competition among *L. ahaetulla* males. Though larger size in females allows greater reproductive capacity, it is common in many Colubrid genera for males to be the larger sex (Bonnet et al., 1998), but this is the first report in this genus. Such activities are generally associated with active male combat (Shine, 1978), which would appear congruent with the reproductive patterns observed here for *L. ahaetulla*.

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## Two types of defensive behaviour recorded in *Bufotes pewzowi* (Bufonidae)

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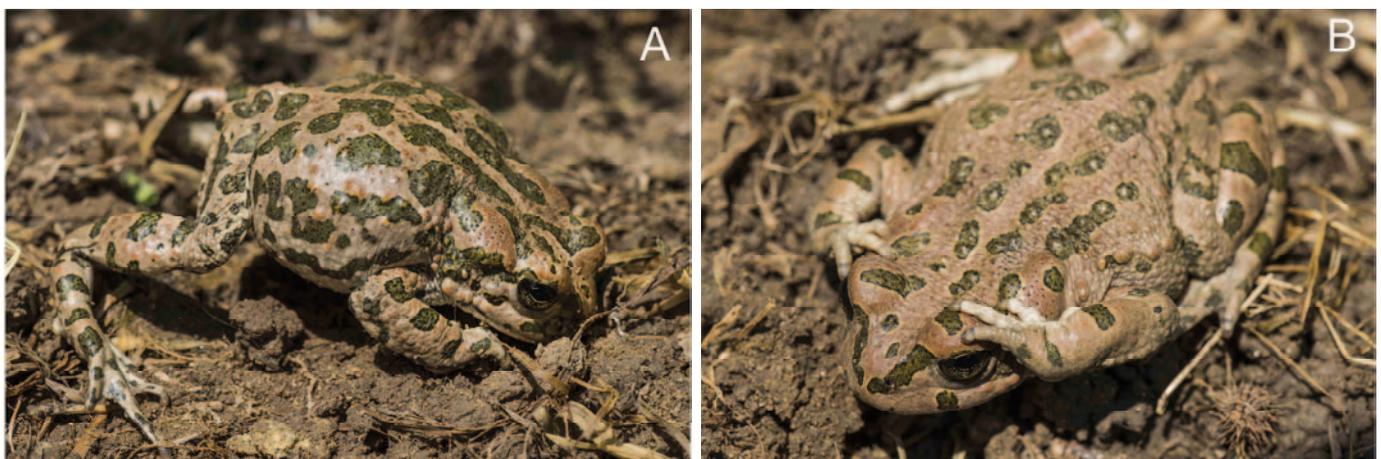
As a common prey for a variety of predators (Duellman & Trueb, 1994; Toledo et al., 2007), amphibians employ several defence strategies (Toledo et al., 2011 and literature therein). These mechanisms, which evolved through increased survival rate, may be displayed together with other related antipredator strategies, such as cryptic coloration or skin toxins. The Xinjiang toad, *Bufotes pewzowi* (Bedriaga, 1898), is distributed in Central Asia from Uzbekistan, possibly Afghanistan and Tajikistan, eastwards to eastern Kazakhstan, western Mongolia and western Xinjiang and Xizang provinces in China (Stöck et al., 2001; Litvinchuk et al., 2011). This species is a member of the Western Palearctic green toads of the *Bufo viridis* subgroup and is relatively common and ecologically plastic, with the ability to survive in very disturbed habitats (Stöck et al., 2015). However, little is known about its ecology and behavioural patterns. Here I describe two new types of defensive behaviour for *B. pewzowi* observed in Fergana valley, Kyrgyzstan.

During fieldwork on 5th May 2015 between 1200-1300h, two adult females of *B. pewzowi* were located under stones in warm, grassy habitat to the east of Jalal-Abad town, Kyrgyzstan (40.9405°N, 73.0363°E, WGS84, 967 m elev., Fig. 1). The first individual initially tried to escape but, when surrounded from all sides, it quickly inflated and vertically raised its body on its hind legs. The head (snout) remained on the ground (Fig. 1A). When the disturbance (a camera) was removed the individual resumed a normal posture, although it resumed the defensive posture when it was again disturbed. This behaviour (named body-raising with

legs vertically stretched) is very well known in amphibians, mostly in toxic species (e.g. *Bufo bufo*, *Rhinella marina*; Toledo et al., 2011). According to Toledo et al. (2011), this body-raising type is displayed in two forms where this case represents partial body-raising in which the individual stretches the legs vertically and keeps its snout close to, or touching, the ground (Fig. 1A).

The second animal immediately took up the defensive posture after direct touch. The individual flattened and slightly arched the body, closed its eyes, and lifted its front limbs to position them alongside the head (Fig. 1B). The specimen remained in such a posture for a couple of minutes, and then returned to a normal position. This behaviour is described as eye-protection (Toledo et al., 2011). During which individuals cover their head, eyes and tympanum with the forearms. In this case the eyes were open but some species close them during the defence.

Eye-protection (most common among species that display body-raising of the first type) may co-occur with body-raising. Some authors discussed this behaviour as a response on hypothermia of individuals during disturbing (Haberl & Wilkinson, 1997) but this not to be an initiating factor in general (Jablonski & Balej, 2014 and literature therein). Both defence strategies are known in the family Bufonidae (Brusquetti et al., 2007; Sharma et al., 2011; Toledo et al., 2011) but, according to the available literature, eye-protection is very rare in this family. To my best knowledge these defense behaviours have never been recorded in *B. pewzowi*.



**Figure 1.** Two individuals of *B. pewzowi* displaying defensive behaviour: body-raising with legs vertically stretched (A) and eye-protection (B)

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# Probably first reported case from Indonesia of a lizard (Scincidae: *Lipinia* sp.) being preyed upon by a spider (Sparassidae: *Pandercetes* sp.)

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Arthropods such as spiders have occasionally been documented as predators of lizards. The majority of observations have been made in Europe, only very few reports exist from Asia (e.g. Priyadarshana & Wijewardana, 2016). We here report on the first documented case of predation by an arachnid on a lizard in Indonesia. This occurred on Batudaka Island (0°24'43"S, 121°51'54"E), Togian Islands, in Central Sulawesi Province. During field work on 9 July 2006 around 17.00h, we observed a palm-sized spider (Sparassidae: *Pandercetes* sp.) at about eye-level on a lichen-covered trunk in a rather wild cacao (*Theobroma cacao*) plantation. It had caught a small skink (Scincidae: *Lipinia* sp.) whose head was held in the spider's chelicerae with the rest of the body hanging motionless down the trunk (Fig. 1). The hairy and whitish spider was well-camouflaged on the bright lichens that covered large areas of the tree bark.

After taking some photographs of both specimens *in-situ*, capture was attempted but the spider escaped and the presumed dead or numb lizard fell to the ground. Despite thorough attempts to trace the skink it could not be found. From the photographs taken, however, the specimen can at least be assigned to genus level. The colour pattern consisting of a prominent golden-yellow longitudinal stripe along the back is typical for most members of *Lipinia* skinks (Greer, 1974). The second half of the tail is orange. On Sulawesi, only two species of this Asian lizard genus are known with certainty (Koch, 2012), viz. *L. subvittata* and *L. infralineolata*. A third species, *L. quadrivittata*, has been listed for that island (Iskandar & Tjan, 1996) but is probably restricted to the Philippines (Brown & Alcala, 1980; Das & Austin, 2007).

This observation adds to our knowledge of the predatory interactions between spiders and vertebrates. The fact that this report is probably the first documented predation of a lizard by a spider in Indonesia highlights the need for increased efforts to study in greater depths the ecological relationships of the diverse herpetofauna of this tropical island nation.

## ACKNOWLEDGEMENTS

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**Figure 1.** A sparassid spider (*Pandercetes* sp.) on the lichen-covered trunk of a cacao tree with its reptilian prey, a *Lipinia* skink, on Batudaka Island, Togian Islands, Central Sulawesi, Indonesia

Pak Tarmin (Conservation International), his family, and the children of the village Langger for hospitality and manifold support during our stay on the Togian Islands. Peter Jäger (Frankfurt) helped to identify the lizard-preying spider. Rafe Brown (Lawrence) kindly shared his observations from the field with us. An earlier draft of this paper benefited from critical comments by Robert Neal (Brisbane) and one anonymous reviewer. Field work in Sulawesi and the Togian Islands was supported by a PhD grant (AK) from the Evangelisches Studienwerk e.V. Villigst.

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