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**Front Cover:** A Persian horned viper (*Pseudocerastes persicus*) found in Wadi Qada'a, United Arab Emirates.  
See article on page 28. Photographed by Oliver Thomas



# Anomalous colour in a Cuban cave-dwelling frog: First record of piebaldism in *Eleutherodactylus zeus* (Anura: Eleutherodactylidae)

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**ABSTRACT** - Pigmentation anomalies may occur due to genetic or environmental factors and can affect restricted parts of the body or the entire surface. *Eleutherodactylus zeus* is frog endemic to western Cuba where it is adapted to life in caves, rock crevices, and other sheltered sites in limestone landscapes associated with forest habitats. We observed 43 frogs in Santo Tomás cave, in Viñales National Park, of which 26 % showed depigmented blotches, typical of piebaldism, along their bodies. No unusual behaviour was detected in any of these frogs. This is the first reported case of piebaldism in frogs of the West Indies and consequently of Cuba. Records of piebaldism in amphibians are very scarce in the literature, not necessarily as a consequence of its rarity in nature but possibly due to inconsistencies in the classification of pigmentation abnormalities.

## INTRODUCTION

In spite of their largely nocturnal habits, amphibians exhibit an impressive diversity and complexity in their colour patterns (Hoffman & Blouin, 2000). Amphibian chromatophores are located in either the epidermis or dermis, and they contain pigment granules that vary in chemical composition (e.g. carotenoids, pteridines) resulting in different colours and patterns (Bagnara et al., 1968). However, chromatic disorders occur due to genetic or environmental factors, resulting in pigmentation anomalies that are restricted to a part, or the entire, body surface. Among the main recognised types of pigmentation anomaly are albinism, axanthism, leucism and piebaldism (Bechtel, 1995; Lucati & López-Baucells, 2016). Piebaldism is defined by Lucati & López-Baucells (2016) as “all-white fur/skin patches and eyes normally colored”. It differs from leucism where the entire body is white but the eyes are normally coloured or albinism where there is an unpigmented body with reddish or pink eyes (Lucati & López-Baucells, 2016).

*Eleutherodactylus (Syrrophus) zeus* Schwartz, 1958 is a frog endemic to western Cuba. It is a cave-dwelling species adapted to life in caves, rock crevices, and other sheltered sites in limestone landscapes associated with forest habitats of the Cordillera de Guaniguanico (Alonso Bosch & Rodríguez Gómez, 2003; Díaz & Cádiz, 2008; Henderson & Powell, 2009). The species has been IUCN listed as endangered because it is restricted to an area of less than 5000 km<sup>2</sup>, its distribution is severely fragmented, and there is continuing decline in the extent and quality of its habitat in western Cuba (Hedges & Díaz, 2004). The Red Book of Cuban Vertebrates (Gonzalez et al., 2012) re-evaluated the conservation status of 61 species of amphibians in Cuba and lists 27 threatened species but failed to include *E. zeus*.

The normal colour pattern of live adults of *E. zeus* (Fig. 1) is an olive brown with a mottled black dorsum and darker brown snout. The upper eyelids of most specimens are clear green. Postscapular spots are yellowish, arms and thighs have lichenous greyish markings. Concealed surfaces of thighs are dull brownish purple, dorsum of thighs mottled brown, tips of dorsal rugosities greenish or yellowish, giving a somewhat speckled and mottled appearance (Schwartz, 1958; Estrada et al., 1986). In this report we document piebaldism in *E. zeus*.

## METHODS

As part of monitoring initiative of *E. zeus*, we visited Santo Tomás cave, El Moncada, Viñales, Pinar del Río (22.544496°N, 83.846895°W, WGS 84, 230 m a.s.l.) in April and August of 2017. It is the only large *Eleutherodactylus* species present in this area, and in recent years some natural history observations on this frog have been collected by our team (Alonso Bosch et al., 2007; García, 2012; Alonso Bosch et al., 2015). The cave gallery we visited had a small entrance (2 m high and 4 m wide) that was shaded by the forest so that only limited day light (visible to human eye) reached into the first 5 m of the gallery. Observations were made from the entrance to 220 m inside the cave, during the day (11:00h-14:00h) and night (20:30h-01:00h), using headlamps to locate active animals. When an individual was sighted, it was collected and marked with a unique combination of toe clips (Ferner, 2009) to prevent duplicate counts and afterwards it was released where captured. We paid attention to any unusual behaviour of the animals and their general body conditions. Photo vouchers were deposited in the herpetological collections of the Museo de Historia Natural “Tranquilino Sandalio de Noda” from Pinar del Río, and Museo de Historia Natural “Felipe Poey” from University of Havana, Cuba.



**Figure 1.** Colour variation in *E. zeus* from Santo Tomás cave, Cuba. Adult male with normal colour pattern (top left), adult female with piebaldism in the loreal area (top right), adult female with the loreal, eyelid, dorsal head, ear and dorsal body areas affected (lower left), and adult male with dorsal and lateral body areas affected (lower right).

## RESULTS

We captured a total of 56 *E. zeus* which amounted to 43 unique specimens after correction for repeat captures (Table 1). Eleven of these captures (5 adult females, 3 adult males, 2 adults of uncertain gender, and 1 juvenile) lacked normal colour patterning with depigmented blotches along their bodies (Fig. 1; Table 1). The affected individuals were observed from the entrance up to 120 m inside the cave. Most of the affected frogs were depigmented in more than one area, the head was the most affected area, ten frogs were depigmented in the loreal region, four frogs on the eyelids, three in the dorsal part of the head, and two in the tympanum (Fig. 1). Additionally, two frogs presented loss of pigmentation in the dorsal and lateral areas of their bodies (Fig. 1), and the fore and hind limbs of one frog were also affected. Our observations show a high rate of piebaldism with at most 11 out of 43 individuals (26 %) affected, with no obvious distinction between the sexes. However, no alteration in behaviour was detected in any of these frogs. All individuals were active during both day and night inside the cave, jumping, climbing, or vocalising. No signs of rash, itch, weight loss or fatigue were observed, all individuals were in good physical condition.

## DISCUSSION

This is the first reported case of piebaldism in frogs of the West Indies and consequently of Cuba. The piebald condition has been described as an intermixture of a pattern of localised irregular patches with an absence of pigment in an otherwise normally pigmented individual (Acevedo et al., 2009). Records of piebaldism in amphibians are very scarce (Dyrkacz, 1981; Bechtel, 1995; Jablonski et al., 2014), not necessarily as a consequence of its rarity in nature but possibly due to inconsistencies in the classification of pigmentation abnormalities. Nevertheless, piebaldism has been reported in Urodela: *Ambystoma mexicanum*, *Plethodon cinereus* and *Dicamptodon tenebrosus* (Neff et al., 2015) and in Anura it is known from a wild population of *Lithobates catesbeianus* (Whipple & Collins, 1990).

Active individuals have been sighted inside the caves during the day, while at night they usually vocalise from the ground and rocks both inside and outside caves. We found individuals of both sexes outside and inside (at different distances from the entrance) of the cave, with either normal or abnormal colour patterns. Consequently, we consider that darkness does not seem to be a determinant of the frequency



**Table 1.** Number of *Eleutherodactylus zeus* captured, recaptured and affected by piebaldism, M = males; F = females, J = juvenile, UD undetermined gender

|        |    |    |    |   | Recaptures |   |    | Affected |   |    |   |
|--------|----|----|----|---|------------|---|----|----------|---|----|---|
|        | M  | F  | UD | J | M          | F | UD | M        | F | UD | J |
| April  | 17 | 10 | 0  | 8 | 0          | 0 | 0  | 2        | 3 | 0  | 1 |
| August | 8  | 9  | 2  | 2 | 6          | 6 | 1  | 1        | 2 | 2  | 0 |

of the appearance of piebald individuals. Further studies are needed to clarify the frequency of this abnormality across western Cuba and to evaluate any biological implications that would be negative for the survival of this Cuban endemic cave-dweller.

## ACKNOWLEDGEMENTS

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## Do female northern vipers (*Vipera berus*) really stop feeding during pregnancy?

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**ABSTRACT** – A temporary reduction or even cessation of feeding has been documented in a wide range of vertebrate species and is usually attributed to conflicts between foraging and other activities. It is generally recognised that females of Old World vipers (*Vipera* spp.) reduce or even stop feeding during pregnancy, even though detailed quantitative information for most species is limited. We conducted a long-term (2000 – 2017) and intensive mark–recapture study in a large population of northern vipers (*Vipera berus*) and employed two indices of feeding frequency in adult females during their breeding years. The first index uses cross-sectional data and estimated that a strict minority (7 %) of captures of females in breeding years revealed detectable signs of recent food intake (swelling of the mid-body, voiding of solid faeces). A second index is based on the magnitude of increases in body mass over recapture intervals of individual females, which can be attributed to the intake of food. Depending on the criteria used to consider an increase in body mass as a consequence of prey consumption, about 12 % (range: 2 – 29 %) of recaptures revealed indications of prey consumption. Overall, we estimated that ca. 20 % of the reproductive females fed at least once before parturition. We thus confirm that most pregnant female *V. berus* fail to feed, but also point out that the number of females that feed occasionally during pregnancy is higher than has often been assumed. We suggest that reduced foraging by pregnant northern vipers is, presumably in part, a consequence of their behaviour of residing in habitats where feeding opportunities are rare because prey encounter rates are low.

### INTRODUCTION

Organisms feed to secure the resources needed for fuelling the metabolic demands of maintenance, growth and reproduction. Hence, organisms dedicate considerable amounts of time and effort to foraging activities. However, conflicts between foraging and other activities can result in the temporary interruption of feeding. For instance, reduction of food intake during the reproductive season has been observed in a wide range of vertebrates (Mrosovsky & Sherry, 1980; Sherry et al., 1980; Shine, 1980; Brivio et al., 2010), including males and females of both oviparous and viviparous species of snakes (Shine, 1980; Gregory et al., 1999; Lourdais et al., 2002a; O'Donnell et al., 2004; Brischox et al., 2011; Webber et al., 2012). Two broad categories of proximate mechanism may reduce feeding in reproductive animals: intrinsic factors that trigger anorexia (i.e., a loss of appetite; Mrosovsky & Sherry, 1980), or extrinsic conditions that reduce feeding occasions (i.e., spatial or temporal fluctuations in food availability; Shine et al., 2003; O'Donnell et al., 2004).

Reduced feeding by reproductive female snakes seems to be a general condition in Old World vipers (*Vipera* spp., Bea et al., 1992). These are typical live-bearing 'capital' breeders (Drent & Daan, 1980) that alternate breeding with non-breeding years. During reproductive years, females initially invest their energy reserves in vitellogenesis and subsequently use any remaining lipids to cope with metabolic costs as they diminish, or even cease feeding during pregnancy. After

giving birth females are emaciated, must restore their fat reserves before they can reproduce again, and therefore produce litters only biennially or even less frequently (Bea et al., 1992; Madsen & Shine, 1993; Bonnet et al., 2001; Lourdais et al., 2002a; 2002b; Pleguezuelos et al., 2007).

Reproductive investments and costs, and prey consumption by reproductive females have been particularly well studied in the aspic viper (*Vipera aspis*). Field data indicate that, depending on prey availability, females may feed during the egg production phase in spring, but they tend to cease feeding during the two months of pregnancy (Bonnet et al., 2001; Lourdais et al., 2002a). However, pregnant females readily accept prey in captivity, indicating that their appetite was not completely lost, so that there are other reasons for their failure to feed in the wild (Lourdais et al., 2002a).

Reduced feeding rates in gravid females have also been reported for the northern viper (*Vipera berus*). Examination of specimens in collections indicate that reproductive females feed occasionally during the period of vitellogenesis, but do not eat during pregnancy (Prestit, 1971; Nilson, 1981; Bea et al., 1992). However, published data from field studies are fragmentary, such that quantitative information on the extent of food intake by pregnant females is largely lacking. Moreover, pregnant females readily take food in captivity (Madsen & Shine, 1992; Völkl & Thiesmeier, 2002) and during our own field study we have sometimes noted that pregnant females exhibited considerable increases in body mass, which we tentatively attributed to food intake.

These considerations warrant a detailed and comprehensive examination of information gathered during our intensive field campaigns.

We present here data on the frequency of feeding by reproductive female *V. berus*, collected during a long-term (2000 – 2017) field study in a large population in northern Belgium. We used cross-sectional and longitudinal data to estimate the frequency of food intake during different phases of the reproductive cycle. Firstly, we recorded instances of food intake on the basis of visible signs (e.g., distension of the mid-body, voiding of solid faeces). However, the detection of prey remnants in living snakes is restricted in time, depending on the digestion rate, which would tend to underestimate the frequency of feeding occasions. We therefore also examined changes in body mass during recapture intervals of individual females. Increases in body mass during pregnancy have been shown to be the result of prey consumption and can thus be used as an index of food intake (Bonnet et al., 2001; Lourdais et al., 2002a).

## MATERIAL AND METHODS

### Study species and phenology

*Vipera berus* is a small, stout-bodied venomous snake that has a huge distribution area covering large parts of Europe and Asia. At our study site males and females reach maximal sizes of 55 cm and 60 cm respectively. Females are live-bearing; litter size varies between 4 and 12 and increases with female size and newborn snakes measure 13 – 16 cm SVL (Prestit, 1971; Madsen & Shine, 1992; own observations).

The annual cycle of the vipers in our study area coincides generally with that observed in other regions (Prestit, 1971; Nilson, 1980; Andr  n, 1985; Madsen & Shine, 1993; Madsen et al., 1993; V  lkl & Thiesmeier, 2002; Phelps, 2004). Adult females emerge from hibernation around mid-March, and those that have accumulated enough reserves initiate vitellogenesis during April. Follicular growth involves an intensive mobilisation of maternal reserves and is finalized upon ovulation around mid-May. The reproductive females stay near the overwintering areas, where they spend the summer carefully thermoregulating to enhance development of the embryos. The fully developed young are born during the second half of August or the beginning of September. Hibernation starts during October.

### Study area and data collection

Data were collected during a long-term citizen science population study (2000 – 2017) of northern vipers in the "Groot Schietveld" (ca. 1570 ha; N 51   20'22" – E 4   32'37", province of Antwerp, Belgium; detailed description in Bauwens & Claus, 2018).

Snakes were located by sight while walking slowly and erratically through the terrain, captured by hand and released immediately after handling. A digital photograph of the upper side of the head allows individual identification of vipers, on the basis of the number, shape and arrangement of the head scales (Bauwens et al., 2018). At every encounter we recorded date, time, exact location (GPS coordinates), sex, snout-vent length (SVL) (to the nearest 5 mm), and body

mass (to nearest 1 g). To obtain an index of body condition (BCI) for females, we first calculated the baseline relation between logMass and logSVL for female vipers, excluding measurements taken after recent food intake or recent parturition ( $\log\text{Mass} = -2.678 + 2.732 \log\text{SVL}$ ,  $r^2 = 0.968$ ). Next we estimated body condition as the difference between the observed mass and the mass predicted by the baseline relation, a procedure that is analogous to the calculation of regression residuals (Schulte-Hostedde et al., 2005).

Reproductive status (reproductive/breeding versus non-reproductive/non-breeding) of potentially mature adult females (in at least their fourth activity season,  $\text{SVL} \geq 38$  cm; D. Bauwens & K. Claus, unpublished data) in a given year was determined by palpation of the abdomen to detect oviductal eggs or developing embryos during May – August and/or by signs of postparturient body condition (i.e., presence of flaccid abdomen and extensive skin folds) in August – October. To avoid false judgements of non-breeding in a given year, this status was assigned only to females that did not show signs of pregnancy during June – August or indications of recent parturition in September – October.

### Evaluating feeding status

We addressed the incidence of food consumption by reproductive females in two complementary ways: 1) by direct indications of food intake, and 2) by examining changes of the body condition index (BCI) at successive measurements of individual females.

Adult vipers feed primarily on prey (mice, voles, frogs) that are large relative to their body width (Prestit, 1971; Bea et al., 1992; V  lkl & Thiesmeier, 2002) such that the recent swallowing of prey can be detected by a conspicuous swelling of the mid-body, by the presence of touchable prey remnants (bones) in the digestive tract and/or by voiding solid faeces. However, the oviductal eggs and developing embryos result in swelling of the abdomen in breeding females, and may hamper detection of prey remnants, especially of small prey, in the gastric tract. This may lead to an underestimation of feeding intake by reproductive females. We therefore also used the difference in BCI between successive recaptures within the same year of individual reproductive females as an indication of food intake (Bonnet et al., 2001; Lourdais et al., 2002a). In a first step, we examined the distribution of changes in BCI in females with clear indications of recent prey consumption and identified the 50 %, 66 % and 75 % percentiles of this distribution. These P50, P66 and P75 values were considered as thresholds to judge on the likelihood that changes in BCI resulted from a recent feeding event. Next, the changes in BCI of reproductive females that were recaptured before giving birth and that did not show marked signs of prey ingestion, were compared to these threshold values to detect presumed unnoticed feeding events. Increases in BCI that surpassed the above defined P50, P66 and P75 values were categorized as "possibly", "likely" and "very likely" to result from an otherwise unnoticed feeding event.

To estimate the timing of the presumed feeding events, we assumed that they occurred during the half-month corresponding to the central date within the interval between two successive captures.

A considerable proportion (28 % of  $n = 263$ ) of the recaptured reproductive females were caught more than twice in a given year and thus yielded 2 - 4 observed changes in BCI in that year. To avoid pseudoreplication, we randomly excluded duplicate records from these animals to retain only a single change in BCI per combination of female and year. This procedure was repeated 50 times. Comparison of the full with these reduced datasets revealed no noticeable differences (Chi<sup>2</sup>-tests, all  $P > 0.20$ ), so that we report results from the complete dataset only.

## RESULTS

During the period March – August only a minority (7 % of  $n = 1377$ ) of captures of females in breeding years revealed detectable signs of recent food intake. This number increased abruptly to 57 % (of  $n = 90$ ) in the short period after parturition (September – October; Fig. 1). By contrast, captures of adult females that were in their non-reproductive years showed high and equal rates of food intake during March – August (45 % of  $n = 325$ ) and September – October (50 % of  $n = 94$ ).

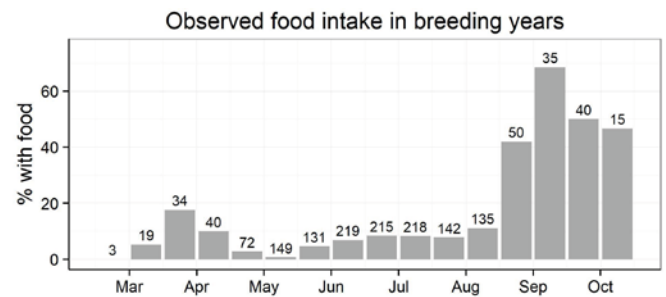
Changes of BCI at successive captures of females with obvious indications of recent prey consumption did not differ between females in reproductive and non-reproductive years (ANOVA:  $F = 1.07$ ,  $df = 1$  and  $39$ ,  $P > 0.30$ ). We therefore combined the data of both groups. Although most of these females showed an increase in BCI, important differences were observed among individual females (range =  $-20.3 - 56.8$  g;  $n = 41$ ; percentiles:  $P50 = 6.0$  g,  $P66 = 10.0$  g,  $P75 = 16.0$  g).

In a vast majority (72 % of  $n = 353$ ) of the recapture events of reproductive females without marked signs of prey consumption, the change in BCI did not exceed the  $P50$  value and, according to our criteria, provided no indication of unnoticed feeding. Increases of BCI that surpassed the  $P50$ ,  $P66$  and  $P75$  thresholds were detected in respectively 16 %, 10 % and 2 % of the recapture events. These were considered respectively as “possibly”, “likely” and “very likely” to result from a putatively unnoticed feeding event. Among these recapture events, the increase in BCI was positively correlated with female SVL ( $r = 0.434$ ,  $df = 95$ ,  $P < 0.001$ ).

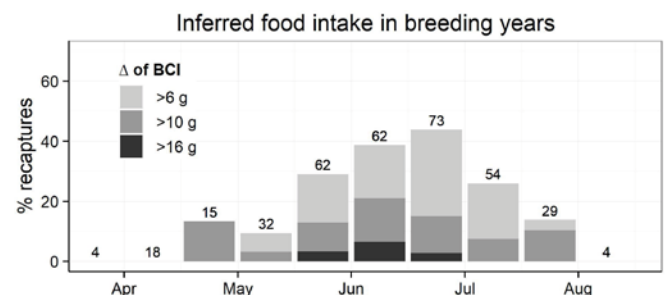
These presumed prey intake events were observed throughout the period May – August and thus included the main periods of ovulation (May) and gestation (June – August) (Fig. 2). There was no correlation between the increase in BCI and the central date within the recapture interval ( $r = -0.011$ ,  $df = 95$ ,  $P > 0.90$ ). We note that due to low recapture probabilities of reproductive females at the onset of the activity period (April) and end of gestation (August), data availability is truncated and the frequency of presumed feeding events possibly underestimated.

## DISCUSSION

Only a small proportion (7 %) of captures of reproductive female northern vipers revealed direct indications of recent food intake. Thus apparently confirming the widely accepted view that most female northern vipers do not feed during the years that they reproduce (Prestit, 1971; Nilson, 1981; Bea et al., 1992; Madsen & Shine, 1992; Völkl & Thiesmeier,



**Figure 1.** Relative frequency of reproductive females with visible signs of recent feeding in distinct half months throughout the annual activity period. Numbers indicate the number of captures per half month. Labels on the x-axis denote the midpoint of each month.



**Figure 2.** Relative frequency of recaptures of reproductive females that exhibited increases of the body condition index ( $\Delta$  of BCI) above three threshold values. These thresholds indicate the likelihood of a recent food intake (see text for explanation). Numbers indicate the number of recapture events assigned to each half month. Labels on the x-axis denote the midpoint of each month.

2002). However, examination of changes in BCI suggests that a number of food intake events may have gone unnoticed by the commonly used indications. The frequency of these ‘concealed’ feeding occasions ranges between 2 – 29 % of recapture events, depending on the criteria used to consider an increase in BCI as a result of prey consumption. Thus, conclusions about the extent to which reproductive females are feeding require consideration of the factors that influence changes of body mass.

Changes in BCI of reproductive females should be interpreted cautiously as they may occur due to at least three major processes with opposing effects, namely metabolism-induced mass losses, food intake, and water uptake. These have been studied in detail in the closely related aspic viper (*V. aspis*). First, female aspic vipers experience a decrease in BCI during pregnancy (Lourdais et al., 2002a), due to an increase in metabolic rate and the mobilisation of remaining fat stores and structural proteins (Bonnet et al., 2001; Dupoué & Lourdais, 2014). Hence, decreases in BCI should be ubiquitous in reproductive females while increases in BCI should be attributed to intake of food and/or water.

Prey consumption appears to be the main factor causing increases in body mass of reproductive female aspic vipers both before (Bonnet et al., 2001; Lourdais et al., 2002a) and after ovulation (Lourdais et al., 2002a). Particularly relevant are the results of the experiment by Lourdais et al. (2002a)



who offered pregnant aspic vipers none, one or two mice and recorded the vipers' body mass at the onset and end of gestation. The feeding regime clearly influenced the change in body mass: it equalled -13.0 g for snakes fasting during pregnancy, -1.9 g for snakes that consumed one prey, and +5.6 g for snakes that consumed two prey items. These results are consistent with the hypothesis that mass changes reflect feeding rate. We note that the increase in body mass in aspic vipers that ate two prey items (i.e., 5.6 g) is very similar to the median value (i.e.,  $P50 = 6.0$  g) for the increase in BCI in our field-sampled females with indication of food intake. This coincidence would justify using the latter value as a threshold for 'concealed' prey consumption in our northern viper population.

Pregnant snakes may increase their body mass due to water uptake which is allocated to the developing embryos as embryonic sac fluids (Bonnet et al., 2017). In food-restricted aspic vipers, Lourdaïs et al. (2015) found an average increase in body mass of 4.4 g during pregnancy, but important differences were observed among individual females (range = -11.2 g – 30.2 g). Most embryonic water uptake takes place during the second half of pregnancy and increases with the number of developing embryos (Lourdaïs et al., 2015; Bonnet et al., 2017). In our study, the largest increases in BCI were found in the biggest females, which generally produce the largest litters (Prestit, 1971; Nilson, 1981; Madsen & Shine, 1992), but did not occur near the end of pregnancy. Assuming that the findings for aspic vipers apply to our northern viper population, then we cannot rule out that increases of BCI in some of our breeding females resulted from embryonic water uptake.

Considering the possible effects of both food consumption and water uptake, we take a conservative view and consider a relatively high value (i.e.,  $P66 = 10.0$  g) as the set-point for 'concealed' food intake in our viper population. Accordingly, we estimate that some 12 % of recapture events of breeding and apparently unfed females revealed an increase in BCI that was indicative of prey consumption. Including the females with obvious signs of food intake, we estimate that ca. 20 % of the reproductive females fed at least once before parturition. Our data thus confirm that most pregnant northern vipers fail to feed, but also indicate that the number of females that feed occasionally during pregnancy is higher than has often been assumed (Prestit, 1971; Nilson, 1981; Bea et al., 1992; Völkl & Thiesmeier, 2002). Moreover, the ingestion of small prey such as the common lizard (*Zootoca vivipara*; adult body mass = 3 – 5 g), will go unnoticed by the methods used here and may result in further underestimation of feeding frequency.

Reduction of feeding in gravid snakes has been interpreted as being proximally caused by a loss of appetite that is intrinsically associated with gestation (Gregory et al., 1999). This may ultimately be a consequence of morphological constraints (i.e., abdominal space limitation to accommodate both embryos and prey items (Bea et al., 1992) or as an adaptation to reduce susceptibility to predation (Seigel et al., 1987; Brischoux, Bonnet & Shine, 2011). However, a reduced food intake may simply be a consequence of low prey encounter rates during specific periods or in particular habitats. Male red-sided garter snakes (*Thamnophis sirtalis*

*parietalis*) provide a well-documented instance. They do not feed while at their courting habitats, where food is scarce, but readily accept experimentally offered prey items, indicating that the cessation of feeding in the reproductive habitats is a facultative response (Shine et al., 2003). In addition, the males initiate foraging as soon as they disperse to their food-rich feeding grounds, after crossing distances as short as 250 meters. Hence, the lack of feeding by reproductive males is due to a scarcity of prey items and/or a cessation of foraging behaviour, rather than by some intrinsic anorexia (Shine et al., 2003; O'Donnell et al., 2004).

Old World vipers typically catch prey that are large relative to their own size and consequently feed at low frequency (Bea et al., 1992). An exception is the meadow viper (*Vipera ursinii*) that forages almost exclusively on grasshoppers, a highly abundant food resource during the summer months. Gravid females of the latter species exploit this unique condition by feeding frequently throughout the gestation period (Baron et al., 2013). By contrast, northern vipers are ambush (i.e., sit-and-wait) predators that feed on large prey, a strategy that precludes a high feeding rate (Greene, 1983). They will take prey that moves within striking range, such that the rate of food ingestion is highly dependent on prey availability (Andrén, 1982; Andrén & Nilson, 1983; Forsman & Lindell, 1997). Of particular relevance in this respect is the spatial separation between breeding and foraging habitats. Northern vipers of all age and sex-classes forage predominantly or even exclusively in the 'summer' or foraging grounds (Prestit, 1971; Völkl & Thiesmeier, 2002). For instance, adult males do not feed during the period March – half May when they stay in the winter habitats; they only start feeding upon arriving in the summer habitats. These are spread out over a large area, include diverse habitat types, and provide more abundant food to the vipers (Viitanen, 1967; Prestt, 1971; Luiselli et al., 1994; Völkl & Thiesmeier, 2002; Phelps, 2004). Pregnant females typically reside in the winter habitats that are favourable for thermoregulation, but have low primary productivity and hence low food availability. Thus, we suggest that the reduced and opportunistic foraging by pregnant vipers is, presumably in part, a consequence of their behaviour of residing in habitats where prey encounter rates are low so that feeding opportunities are rare.

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# Brown frog breeding phenology in south Sweden 1990 – 2017: are data subsets robust?

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**ABSTRACT** – Field data on breeding phenology of *Rana arvalis* and *R. temporaria* for the period 1990- 2017 in Scania, Sweden are presented and analysed. There was no temporal trend for *R. temporaria*. For *R. arvalis* the results were mixed, one out of two approaches found a trend to earlier breeding. The lack of a consistent trend in breeding phenology is unsurprising considering that local mean air temperatures in February, March and April showed no significant trends over the study period. To test the robustness of the conclusion, different subsets of the data (years or ponds) were also analysed for trends. For most subsets the conclusions remained the same. Possible bias arising from the choice of which years to include in an analysis of trends is discussed.

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## INTRODUCTION

Phenology, especially the progress of spring, has always fascinated people. Arrival of migrating birds (Ekström, 1826), flowering of early plants (Lappalainen, Linkosalo & Venäläinen, 2008) and also the breeding of frogs, for which Terhivuo (1988a) has compiled data collected since 1846, are examples. Spring breeding frogs in temperate areas are particularly suited for such studies (Richter-Boix et al., 2006). Different signs have been used to record the start of spring activities; breeding migration (Gittins et al., 1980; Reading, 1998; Miwa, 2007; Todd et al., 2011; Arnfield et al., 2012; Klaus & Loughheed, 2013; Green, 2017), first calling (Strömberg, 1988; Elmberg, 1990; Blaustein et al. 2001a; Hartel, 2008; Lappalainen et al., 2008; Scott et al., 2008; Klaus & Loughheed, 2013; Steen et al., 2013), or time of spawning (Elmberg, 1990; Beebee, 1995; Gollmann et al., 1999; Blaustein et al., 2001a; Tryjanovski et al., 2003; Hartel, 2008; Scott et al., 2008; Carroll et al., 2009; Neveu, 2009; Loman, 2014). Sensibly, the choice of variable depends mainly upon what is more practical with different species. The subject has received fresh attention as global warming has become a major debate (Beebee, 1995; Blaustein et al., 2001; While & Uller, 2014). Many long-term studies have been compiled over the years and analysed for trends in breeding phenology. These are summarised and discussed below.

The data and analysis in this report are similar to that of Loman (2014) but extend the period from 2010 until the end of 2017 but omit an analysis of pond effect on phenology as previous results were clear enough and were also elaborated (including data until 2015) in Loman (2016).

A problem that is seldom addressed with time series studies is that the outcome partly relies on the, usually random, choice of start year and (hopefully) random choice of end year. A possible solution to this problem is suggested. In addition, an analysis of the robustness of the results is included that considers what the outcome might be if only

shorter time series were included or only single ponds. This study is based on a large number of ponds. But would the same conclusions have been reached had only one pond been studied? Analysis of data began after the 2017 field season. Results from the 2018 field season are also included in Figure 2 but are not included in the analyses. This decision is discussed in light of data selection and biases.

## METHODS

### Field methods

The breeding of *R. arvalis* and *R. temporaria* was monitored from 1990 to 2017 in south-central Scania, the southernmost province of Sweden (Table 1; Fig. 1). In this period the number of ponds varied (maximum 120 but some with no spawn) and most ponds were monitored over several years, occasionally interrupted by a single year when the focal pond was dry in spring.

Within pond 'breeding sites' were identified. A breeding site consists of spawn clumps separated by no more than 1 m although usually all clumps at a site were in physical contact. If breeding took place at the same place but was interrupted by at least 6 days of non-breeding (very rare) this was considered to be two 'breeding sites'. Most ponds had only one or two breeding sites but the number was in some cases much larger. For *R. arvalis* the number of breeding sites per pond ranged from 1 to 17, with a median of 2.5 and for *R. temporaria* the corresponding numbers were 1 to 15 and 1.9. For each site and species the first date of breeding was recorded. This date usually was a good approximation of the time of breeding for all frogs at the site because at any one site most frogs bred in the first two days (pers. obs.; Loman & Håkansson, 2004). The ponds were visited at least every 5 days during the breeding period, often more frequently. Time for the earliest spawn at a site could therefore be extrapolated from the condition of the spawn at the time of visits, taking the effect of water temperature on development



**Table 1.** Number of ponds monitored. Only ponds where spawn was found are included. Ponds listed under 'Both' are a subset of those listed under either species.

|      | <i>R. arvalis</i> | <i>R. temporaria</i> | Both | Ponds with spawn |
|------|-------------------|----------------------|------|------------------|
| 1990 | 8                 | 25                   | 7    | 26               |
| 1991 | 7                 | 27                   | 6    | 28               |
| 1992 | 8                 | 30                   | 8    | 30               |
| 1993 | 10                | 28                   | 8    | 30               |
| 1994 | 25                | 65                   | 24   | 66               |
| 1995 | 26                | 60                   | 18   | 68               |
| 1996 | 28                | 70                   | 28   | 70               |
| 1997 | 32                | 79                   | 30   | 81               |
| 1998 | 43                | 80                   | 38   | 85               |
| 1999 | 40                | 86                   | 40   | 86               |
| 2000 | 42                | 83                   | 38   | 87               |
| 2001 | 46                | 81                   | 39   | 88               |
| 2002 | 44                | 82                   | 39   | 87               |
| 2003 | 34                | 69                   | 29   | 74               |
| 2004 | 42                | 71                   | 33   | 80               |
| 2005 | 47                | 76                   | 41   | 82               |
| 2006 | 20                | 40                   | 19   | 41               |
| 2007 | 26                | 47                   | 25   | 48               |
| 2008 | 23                | 45                   | 23   | 45               |
| 2009 | 7                 | 29                   | 6    | 30               |
| 2010 | 10                | 31                   | 10   | 31               |
| 2011 | 13                | 31                   | 12   | 32               |
| 2012 | 13                | 30                   | 13   | 30               |
| 2013 | 10                | 26                   | 9    | 27               |
| 2014 | 7                 | 25                   | 6    | 26               |
| 2015 | 9                 | 28                   | 9    | 28               |
| 2016 | 4                 | 7                    | 4    | 7                |
| 2017 | 3                 | 4                    | 3    | 4                |

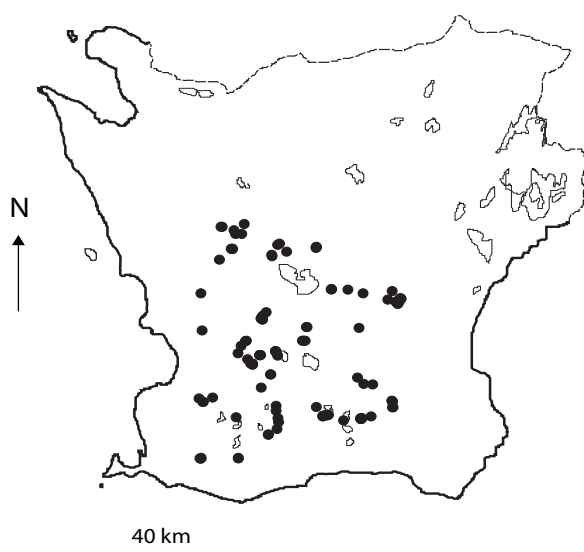


Figure 1. Map of study ponds in the province Scania, south Sweden. Lakes are outlined for easier orientation.

into account. This can usually be done with a certainty of one or at most two days. A similar approach was adopted by Tryjanowski et al. (2003). Frequent visits, observing fresh spawn, also made it easier to separate the spawn of the two species. After a few days this tends to be difficult. The number of spawn clumps at each breeding site was counted or estimated from the area of the egg mass at the breeding site (Loman & Andersson, 2007).

### Analysis of trends

For each pond, breeding time was computed as the average breeding time for all sites in the pond, weighted by the number of female frogs breeding (assumed equal to number of spawn clumps) at each site. Thus the measure approximated to the actual average breeding time for all frogs at each pond. This use of breeding sites in the present context explains why a breeding site was counted as 'new' after 6 days of interrupted breeding (a rare occurrence).

For each year, breeding time was calculated as the average time for all ponds studied in that year (raw means). This suffered from the fact that different ponds were studied in different years. To account for this an alternative measure of yearly breeding time was also used. The ponds were subject to an ANOVA (with ponds and year as categories). Data was weighted by number of frogs breeding at a pond and the least square mean was used to represent each year. Trends were analysed as the correlation between year and breeding time. For an alternative approach, the combined effect of pond and year on breeding time was also analysed as an ANCOVA.

### Subset analysis

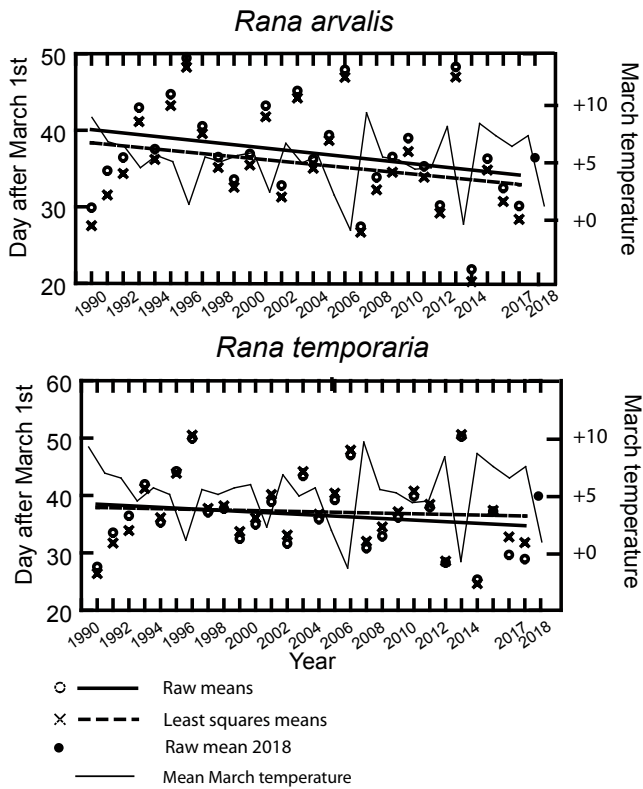
Trend analyses depend much on the starting year. If data collection starts in a year with late breeding there is a bias for a negative trend (earlier breeding in later years), and vice versa. Analyses were therefore done for subsets of the complete data set, assuming data collection had started in 1991 (rather than 1990), 1992, 1993 etc. The shortest data set analysed used 5 years of data, starting in 2013. Subsets based on single ponds were also analysed. Trends for all ponds monitored for at least 20 years and starting no later than 1995 were therefore also analysed.

## RESULTS

### Trends?

There was no significant trend in the time for breeding for either species (Fig. 2). This was true both using raw year means (Both  $n=28$ : *R. arvalis*:  $r=-0.28$ ,  $p=0.16$ ; *R. temporaria*:  $r=-0.18$ ,  $p=0.37$ ) and least squares means (Both  $n=28$ : *R. arvalis*:  $r=-0.25$ ,  $p=0.20$ ; *R. temporaria*:  $r=-0.07$ ,  $p=0.72$ ) as a measure of the start to breeding. For both species there were non-significant negative slopes. Although the slope was steeper for *R. arvalis* there was no significant difference between the species (ANCOVA, Year\*Species interaction; Raw means: d.f. = 1:52,  $F=0.14$ ,  $p=0.71$ ; Least squares means: d.f. = 1:52,  $F=0.46$ ,  $p=0.50$ ).

The ANCOVA found no interaction between pond and time (year) on breeding date for either species (*R. arvalis*: d.f. = 16:199,  $F=1.32$ ,  $p=0.19$ ; *R. temporaria*: d.f. = 42:577,



**Figure 2.** Mean breeding date and linear trends for years 1990 to 2017. The computation of the two types of yearly mean is explained in the Methods. Data for 2018 are not included in the regression lines.

$F = 1.36$ ,  $p = 0.07$ ). However removing the interactions, both pond and time (year) had a significant effect on breeding date in *R. arvalis* (Pond: d.f. = 16:215,  $F = 2.52$ ,  $p < 0.001$ ; Year: d.f. = 1:215,  $F = 7.61$ ,  $p = 0.006$ ). For *R. temporaria* only pond but not year had a significant effect on breeding date (Pond: d.f. = 42:619,  $F = 3.46$ ,  $p < 0.001$ ; Year: d.f. = 1:619,  $F = 1.53$ ,  $p = 0.217$ ).

#### Temperature effects

Among years, the trend in phenology for both species were negatively correlated to February, March and (almost so to) April temperatures ( $n=28$ ; *R. arvalis*:  $r=0.43$ ,  $p = 0.02$ ,  $r = 0.84$ ,  $p < 0.001$ ,  $r = 0.48$ ,  $p = 0.11$ ; *R. temporaria*:  $r=0.46$ ,  $p = 0.016$ ,  $r = 0.85$ ,  $p < 0.001$ ,  $r = 0.35$ ,  $p = 0.07$  respectively), with earlier breeding in warmer years. During the study period 1990 to 2017 there were no trends in spring (February, March or April) temperatures (all  $n=28$ :  $r=0.14$ ,  $p=0.48$ ;  $r=0.01$ ,  $p=0.94$ ;  $r=0.15$ ,  $p=0.43$ , respectively) in the study region.

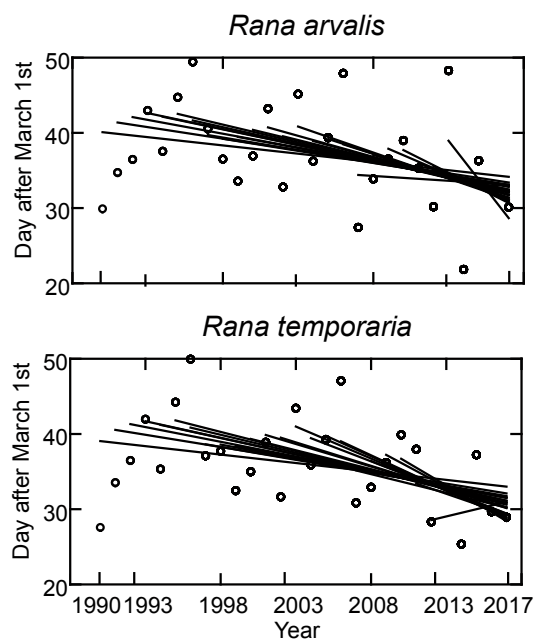
#### Subsets

Had the data collection started in any of the years 1992, 1993, 1994 or 1995 (rather than in 1990, analyzed above) one would have found a significant negative trend ( $p < 0.05$ ) for the breeding start of *R. arvalis* (Table 2). For these years the negative trend was almost significant for *R. temporaria* ( $p = 0.05 - 0.10$ ). Had the study started in 1996 or later, the conclusions were the same as for the present study; with no significant trend for either species.

The results from the ANCOVA for successive subsets of years are similar (Table 3). If the analysis started from any of

**Table 2.** Tests for significance of trends in phenology, using alternative starting year. All sequences end with 2017. Trends are based on raw pond breeding dates within years.

|             | <i>R. arvalis</i> |       |       | <i>R. temporaria</i> |       |       |
|-------------|-------------------|-------|-------|----------------------|-------|-------|
|             | n                 | r     | p     | n                    | r     | p     |
| 1990 - 2017 | 28                | -0.28 | 0.16  | 28                   | -0.17 | 0.37  |
| 1991 - 2017 | 27                | -0.37 | 0.055 | 27                   | -0.29 | 0.14  |
| 1992 - 2017 | 26                | -0.42 | 0.03  | 26                   | -0.35 | 0.081 |
| 1993 - 2017 | 25                | -0.46 | 0.02  | 25                   | -0.38 | 0.064 |
| 1994 - 2017 | 24                | -0.44 | 0.032 | 24                   | -0.35 | 0.095 |
| 1995 - 2017 | 23                | -0.46 | 0.026 | 23                   | 0.39  | 0.066 |
| 1996 - 2017 | 22                | -0.42 | 0.052 | 22                   | -0.34 | 0.12  |
| 1997 - 2017 | 21                | -0.32 | 0.16  | 21                   | -0.21 | 0.36  |
| 1998 - 2017 | 20                | -0.29 | 0.22  | 20                   | -0.21 | 0.37  |
| 1999 - 2017 | 19                | -0.31 | 0.2   | 19                   | -0.2  | 0.41  |
| 2000 - 2017 | 18                | -0.37 | 0.13  | 18                   | -0.28 | 0.27  |
| 2001 - 2017 | 17                | -0.4  | 0.12  | 17                   | -0.32 | 0.21  |
| 2002 - 2017 | 16                | -0.33 | 0.21  | 16                   | -0.3  | 0.26  |
| 2003 - 2017 | 15                | -0.42 | 0.12  | 15                   | -0.41 | 0.12  |
| 2004 - 2017 | 14                | -0.32 | 0.26  | 14                   | -0.34 | 0.24  |
| 2005 - 2017 | 13                | -0.34 | 0.26  | 13                   | -0.37 | 0.21  |
| 2006 - 2017 | 12                | -0.3  | 0.34  | 12                   | -0.35 | 0.26  |
| 2007 - 2017 | 11                | -0.06 | 0.86  | 11                   | -0.16 | 0.65  |
| 2008 - 2017 | 10                | -0.26 | 0.46  | 10                   | -0.28 | 0.44  |
| 2009 - 2017 | 9                 | -0.32 | 0.39  | 9                    | -0.38 | 0.31  |
| 2010 - 2017 | 8                 | -0.32 | 0.44  | 8                    | -0.41 | 0.31  |
| 2011 - 2017 | 7                 | -0.22 | 0.63  | 7                    | -0.34 | 0.46  |
| 2012 - 2017 | 6                 | -0.2  | 0.7   | 6                    | -0.27 | 0.6   |
| 2013 - 2017 | 5                 | -0.42 | 0.48  | 5                    | -0.61 | 0.27  |



**Figure 3.** Trends in breeding, based on raw means of breeding date within ponds and years. The starting year for each trend is evident from the left end of each line. The shortest trend analysed was 2013 to 2017 inclusive.

**Table 3.** As Table 2 but analysis based on a 2-way ANCOVA; pond and year effects on breeding date. Only year effects are accounted for in the table. All interactions are non-significant and were removed before final analysis.

|              | <i>R. arvalis</i> |       |        | <i>R. temporaria</i> |       |        |
|--------------|-------------------|-------|--------|----------------------|-------|--------|
|              | d.f.              | F     | p      | d.f.                 | F     | p      |
| 1990 - 2017  | 1:227             | 7.7   | 0.006  | 1:679                | 0.92  | 0.34   |
| 1991 - 2017  | 1:221             | 15.1  | <0.001 | 1:660                | 8.59  | 0.004  |
| 1992 - 2017  | 1:216             | 20.5  | <0.001 | 1:639                | 17.7  | <0.001 |
| 1993 - 2017  | 1:208             | 27.7  | <0.001 | 1:613                | 26.8  | <0.001 |
| 1994 - 2017  | 1:200             | 21.9  | <0.001 | 1:591                | 23.8  | <0.001 |
| 1995 - 2017  | 1:188             | 26.3  | <0.001 | 1:553                | 36.1  | <0.001 |
| 1996 - 2017  | 1:175             | 16    | <0.001 | 1:519                | 18.9  | <0.001 |
| 1997 - 2017  | 1:163             | 5.14  | 0.025  | 1:485                | 0.36  | 0.55   |
| 1998 - 2017  | 1:149             | 2.32  | 0.13   | 1:446                | 0.17  | 0.68   |
| 1999 - 2017  | 1:134             | 2.75  | 0.1    | 1:407                | 0.081 | 0.78   |
| 2000 - 2017  | 1:121             | 6.2   | 0.14   | 1:368                | 3.22  | 0.073  |
| 2001 - 2017  | 1:108             | 7.81  | 0.006  | 1:330                | 6.66  | 0.01   |
| 2002 - 2017  | 1:94              | 3.48  | 0.065  | 1:293                | 1.66  | 0.199  |
| 2003 - 2017  | 1:78              | 7.87  | 0.006  | 1:257                | 10.6  | 0.001  |
| 2004 - 2017  | 1:66              | 2.35  | 0.13   | 1:225                | 2.1   | 0.15   |
| 2005 - 2017  | 1:52              | 2.72  | 0.11   | 1:188                | 5.51  | 0.02   |
| 2006 - 2017  | 1:42              | 0.56  | 0.45   | 1:164                | 2.65  | 0.11   |
| 2007 - 2017  | 1:35              | 1.68  | 0.2    | 1:145                | 1.42  | 0.23   |
| 2008 - 2017* |                   |       |        | 1:121                | 0.17  | 0.68   |
| 2009 - 2017  | 1:20              | 0.58  | 0.46   | 1:102                | 1.21  | 0.27   |
| 2010 - 2017  | 1:18              | 0.53  | 0.48   | 1:85                 | 2.63  | 0.11   |
| 2011 - 2017  | 1:15              | 0.13  | 0.72   | 1:68                 | 0.53  | 0.47   |
| 2012 - 2017  | 1:11              | 0.048 | 0.83   | 1:52                 | 0.051 | 0.82   |
| 2013 - 2017  | 1:7               | 1.6   | 0.25   | 1:36                 | 9.73  | 0.004  |
| 2014 - 2017  | 1:4               | 1.68  | 0.26   | 1:22                 | 3.46  | 0.076  |

\* This test for *R. arvalis* signalled 'Lost degrees of freedom'

the years 1990 to 1996 then there was a significant effect of YEAR (with earlier breeding in later years) for *R. arvalis* (Fig. 3). For *R. temporaria* this was only true for the series starting from 1991 to 1996. For shorter time series (starting later than 1997 or 1996 respectively), usually no year effect could be detected. An exception (for both species) was starting in 2003, a year in which breeding started very late, when a year effect was found.

A study was also made to investigate what conclusions would be reached if only one pond was sampled. For this analysis it is assumed that the study started in 1993. This choice is motivated by the fact that this starting year yielded 'the most significant' result ( $p < 0.02$ ) for *R. arvalis* and was almost significant ( $p = 0.064$ ) for *R. temporaria* (Table 2). This analysis included all ponds for which monitored starting no later than 1995 and continued until at least 2015. For only one of these 20 ponds there was a trend to earlier breeding (*R. arvalis*:  $n = 23$ ,  $r = -0.53$ ,  $p = 0.009$ , all others ponds  $p > 0.11$ ; *R. temporaria*:  $n = 23$ ,  $r = -0.44$ ,  $p = 0.035$ , all other ponds  $p > 0.08$ ). The fact that the ANCOVA test never found any interactions between pond and year confirms that the conclusions are robust with respect to the choice of ponds.

## DISCUSSION

### Trends

In the previous analysis of this data set up to 2010, a significant trend to earlier breeding could not be detected with certainty. Adding another 7 years of data has allowed a more powerful and/or reliable analysis. The more simplistic analyses still do not find any trends towards earlier breeding, using the full data set. However, taking advantage of the large number of ponds included in the study and with pond identity included in the analysis, accounting for possible concordance among pond trends (by means of an ANCOVA), there is a significant trend to earlier breeding for *R. arvalis*. However, not even this approach finds any trend in breeding phenology in *R. temporaria*. The weak trends come as no surprise as there were no trends in spring temperatures during the study period.

### Causes for lack of trends

Several other studies have documented trends to earlier breeding in frogs but there are also exceptions (Table 4). If no trend is found, it could simply mean that there has been no climate trend in the study area. This seems to be the explanation in the present case. Actually, one of the main lessons from this study is that climate trends are geographically very heterogeneous, and one should not automatically assume everything is happening earlier in all areas! Another reason could be that the organisms are date conservative, date is as important a clue to phenology as short term weather, as shown by Harri & Koskela (1997) in a common garden study of *R. temporaria*. There are two reasons. Although early breeding is the key to a head start for the offspring, eventually resulting in a larger size of young frogs at the time of first hibernation, the effect is not as strong as one might suspect; a few days difference in egg laying and hatching make for even less difference later in the season (Loman, 2009, Steen et al., 2013, Benard, 2015). There is also a strong incentive not to respond too strongly to an occasional warm spring as the likelihood of a set back with later low temperatures is not to be neglected (Loman, 2009). The price to pay in these cases is high as the spawn risks destruction by freezing of the breeding pond.

### Robustness

Shortening the study period did not affect the conclusion reached for *R. temporaria*. For *R. arvalis* the outcome was ambiguous. By chance (starting the study one or a few years later) it could have been concluded that there was indeed a trend for earlier breeding. The large number of years for this study gave an opportunity to put the main conclusion in perspective. Also, studying only one pond had in almost all cases resulted in reaching the same conclusion. Again, the large number of ponds studied gave an opportunity to test the robustness of the conclusions in this respect.

There are two reasons for not redoing the analyses after the 2018 field season. First, it had meant more work and sometimes you have to decide that something is finished, no more data collection and time to start compilation. This decision should be (and was) taken regardless of the last data point (2017) being late or early. Second, 2018 was a late year



**Table 4.** Results from long-term studies of frog phenology

| Species                             | Site            | Measure   | Time span                         | Trend                | Ref.                                      |
|-------------------------------------|-----------------|-----------|-----------------------------------|----------------------|---|
| <i>R. temporaria</i>                | Finland         | Spawn     | 1846 - 1986                       | Earlier              | Terhivuo, 1988a, Terhivuo, 1988b          |
| <i>B. bufo</i>                      | S England       | Migration | 1980 - 1998                       | No trend             | Reading, 1998                             |
| <i>Rana cascadae</i> (site 1 and 2) | Oregon, USA     | Spawn     | 1982 - 1999                       | No trend             | Blaustein et al., 2001                    |
| <i>Bufo boreas</i> (site 1)         | Oregon, USA     | Spawn     | 1982 - 1999                       | Dubious earlier      | Blaustein et al., 2001                    |
| <i>Bufo boreas</i> (site 2 and 3)   | Oregon, USA     | Spawn     | 1982 - 1999                       | No trend             | Blaustein et al., 2001                    |
| <i>Pseudacris crucifer</i>          | Michigan, USA   | Call      | 1967 - 1994                       | No trend             | Blaustein et al., 2001                    |
| <i>Bufo fowleri</i>                 | Oregon, USA     | Call      | 1980 - 1998                       | No trend             | Blaustein et al., 2001                    |
| <i>Pseudacris crucifer</i>          | New York, USA   | Call      | 1900-1912 comp. to 1990-1999      | Early trend          | Gibbs & Breisch, 2001                     |
| <i>Rana sylvatica</i>               | New York, USA   | Call      | 1900-1912 comp. to 1990-1999      | Early trend          | Gibbs & Breisch, 2001                     |
| <i>Rana catesbeiana</i>             | New York, USA   | Call      | 1900- 1912 comp. to 1990-1999     | Possibly early trend | Gibbs & Breisch, 2001                     |
| <i>Hyla versicolor</i>              | New York, USA   | Call      | 1900 - 1912 comp. to 1990-1999    | Early trend          | Gibbs & Breisch, 2001                     |
| <i>Bufo americanus</i>              | New York, USA   | Call      | 1900 - 1912 comp. to 1990-1999    | No trend             | Gibbs & Breisch, 2001                     |
| <i>Rana clamitans</i>               | New York, USA   | Call      | 1900-1912 comp. to 1990-1999      | No trend             | Gibbs & Breisch, 2001                     |
| <i>R. temporaria</i>                | Poland          | Spawn     | 1978 - 2002                       | Earlier              | Tryanowski, Rybacki & Sparks, 2003        |
| <i>Bufo bufo</i>                    | Poland          | Spawn     | 1978 - 2002                       | Earlier              | Tryanowski, Rybacki & Sparks, 2003        |
| <i>R. temporaria</i>                | Central Finland | Call      | 1952 - 2005                       | Earlier              | Lappalainen, Linkosalo & Venäläinen, 2008 |
| <i>Rana temporaria</i>              | Eastern England | Spawn     | 1978-2005                         | Weak early trend     | Sparks et al. 2007                        |
| <i>Rana temporaria</i>              | Wales           | Spawn     | 1978-2005                         | No trend             | Sparks et al., 2007                       |
| <i>Bufo bufo</i>                    | Eastern England | Migration | 1978-2005                         | Early spawning       | Sparks et al., 2007                       |
| <i>Rana ornativentris</i>           | Tokyo, Japan    | Spawning  | 1992 - 2007                       | Earlier              | Kusano & Inouse, 2008                     |
| <i>Rhacophorus arboreus</i>         | Tokyo, Japan    | Spawning  | 1992 - 2007                       | Earlier              | Kusano & Inouse, 2008                     |
| <i>R. temporaria</i>                | United Kingdom  | Hatching  | Starting 1994-99, lasting 5-12 y. | No trend             | Scott, Pithart & Adamson, 2008            |
| <i>R. temporaria</i>                | United Kingdom  | Spawning  | Starting 1994-99, lasting 5-12 y. | Earlier              | Scott, Pithart & Adamson, 2008            |
| <i>R. temporaria</i>                | NW France       | Spawn     | 1984 - 2007                       | Earlier              | Neveu, 2009                               |
| <i>Bufo americanus</i>              | Ontario         | Call      | 1970 - 2010                       | Earlier              | Klaus & Loughheed, 2013                   |
| <i>Rana pipiens</i>                 | Ontario         | Call      | 1970 - 2010                       | Earlier              | Klaus & Loughheed, 2013                   |
| <i>Rana sylvatica</i>               | Ontario         | Call      | 1970 - 2010                       | Dubious earlier      | Klaus & Loughheed, 2013                   |
| <i>Hyla versicolor</i> ,            | Ontario         | Call      | 1970 - 2010                       | No trend             | Klaus & Loughheed, 2013                   |
| <i>Pseudacris crucifer</i> ,        | Ontario         | Call      | 1970 - 2010                       | No trend             | Klaus & Loughheed, 2013                   |
| <i>Rana catesbeiana</i>             | Ontario         | Call      | 1970 - 2010                       | No trend             | Klaus & Loughheed, 2013                   |
| <i>R. arvalis</i>                   | South Sweden    | Spawn     | 1990 - 2010                       | Dubious earlier      | Loman, 2014                               |
| <i>R. temporaria</i>                | South Sweden    | Spawn     | 1990 - 2010                       | No trend             | Loman, 2014                               |

and thus supports the main conclusion; there is no or a very weak trend to earlier breeding for these species in this region. Adding the 2018 data, knowing the outcome of the season, could justifiably be considered a bad statistical practice. But it is worthwhile to present the data (Fig. 2) and point out that this season certainly does not weaken the conclusion!

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## New localities for *Tylototriton uyenoi*, *T. panhai* and *T. anguliceps* in Thailand with remarks on the southernmost distribution of the genus

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### INTRODUCTION

The genus *Tylototriton* inhabits tropical and subtropical, moist broad-leaf forests close to waterbodies at moderate to high elevations between 1,250 and 1,900 m above sea level (m a.s.l.) in Thailand (Taylor, 1962; Wongratana, 1984; Nabhitabhata & Chan-ard, 2005; Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2016a,b; Dowwiangkan et al., 2018). Three species were reported in the region, *Tylototriton uyenoi*, *Tylototriton panhai* and *Tylototriton anguliceps* (Nishikawa et al., 2013; Le et al., 2015). These crocodile newts are distributed throughout northern Thailand but their occurrence and ecological requirements are poorly known (Hernandez, 2015, 2016a,b; Hernandez et al., 2018). *Tylototriton uyenoi* occurs from the Dawana and the Daen Lao Hills and from the Phi Pan Nam to the Inthanon Range (Gerlach, 2012; Michaels, 2015; Hernandez, 2015, 2016a,b, 2017; Dowwiangkan et al., 2018). The Daen Lao Hills are a mountain network that extends to the southern Shan states and Karen Hills (Kayah and Kayin States bordering Mae Hong Son) in Myanmar. This mountain system is connected to the north-western mountains of Thailand where *T. uyenoi* can also occur (Hernandez, 2017). *Tylototriton panhai* inhabits mixed deciduous, dry dipterocarp, dry evergreen, and hill evergreen forests at elevations between 1,285 to 1,688 m a.s.l (Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2016a,b, 2017). Its presence is known from Phitsanulok, Uttaradit, Phetchabun and Loei Provinces in north-eastern Thailand, and east into Sainyabuli Province, Botene District, Laos (Wongratana, 1984; Nabhitabhata & Chan-ard, 2005; Pomchote et al., 2008; Nishikawa et al., 2013; Hernandez, 2015, 2016a,b; Phimmachak et al., 2015). *Tylototriton anguliceps* inhabits evergreen forests at elevations of 1,300–1,800 m a.s.l in north-western Vietnam, northern Laos and north-eastern Thailand (Le et al., 2015; Phimmachak et al., 2015). Here we report four new records for these *Tylototriton* species in northern and north-eastern Thailand and we discuss their biogeographical preferences (Fig. 1).

### MATERIALS AND METHODS

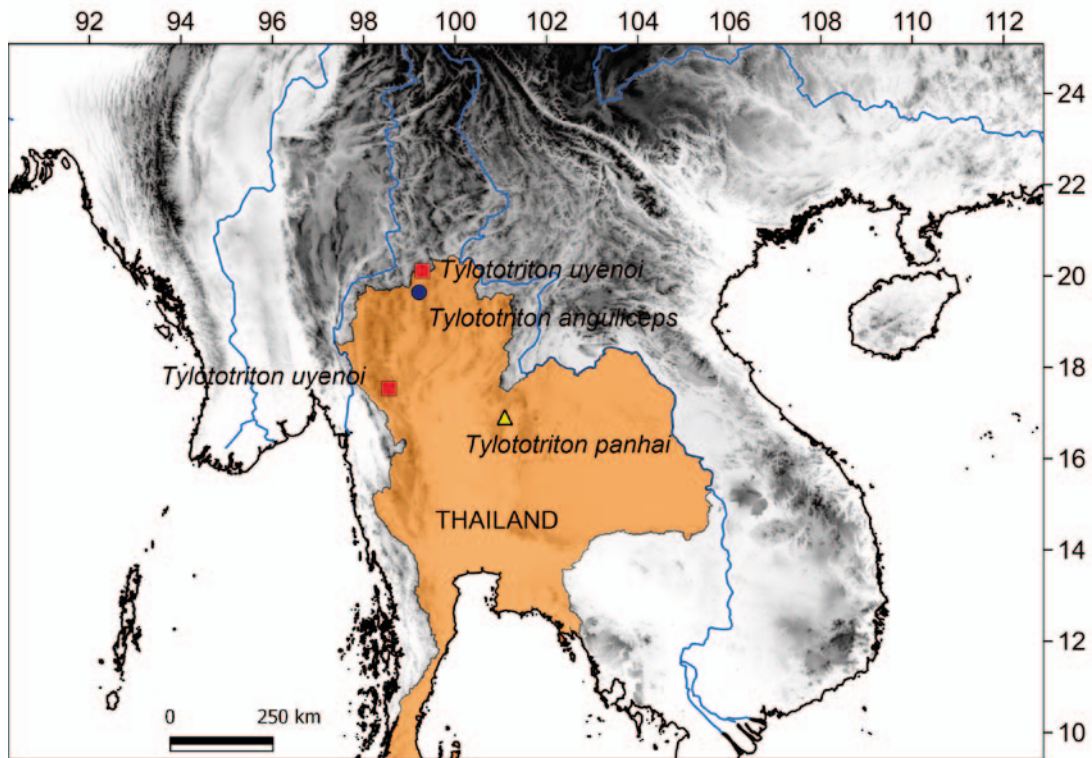
We conducted field work over a three-year period 2014–2016 and 2018 respectively, during the monsoon season (April to September) in north-western, northern and north-eastern Thailand.

Observations of the vegetation and habitat of salamanders were made during field trips both on sunny and on rainy days from approximately 08:00 h to 23:30 h. We surveyed almost all types of habitat which included permanent and temporary streams but also ponds (including artificial reservoirs and irrigation canals), and surrounding terrestrial habitats, stumps, stones and litter. For identification purposes, we photographed using a digital camera (Sony Nex-5; Sony Ltd., Japan) each taxon observed, including plant and tree species. Coordinates, geographic and elevational data were collected in situ using a Global Positioning System (Garmin Montana 680; Garmin Ltd., Olathe, KS, USA) and located on maps. Water pH and temperature was recorded in situ using a Expresstech @ LCD PH Medidor Digital (Expresstech; Kingpow Company Limited; Hong-Kong; China). We also assessed the relative positions of the species on environmental gradients using principal component analysis (PCA). This allows us to visualise if these new localities are within the ecological range (95 % confidence ellipses) expected for these species (Hernandez et al., 2018). For this we followed the protocol of Hernandez et al. (2018), including the mean annual temperature (°C), temperature seasonality, annual precipitation (mm), and precipitation seasonality, from the WorldClim database (Hijmans et al., 2005). We also included a descriptor of the topography (index of terrain ruggedness) and the percentage of forest cover (Tuanmu & Jetz, 2014). The variables were normalised before the PCA was implemented using the Paleontological Statistics package (PAST; Hammer, 2015).

### RESULTS

We found *T. uyenoi* at Doi Mon Jong, Tak province, north-western Thailand (17°32'12.18" N, 98°31'47.30" E) 1,597 m





**Figure 1.** Map of Thailand (shaded orange) and neighbouring countries, showing the four new locations described in this study: Blue circle, *T. anguliceps*; Red squares, *T. uyenoi*; Yellow triangle, *T. panhai*

a.s.l. One adult male (Fig. 2A) was found near a small pond in a montane evergreen forest. Four eggs were also recorded from the same pond. They were deposited one by one in the submerged vegetation. The finding confirmed that this species lays eggs underwater like other species of the subgenus *Tylototriton* (Raffaëlli, 2013; Hernandez, 2016a,b). We found *T. uyenoi* at Doi Mak Lang, Mae Ai district, Chiang Mai province, northern Thailand (20°6'47" N, 99° 15'37" E) 1,456 m a.s.l. One adult male was observed under a large rotten tree in a hill evergreen forest dominated by *Ostodes paniculata*, *Canarium bengalense* and *Polyspora axillaris* (Fig. 2B). The air temperature was 21.5 °C with a relative humidity of 76 %. This specimen was blackish-to brown in colour and showed robust parotoid glands, being similar to topotypic *T. verrucosus*. It measured a total length of 168 mm (TL).

Furthermore, on 12 July 2014, three adults of *T. panhai* were found at Phu Thap Boek, Lom Kao district, Phetchabun province, north-eastern Thailand (16°54'23"N, 101°5'14"E) 1,663 m a.s.l. These individuals were observed in a small stream. One adult male measured 132 mm (TL) showing the typical traits of the "type III" (Fig. 3A; see Hernandez 2016a,b, 2017): it showed a dark reddish brown colouration with 11-13 small reddish dorsolateral glandular warts (sometimes indistinct in adult specimens). These warts were placed on the dorsum, laterally, very close to each other. The shape of the head and the cephalic edges were also more angular than in other phenotypes. The vegetation around the stream included trees and giant perennial grasses of the genus *Musa*. The air temperature was 26.4 °C, water temperature 20.2 °C with a relative humidity of about 80.9 %. The water was slightly acidic (pH 6.62). We recorded *T. anguliceps* at Si Dong Yen, Chai Prakan district, Chiang Mai province, northern Thailand during

August in the same year 2014 (19°38'31.8"N 99°12'44.4"E) at 1,260 m a.s.l. We found one adult male hiding under large rocks in a dry evergreen forest near a slow-flowing stream (Fig. 3B). The air temperature was 22.1 °C with a relative humidity of 73.2 %.



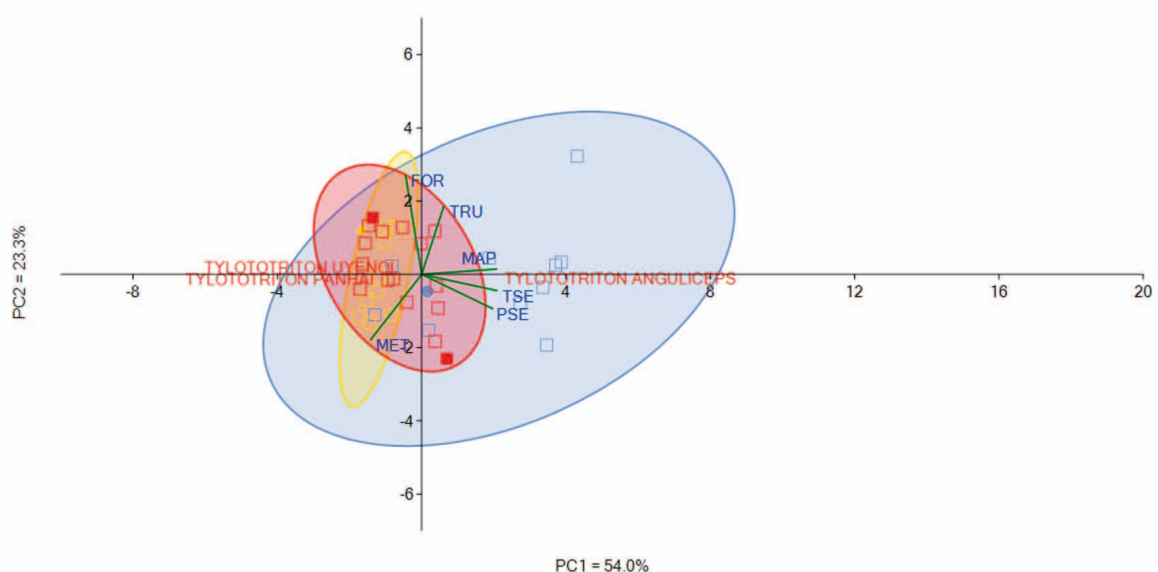
**Figure 2.** **A.** Adult male of *T. uyenoi* found at Doi Mon Jong, Tak province, north-western Thailand **B.** Specimen of *T. uyenoi* at Doi Mak Lang ("group II" from Hernandez, 2016a,b) which is more related to *T. verrucosus* topotypic from Husa, Longchuan county, Yunnan province, China



**Figure 3.** A. One male individual of *T. panhai* found in 2014 at Phu Thap Boek, Phetchabun province, north-eastern Thailand B. Specimen of *T. anguliceps* from Doi Lahnga range which is located 100 km south from our new locality in Si Dong Yen, Chiang Mai province, northern Thailand

## DISCUSSION

These important new records confirm that crocodile newts of the genus *Tylototriton* inhabit mountainous moist evergreen forests at medium to high altitudes (Hernandez, 2016a,b, 2017; Hernandez et al., 2018). In the PCA, the first axis explained 54.0 % of the variance and the second axis the 23.3 %. In the first axis the localities were segregated in a gradient of climate seasonality, precipitation and temperatures (factor loadings: temperature seasonality = 0.54, precipitation seasonality = 0.51, mean annual precipitation = 0.53 and mean temperature = -0.37). In the second axis, the localities were segregated in a topographic and forest cover gradient (factor loadings: terrain ruggedness = 0.48, forest cover = 0.70), negatively associated with temperatures (mean temperature = -0.46). The PCA showed that these new sites were within the expected macroecological niche for these species, although in the case of *T. uyenoii*, these localities occupied two extremes within the environmental gradient (Fig. 4). However, macroecological models could fail to describe the niche of species that are closely linked to densely forested habitats (Scheffers et al., 2014); for this reason future studies should be directed to confirm this preliminary result, based on fine habitat characterisation. Moreover, the northernmost localities found in Thailand are Doi Mak Lang, Chiang Mai province for *T. uyenoii* (this paper), Phu Soi Dao, Uttaradit province for *T. panhai* (Hernandez, 2017) and Si Dong Yen for *T. anguliceps* (this paper). However, phylogenetic studies are needed to confirm the taxonomic status of these new populations. The Doi Mak Lang type differs in colour pattern and external morphology by having large cephalic edges, pronounced parotoid glands, skin finely granular, general ground colour dark brown above, glands on the neck and in the dorsolateral regions lighter brown to reddish orange. Indeed, *Tylototriton* species show a



**Figure 4.** Crocodile newts ecological space, based on PCA. The 95 % confidence ellipses are shown. FOR: Forest cover; MAP: Mean Annual Precipitation; MET: Mean annual temperature; PSE: Precipitation seasonality; TSE: Temperature seasonality; TRU: Terrain ruggedness. Blue, *T. anguliceps*; Red, *T. uyenoii*; Yellow, *T. panhai*. The new records are shown as filled figures, those described in Hernandez et al., 2008 as empty squares.



conservative morphology that makes it difficult to differentiate between some species (Nishikawa et al., 2013; Le et al., 2015; Phimmachak et al., 2015). Genetic and ecological studies can provide new clues to classify these cryptic species (Hernandez et al., 2018).

Concerning *T. panhai*, we found three main phenotypes located in north-eastern Thailand (see Hernandez, 2016a,b) while for *T. uyenoi*, we found two main phenotypes including: (i) a northern and north-western type (including the type locality: Doi Suthep, Chiang Mai province); (ii) a north-eastern type which is similar to *T. verrucosus sensu stricto*. However, this latter group would require phylogenetic analyses to determine its true taxonomic affinities (Hernandez, 2015, 2016a,b, 2017). Regarding *T. anguliceps* we confirmed its presence in the three previously known localities in Thailand: Doi Lahnga, Doi Wiang Pha (Hernandez, 2015, 2016a; Le et al., 2015) and Si Dong Yen (this paper). All of these localities are within the same mountain range (pers. obs.). Furthermore, three other known localities of *T. uyenoi* are distributed through Tak province along the Dawana hills comprising: Doi Soi Malai, Umphang (Hernandez, 2015, 2016a,b, 2017) and Doi Mon Jong (this paper). These records constitute the southernmost localities for the species but also, for the whole genus *Tylototriton* extending 200 km southwards of its known distribution (Hernandez, 2017; Hernandez et al., 2018). Moreover, nearby mountains also have suitable habitats and could harbour undiscovered populations. More surveys are needed to improve knowledge about the distribution of these threatened crocodile newts and to establish conservation priorities

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## Defensive behaviour in *Rana graeca*

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Defensive behaviours in frogs and toads have been well reviewed (e.g. Toledo et al., 2011; Jablonski & Balej, 2014; Jablonski, 2017) indicating different families of amphibians are reported to show different types of defense against potential threats. This includes the Ranidae (Toledo et al., 2011; Carretero et al., 2011) and here we describe the first observation of defensive behaviour in *Rana graeca*. This species is endemic to the Balkan Peninsula ranging from Bosnia and Herzegovina through central and southern parts of the peninsula to eastern Bulgaria (Speybroeck et al., 2016). Except in distribution, only scattered information is known about this species.

On 12 October 2014 (near the village of Chora Getson, Peloponnese, Greece; 36.948°N, 22.254°E, WGS84, 605 m elev.; Fig. 1A) and on 29 October 2018 (near the village of Krini, Peloponnese, Greece; 38.179°N, 21.749°E, WGS84, 206 m elev.; Fig. 1B) we observed defensive behaviour known as “eye-protection” in three adult individuals of *R. graeca*. These individuals were captured from the surface of the water in a small stream. The animals were captured by hand around the noon and immediately took up the defensive posture after direct touch. They remained in this posture for up to 5 minutes. The individuals were released back to their habitat after photography. After release, they remained in this defensive posture for a few seconds until it reached the bottom of the stream and then moved normally. In all cases the eyes were open (Fig. 1). No defensive call was heard or defensive odour detected. During field work from spring to autumn the first author examined approximately 100 individuals of the species and recorded this behaviour only once.

This defensive behaviour is known in different members of the Ranidae as well as other families of amphibians (Toledo et al., 2011; Schlüpmann, 2000; Carretero et al., 2011) and it is hypothesised that the main function is protection of the eyes that could be damaged during possible regurgitation (Toledo et al., 2011). However, such explanation may be misleading and observed behaviour could be only a part of complex defensive mechanisms that are currently understudied. Similar behaviour is well known for the genus *Bombina* (unkenreflex) but is usually attributed to warning colouration of skin toxins when on land and to fish swimming below when floating on the surface (Jablonski



**Figure 1.** Individuals of *R. graeca* displaying defensive posture- Chora Getson (A), Krini (B)

& Balej, 2014; Bordinon et al. 2018). The possibility that the posture would be of benefit if the individual was regurgitated seems improbable. Usually if an amphibian is swallowed, the head is the first (common for snake predators) before the front limbs which are forced backwards away from the eyes. During regurgitation they would be held in this position and unprotected. Hypothetically, this posture may i) make the individual appear more difficult to swallow (especially for snakes), ii) as a variant of thanatosis it could be an attempt to be inconspicuous to increase its survival (Brodie et al., 1998). Our observations as well as in the literature (e.g. Jablonski & Gvoždík, 2009; Carretero et al., 2011) suggest the possibility

that some Palaearctic frogs use this behaviour more often at lower temperature (depending on year, season, time of day, elevation etc.) when they are hypothermic. In this case (middle and late October), our observed individuals displayed slower reactions and adopted the observed behaviour defence instead of attempting to escape. The lower temperatures during autumn may play a role (Haberl & Wilkinson, 1997), but do not have to be the core factor involved.

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## Addition to the snake fauna of Pakistan: Mackinnon's Wolf Snake, *Lycodon mackinnoni* Wall, 1906

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Pakistan harbours around 71 snake species (Khan, 2006). A large snake fauna would be expected in Pakistan given that it covers a wide geographical area that includes three major zoogeographical regions and three large mountain ranges (Himalayan, Hindu Kush, and Karakoram). However, to date this snake diversity has probably been underestimated (Khan, 2006). Moreover, some of the species may represent species complexes with unclear taxonomy (e.g. genus *Platyceps*, *Spalerosophis*). Other species are expected to occur in Pakistan in cases where their known distributions reach close to the borders with neighbouring countries (especially India). A good example are species of the genus *Lycodon* Boie in Fitzinger, 1826 (wolf snakes). This genus represents about 50 Asian species but only three are currently known from the territory of Pakistan: *L. aulicus* (Linnaeus, 1758), *L. striatus* (Shaw, 1802) and *L. travancoricus* (Beddome, 1870). The first two are distributed in Pakistan from Sindh to Khyber Pakhtunkhwa Province whereas the third has been recorded only in one locality near Hyderabad, Sindh (Khan, 2006).

In this study, we provide the first record of the rare Mackinnon's Wolf Snake (*L. mackinnoni* Wall, 1906) from Azad Jammu and Kashmir (AJK), north-eastern Pakistan. This species was described by Wall (1906) from a single specimen collected by P. W. Mackinnon from the neighbourhood

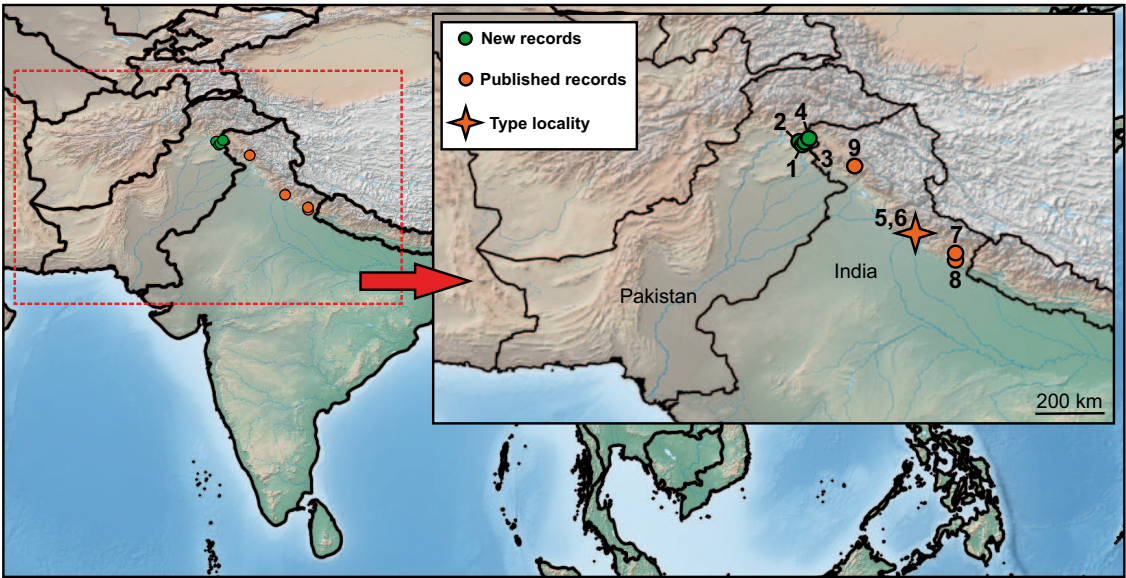
of Mussoorie, Uttar Pradesh, India (holotype BMNH 1946.1.13.81). Unusually for a *Lycodon* species the specimen was described as having no loreal scales although there were reservations about this due to the poor condition of specimen's head. Furthermore it has been noted for *Lycodon* that "aberrations such as the union of the loreal scale with the prefrontal ..... are not unknown" and a subsequent description of *L. mackinnoni* has included loreal scales (Smith, 1943).

We undertook herpetofaunal surveys in the Bagh and Hattian Bala districts of AJK from November 2016 to April 2018. These involved active searching of different microhabitats in the two districts, e.g. in the vicinity of stream and ponds, agricultural fields, forests (pine, moist temperate and sub-tropical deciduous forest) and habitats near to human settlements. Local people were also questioned and some of our observations were based on low quality photos provided by them. The snakes recorded were identified using the keys in Lanza (1999) and Khan (2006). If possible, nine morphological and thirteen meristic characters, altitude, coordinates and valuable ecological data were recorded. Morphological measurements were made using digital callipers and geographic data by GPS (WGS 84). A single specimen (male) of *L. mackinnoni* was collected and preserved in 10 %

**Table 1.** An overview of all published and new distribution records of *L. mackinnoni* from Pakistan and India

| Number                              | Locality   | N        | E        | Elevation (m) | Date              | Observations | Source             |
|-------------------------------------|--|----------|----------|---------------|-------------------|--------------|--------------------|
| 1                                   | Hari Ghel, Bagh District, AJK                                      | 33.9464° | 73.7127° | 830           | April & June 2017 | 3            | This study         |
| 2                                   | Dhirkot, Bagh District, AJK  | 34.0283° | 73.5400° | 1396          | May 2017          | 4            | This study         |
| 3                                   | Bagh city, Bagh District, AJK                                      | 33.9904° | 73.7759° | 1127          | May 2018          | 3            | This study         |
| 4                                   | Hattian, Bala District, AJK  | 34.1143° | 73.8813° | 1100          | May 2018          | 3            | This study         |
| <b>Published records from India</b> |  |          |          |               |                   |              |                    |
| 5                                   | "Neighbourhood of Mussoorie", Uttar Pradesh – <b>type locality</b> | 30.4595° | 78.0715° | ~1860         | -                 | -            | Wall 1906          |
| 6                                   | Mussoorie, Uttar Pradesh   | 30.4595° | 78.0715° | ~1900         | -                 | -            | Smith 1943         |
| 7                                   | Alomar near Nainital, Uttarakhand                                  | 29.5899° | 79.6514° | ~1600         | -                 | -            | Smith 1943         |
| 8                                   | Mukteshar near Nainital, Uttarakhand                               | 29.4619° | 79.6538° | ~2000         | -                 | -            | Smith 1943         |
| 9                                   | Nai, Doda district, Jammu and Kashmir                              | 33.0924° | 75.7084° | 1824          | May 24, 2015      | 1            | Manhas et al. 2015 |





**Figure 1.** Published and new records of *L. mackinnoni* from India and Pakistan. Numbers on the map correspond with Table 1.

**Table 2.** A comparison with published measurements and scale counts of *L. mackinnoni*

| Morphometry   | Measurements (mm)   |  |  |
|---|---|--|--|
|   | This study  | Manhas et al. 2015   | Wall 1906  |
|   | PMNH 3916<br>adult, male<br>Hattian Bala, Azad Jammu and Kash-<br>mir, Pakistan | Unvouchered<br>adult, ?<br>Nai, Doda district, Jammu and Kashmir,<br>India | BMNH 1946.1.13.81<br>adult, female<br>“Neighbourhood of Mussoorie”, Uttar<br>Pradesh, India<br>Type locality |
| Snout-vent length   | 350.0   | 354.0  | -  |
| Tail length   | 88.2  | 80.0   | -  |
| Head length   | 11.4  | 12.0   | -  |
| Head width  | 5.0   | 3.0-5.0 (mouth to neck)  | -  |
| Head height   | 4.1   | -  | -  |
| Eye diameter  | 1.7   | 1.0  | -  |
| Distance b/w eyes   | 3.7   | 4.0  | -  |
| Ventral scales diam-<br>eter (at midbody)   | 7.3   | 8.0  | -  |
| Total length  | 438.2   | 435.0  | ~355.0   |
| Meristics   | Numbers   |  |  |
| Supralabials  | 8   | 8  | 8  |
| Infralabials  | 7   | 7  | 6?   |
| Supraocular   | 1   | 2  | 1  |
| Parietal  | 2   | 2  | 2  |
| Prefrontal  | 2   | 2  | 2  |
| Preocular   | 1   | 1  | 1  |
| Postocular  | 2   | 1  | 2  |
| Loreal  | 1   | 1  | 0  |
| Intranasal  | 2   | 2  | 2  |
| Temporal  | 5 (2+3)   | 5 (2+3)  | 5 (2+3)  |
| Dorsal body scales<br>(one head length, mid-<br>body, one head length<br>before anus) | 17-17-15  | 17-0-15  | 17-17-15   |
| Ventrals  | 180   | 193 (163-187)  | 192  |
| Subcaudals (paired)   | 57 (paired)   | 53   | 54 (paired)  |



**Figure 2.** The preserved specimen PMNH 3916 of *L. mackinnoni* from Hattian Bala, Azad Jammu and Kashmir, Pakistan

formaldehyde while a tissue sample was placed in 96 % ethanol. Both are stored in the Pakistan Museum of Natural History, Islamabad (voucher specimen) and Department of Zoology, Comenius University in Bratislava, Slovakia (tissue sample) under voucher numbers PMNH 3916 and 7944, respectively.

*Lycodon mackinnoni* was recorded from four geographically close localities of the Bagh and Hattian Bala districts with elevation ranging from 830 to 1396 m a.s.l. (Table 1, Fig. 1). The specimen we collected was a freshly-killed adult male from Hattian Bala. In addition, we recorded, but did not collect, 12 additional observations of adults from other localities (Hari Ghel, Dhirkot, and Bagh city). The snakes

observed in Bagh district were found in humid localities with hot summers (about 37 °C) and cool winters (about 4 °C) but where snowfall is recorded only at higher elevations. Snakes from Hattian Bala district were from localities with warm summers but freezing cold winters. Morphometric and meristic data for the individual collected are shown in Table 2 where they are compared with published data.

Mackinnon's Wolf Snake is very rare and known only from a few localities in the western Himalayas (Wall, 1906; Smith, 1943; Fig. 1). This snake has only recently been recorded from Indian Jammu and Kashmir (Manhas et al., 2015), about 100 km from the border with Pakistani Punjab, a lowland province. Only five specimens are known from the published literature or museum collections and one of them (the holotype) has a damaged head. Thus any morphological data are important to present. Individuals observed by us did not differ in colouration, all having a black-yellow dorsal pattern which fades away in the posterior third but there were differences in subcaudal and ventral scales that may correspond with gender. It is worth noting that in our specimen the loreal scales were present otherwise all other characters fall within the original diagnostic and descriptive range for *L. mackinnoni*.

## ACKNOWLEDGEMENTS

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## The type locality of a giant salamander, *Andrias japonicus* (Temminck & Schlegel, 1838)

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Giant salamanders were discovered on the Tokaido trail in Japan approximately 190 years ago by European herpetologists. *Andrias japonicus*, originally named *Triton japonicus*: type locality, Suzuka-toge, Mie prefecture (Temminck, 1836) (Figs. 1, 2). The type locality was described only as coming from Okude in the area of Suzuka-toge, but with detailed locality information not recorded (Temminck, 1836; Hoogmoed, 1978, see Fig. 2). *Andrias japonicus* was first catalogued by Europeans when the resident physician of Dejima Island in Nagasaki, Philipp Franz von Siebold (February 17, 1796 - October 18, 1866), captured two individuals and shipped them back to Leiden in the Netherlands, in the 1820s. Coenraad Jacob Temminck (March 31, 1788 - January 30, 1858) and his colleague described these salamanders as *Salamandra maxima* in Fauna Japonica (Temminck & Schlegel, 1838). The giant salamander can be found on the islands of Kyushu, Honshu, and Shikoku in Japan and are listed as vulnerable (IUCN 2004) and as an endangered species as early as 1952 by the Agency for Cultural Affairs of the Japanese government. It is federally protected in Japan. To confirm habitat types, three known localities were visited, Honshu islands, i.e., Ikuno-cho, Hyogo prefecture in 1995, Wara-cho, Gifu prefecture in 2008 and Nabari-shi, Mie prefecture. The habitats were creeks in woodland areas and/or mountain forests. An important difference between their former habitats and present-day habitat in the Suzuka-gawa creek, as described by Siebold collection in 1826, is that in the latter environment there are now no large flat stones normally used as retreat sites (Fig. 3).

Between 2010 and 2017 searches were made for suitable salamander habitat along the 495 km historic Tokaido trail from Tokyo to Kyoto in an attempt to locate potential sites that could present the type localities for two species of salamander: *Onychodactylus japonicus* (Houttuyn, 1782, Iizuka et al., 2011) and *A. japonicus*. Between May and August 2017, searches were also made for suitable habitats in the Suzuka-toge region, Mie prefecture, which could also represent the potential type locality of *A. japonicus* (Figs. 1, 2). During surveys no *A. japonicus* were found in this locality and the available habitat within the areas searched did not appear suitable for *A. japonicus* (Fig. 3). It is therefore suggested that the true origins of *A. japonicus* were not from the Suzuka-toge region, Mie prefecture but possibly transported there from nearby areas by villagers.



Figure 1. *Andrias japonicus*, photographed at the Museum of Salamanders, Akame, Nabari-shi, Mie prefecture on June 5, 2017



Figure 2. Maps showing the location of search routes in this study. Red lines on the maps are the author's study route of the Tokaido trail, i.e., total approximately 495 km walking from Tokyo to Kyoto. Squares are indicated Suzuka-toge region. The old road and ship route within Seto-naikai Sea are coloured as a red line from Dejima in Nagasaki through Suzuka-toge to Edo (old Tokyo) that was travelled by Siebold in 1826.





**Figure 3.** Riverbed environment in Suzuka-gawa river, that was searched for *A. japonicus* without success

A valuable future study would be a comparative analysis of DNA using the tissues of the type specimen deposited in Naturalis, Netherlands, and tissues of oral cavity epithelium including water samples around their habitats in several populations that seem to indicate the type locality, Mie prefecture. Molecular analysis may need to be undertaken if the type locality of *A. japonicus* is to be determined but this would be dependent on the ability to retrieve genetic material from the preserved type specimens for comparison with samples collected from the proposed region that surrounds the type locality. It is not possible to say how the type series is preserved, as this will dictate how difficult it may be to retrieve genetic material.

## ACKNOWLEDGEMENTS

I would like to thank following colleagues; Kraig Adler, Tamotsu Kusano, Tomoko Takayama, Yoshinori Takeuchi and Edward Hullsieck. I also thank an anonymous referee for reviewing this work.

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## Self-cannibalism in the Aegean wall-lizard, *Podarcis erhardii*

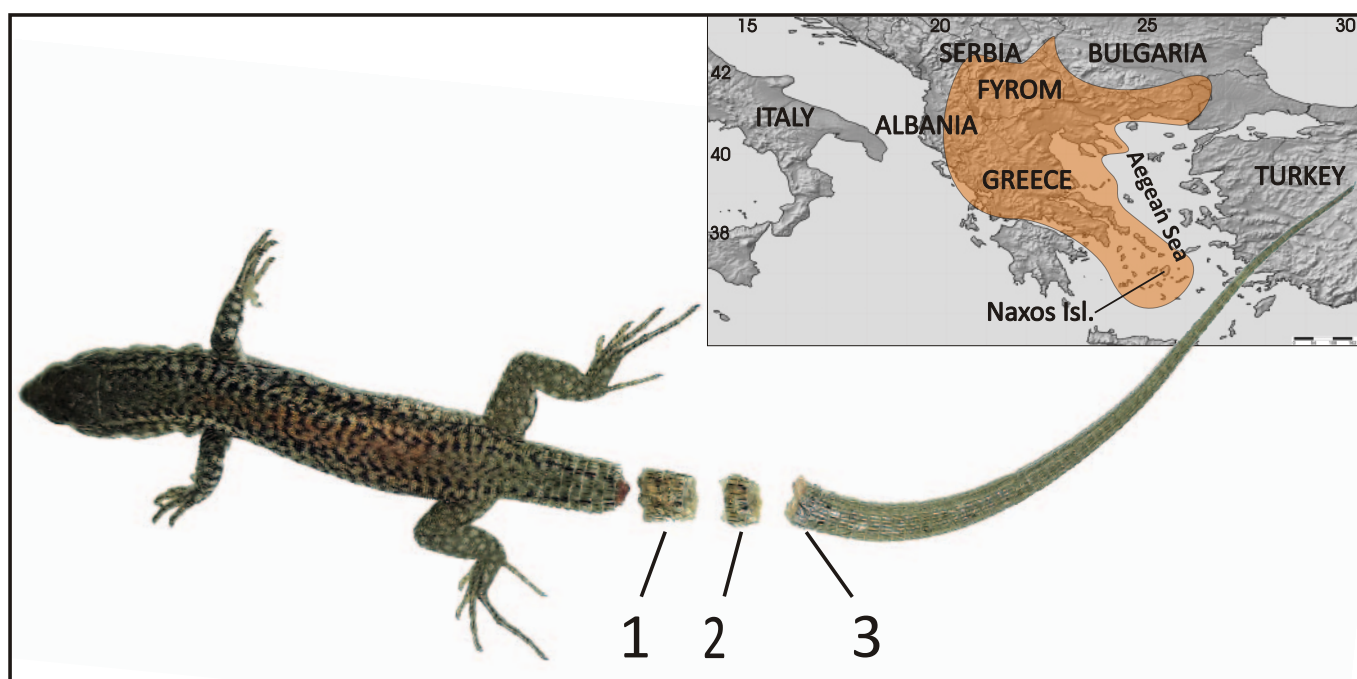
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Autophagy - also called self-cannibalism - is a term used to describe the consumption of an animal's own body parts. Lizards, for example, may both lose and sometimes eat their own tails intentionally. Caudal autotomy or the self-amputation of the tail in lizards is an anti-predation strategy that helps these animals escape when caught by the tail. In some species, tails can also be shed in response to physical grasping during mating, as a result of male fighting (Arnold, 1984), or even in response to a stressful condition (Walter, 1962). Almost all lizards that perform autotomy can regenerate the lost tail after a short period of time (Arnold, 1984). Yet both losing a tail and regenerating it have negative consequences for the individual's locomotion (Lin et al., 2006), social interactions (Salvador et al., 1995), immune system (Argaez et al., 2018), and future body growth (Maginnis, 2006), which affect both survival and reproductive potential (Bateman & Flemming, 2009). In compensation a lizard may return and eat its own tail, to regain some of the sacrificed resources which can then contribute towards the re-growth of the tail (Maginnis, 2006).

The first case of autophagy in the family Lacertidae was recently reported as a field observation on the Iberian rock-lizard, *Iberolacerta monticola* (Iglesias-Carrasco & Cabido, 2016). Here we report autophagy in another mediterranean endemic lacertid, the Aegean wall-lizard, *Podarcis erhardii* (Bedriaga, 1882). During field-work on 21 September 2018 we collected *P. erhardii* from Naxos Island (37.037°N - 25.486°E; Fig. 1) with the use of a rubber band fired at the lizards. This can stun the animals for a short period but they are unharmed and make a full recovery (Bennett, 1999). Captured lizards were placed individually in soft and dark fabric bags and then into a plastic container to allow recovery with as little stress as possible. Lizards were processed the next day for the collection of tissue, stomach-contents and their preparation as museum specimens for the Zoological Museum of the University of Patras (ZMUP).

In this process, we found one adult male specimen (museum voucher ZMUP523) to have shed its tail, more likely during transfer. The tail was actually broken in three parts (Fig. 1) and the two smaller ones were found intact in the animal's



**Figure 1.** The *P. erhardii* specimen that exhibited self-cannibalism. There were no chewing marks on either of the two ingested pieces (marked 1 and 2) and the tail was restored to its full length when all parts (1-3) were aligned. Map shows the approximate distribution of the species and the sampling location of the specimen.

stomach. We believe our observation raises three interesting points. First, autotomy was performed without the triggering effect of physical grasping and was most probably due to the stress of captivity. Secondly, autophagy still occurred despite that stress. Third, our observation corroborates previous suggestions that the consumption of the autotomised tail might be a common behaviour in lacertid lizards, although not an easy one to observe in nature. For *I. monticola*, this behaviour was associated with adaptation to alpine habitats (Iglesias-Carrasco & Cabido, 2016). The Aegean insular populations of *P. erhardii* are also facing harsh environmental conditions, specifically prolonged drought, hot summers and a high rate of predation, and consequently are considered among those species that may readily apply autotomy as an attempt to escape predators (Pafilis et al., 2009). In these conditions, it is possible that regaining lost resources by consuming severed tails could have important survival value.

### ACKNOWLEDGEMENTS

Fieldwork and collection were performed according to international standards and in compliance with the national legislation (Presidential Decree PD 67, 23/A/30-1-81) governing research on animals in Greece.

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## Persian horned viper (*Pseudocerastes persicus*): a record at unusually low elevation from the United Arab Emirates

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*Pseudocerastes persicus* (Duméril, Bibron & Duméril, 1854) is a medium sized, thick bodied snake. Above the eyes it has prominent horn-like structures composed of a series of small scales (Fig. 1), which contrast with the single elongated scale above each eye of *Cerastes* spp (Mallow et al., 2003). *Pseudocerastes persicus* is the most widely distributed species of its genus, ranging from Arabia eastwards to Pakistan. In Arabia, the species is found in northern Oman and the United Arab Emirates (UAE) where its range is limited to the Musandam peninsula and the Hajar mountains (Cunningham 2002; Gardner 2013). While listed as 'Least Concern' for its global range by the IUCN, in Arabia it is classified as 'Vulnerable' (Cox et al., 2012).



**Figure 1.** The head of the *P. persicus* individual encountered, with the horn structures visible

The current lowest elevational records of this species range from 460-487 m a.s.l. (de Pous et al., 2016) but all other published records suggest that this species is restricted to higher elevations (Cunningham 2002; Mallow et al., 2003; Helliyyer & Aspinall 2005; Cox et al., 2012; Feulner 2014). Egan (2007) states more specifically that the snake is found above 600 m. Here I report an encounter with *P. persicus* at much lower altitude.

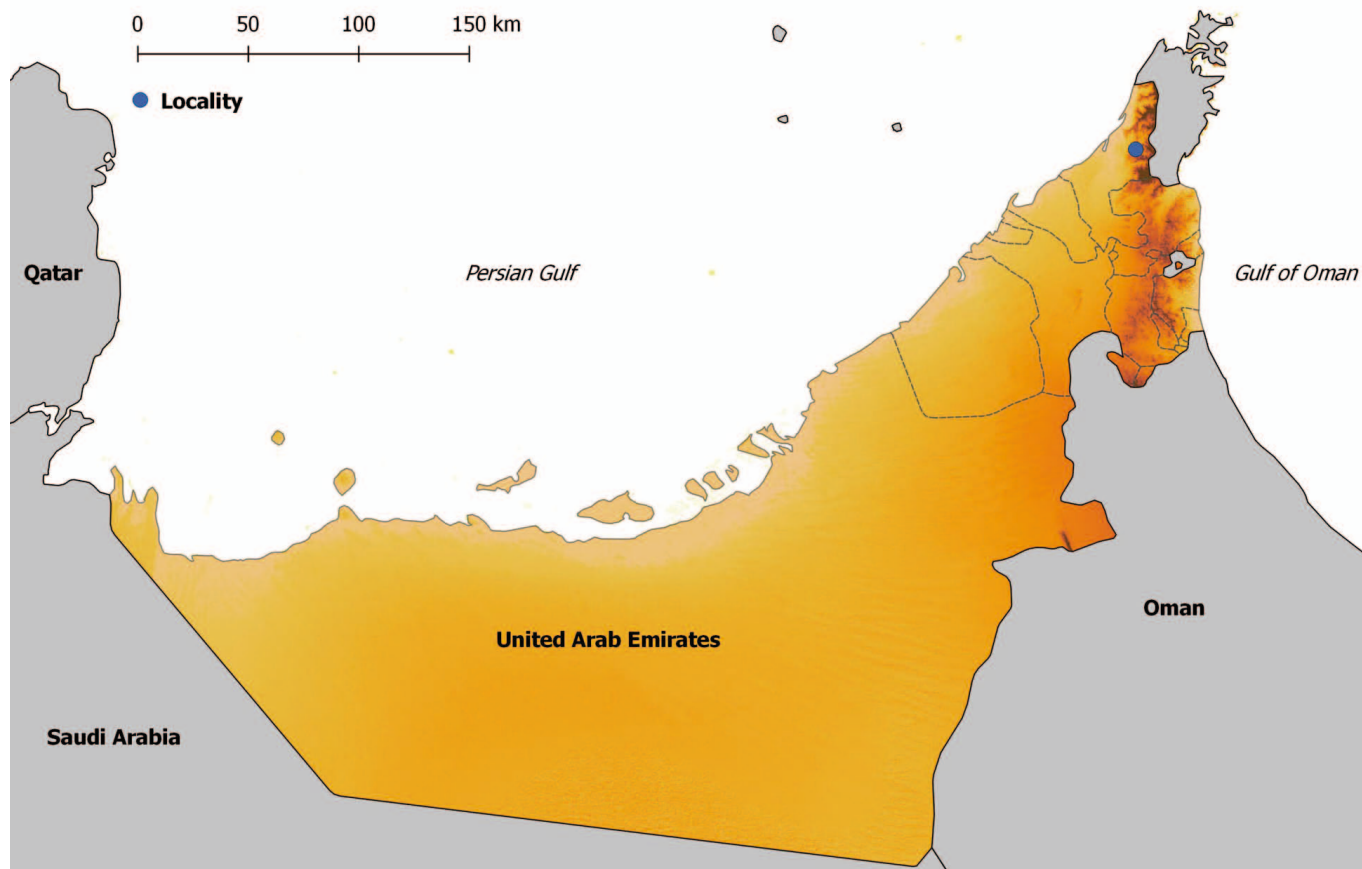
On 12 January 2019 at 11:10 h in the UAE, a *P. persicus* (Fig. 1) was observed in a small, shaded rock formation in a north-west facing, steep sided tributary of Wadi Qada'a, Ras Al Khaimah (Fig. 2) at 25°77' 69" N, 56°08'45" E as

determined by the HerpMapper app. This location is at an altitude of only 195 m a.s.l., roughly 200 m lower in altitude than any previous record. The habitat was composed largely of sharp, small to medium sized limestone boulders with either a hard mud or gravel substrate. A small number of Acacia trees were present in the wadi, as well as other thinly distributed small shrubs and grasses. When found, the snake immediately began defensive displays including loud hissing and coiling into a strike position. The snake was left alone but then on return at 17:36 h, just before dusk, it was observed moving from its shelter directly towards a collection of larger boulders. Next, it entered a burrow with an aperture of approximately 15 cm in diameter and coiled up just inside the entrance. Upon returning to the location again at 20:26 h, the snake was still in the burrow. The combination of directed movement towards the burrow and snake's persistence at this location suggests that it was using an established den. The snake displayed a combination of concertina, rectilinear, and sidewinding movements when outside of the burrow.

There is a paucity of information on the ecology, distribution, and natural history of *P. persicus* (de Pous et al., 2016, Cunningham 2002). New records of locations such as this are important for assessing the conservation status and potential distribution of this species which currently appears to be outside any protected areas in Arabia (Cox et al., 2012). Many environmental variables are altered markedly with changing elevation (Körner 2007). The tolerance of *P. persicus* to greater extremes of temperature and desiccation at lower altitudes in Arabia may be underestimated when data on elevational distribution is incomplete. Nevertheless, the location where this observation was made is within an area identified by Cox et al. (2012) as having the highest reptile species richness (36-52 species) in Arabia suggesting that its environment is actually particularly favourable for reptiles. The collection of additional information on the species distribution and associated environmental variables, especially in Arabia, where the species is Vulnerable, would allow more accurate estimates of the species distribution and a greater understanding of its ecology and potential for conservation.

### ACKNOWLEDGEMENTS

I'd like to thank André Gaspar and Sean Laughlin for accompanying me when we found the snake and when returning in the evening. Thanks also to Chris Michaels and



**Figure 2.** Map depicting the observation locality in the emirate of Ras Al Khaimah, UAE. Darker areas show higher elevation.

Steven Allain for their advice with the manuscript and Rick Hodges for his detailed review and comments.

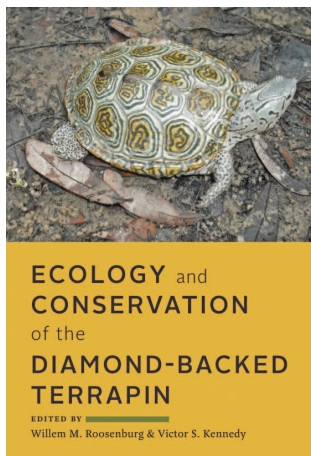
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Accepted: 15 February 2019

## Ecology and Conservation of the Diamond-backed Terrapin

Editors: Willem M. Roosenburg & Victor S. Kennedy  
Johns Hopkins University Press, ISBN 978-1-4214-2626-6. Hardback, 368 pp.



I expected a very readable and informative text from this publisher on a fascinating terrapin, and I was not disappointed. In his introduction, Whit Gibbons summarises exactly how the various topics are dealt with. He includes the sentence “The terrapin story offers insight that can be applied to developing effective conservation practices for any overexploited species” and this would particularly

apply to other chelonians. We need to be quite clear about nomenclature: whereas some emydid turtles (*Pseudemys* species) can be referred to as terrapins, in this book ‘terrapin’ only applies to *Malaclemys terrapin*.

The book is well structured, with individual chapters by researchers on each topic. Each comprises a combination of the expertise of the individual authors, plus information gleaned from a review of relevant literature, and thus there are many references in the text. There are diverse situations to describe, as the diamond-backed terrapin ranges from the Gulf of Mexico up to Cape Cod, and as well as climatic extremes it also inhabits water of varying salinity. The book begins with descriptions of the various search and capture methods in this terrapin’s different habitats. The difficulty in finding this species means observation of secondary clues may be needed, such as the presence of snails showing metacercarial cysts of a trematode whose final host is exclusively the diamond-back. There are details of how to assemble and use the various items of equipment needed for capture, measurement and humane marking, and the experience of the authors even extends to warning readers not to let sticky estuarine mud suck off their shoes.

Moving on to taxonomy, a new 3-tier system of categorising subspecies is suggested to try and address the conflict in nomenclature. There is a wide range of morphological differences in the seven subspecies, but not the expected corresponding genetic variation, with the reasons clearly discussed.

The chapter describing the biology of hatchlings and juveniles, while giving comprehensive information, highlights

more than any other topic the need for further research. As with other chelonian species, the cryptic lifestyle of young terrapins means there are still many gaps in our knowledge which need to be filled to inform conservation strategies. For example, the suitability of various soil types for hibernation, and whether hatchlings prefer to head inland after emergence from the nest, need further investigation. This sort of information can be vital when land is earmarked for development and terrapins may be present.

The thermoregulatory ability of the diamond-back is very clearly explained, including enough biochemical details to show exactly how cryoprotectants allow survival at low temperatures that would be lethal to most other chelonians. Similarly, the homeostatic mechanisms that enable the animals to move between areas of differing salinity are well explained, including behavioural strategies as well as physiological aspects down to cellular level.

The section on temperature-dependent sex determination gives a wealth of information gleaned from the literature, and again, has precise details of where further research is needed. This is of particular importance in a world where climate change threatens the sex ratio of animals whose developmental conditions influence the sex of the offspring.

Foraging and habitat selection are considered together, being linked by prey preferences of the different age classes in their varied environments. Sexual dimorphism is another factor in prey choices, with the wider gape of females allowing larger molluscs to be consumed. There are discussions on the terrapin’s place in the food web, whereby this top predator can spread seeds of, for example, eelgrass *Zostera marina* which it can consume incidentally while feeding on epibiota such as barnacles. Again, there are opportunities for further research, one of which is whether terrapins will consume alien species which are competing with local fauna. I was pleased to note that this section comments on the invasive and possibly unethical methods of investigating ingesta, with suggestions for moving on from these traditional studies.

Turning to toxicology, terrapins’ reproductive cycles and other hormonal systems are described, with reference to endocrine disruptors. From this point onwards, the book becomes a more depressing read, documenting the population crash which has been caused entirely by mankind. All the usual suspects are mentioned: terrapin overfishing; habitat loss due to development; fatal injury from motor vehicles and motorboats; by-catch in crab pots;



and so on. On a brighter note, there is plenty of information in the last few chapters on steps being taken to mitigate these serious threats. It is heartening to learn that in spite of being hampered by political and industrial wrangling, the Diamondback Terrapin Working Group (DTWG), established in 1994, has made great strides in achieving protective legislation, and continues to gather support in pressing for more regulations and better enforcement.

If I have to find a criticism of this book, it would only be the fact that there are no colour illustrations apart from the attractive cover photos. However, the images are clear and serve their purpose, and there are other sources of colour photos if an identification guide of the subspecies is required, such as Ernst & Lovich (2009).

Although each topic has a chapter that stands alone, there are threads running through where the reader is referred to other sections, emphasising the significance of particular issues. The Bermuda mystery, i.e. the question of why it is the only Atlantic island on which diamond-backed terrapins exist in the wild, is a thread that appears in several chapters.

The evidence, or lack of it, is revealed in the sections on field techniques, evolutionary history, palaeontology, molecular ecology and phylogeography, but you'll have to read the book yourself to find out the conclusions.

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*Received: 22 March 2019*

# HERPETOLOGICAL BULLETIN REPORT

February 2019

All four issues of *The Herpetological Bulletin* were published on time during 2018 with 100 % of manuscripts published within 3 months of acceptance. The target for 2019 is a continuation of publication of papers in the next available issue once they have been reviewed and accepted.


During 2018 we received 85 submissions. This represents a decrease compared to 2017 but manuscript submissions remain buoyant and *The Bulletin* score on, for example, Researchgate continues to increase. Full paper submissions remained high at around 36 % of total submissions but with lower acceptance rates than previously, as was overall acceptance compared to 2017. As for 2017, *Short Communications* and *Natural History Notes* (single unusual observation) are grouped together but for simplicity treated distinct from *Short Notes* (single data set). Full details are presented in the table below with comparable figures for 2017 shown in parentheses but note 2017 numbers include manuscripts that were rejected without review.

|                       | Submitted | Accepted | Percent accepted |
|-----------------------|-----------|----------|------------------|
| Full papers           | 31 (34)   | 12 (13)  | 38.7 (55.9)      |
| Short Notes           | 13 (13)   | 7 (7)    | 53.8 (53.8)      |
| Natural History Notes | 41 (52)   | 11 (22)  | 26.8 (36.1)      |
| Total                 | 85 (108)  | 30 (48)  | 35.3 (44.4)      |

From the March 2019 AGM Rick Hodges will assume the main receiving editors role at *The Bulletin* with Roger Meek continuing in a support role for a 1-year period. Roger Avery has given notice of his intention to step down from his editorial role with *The Bulletin*, which will end a long period of service to the BHS as Editor of both *The Herpetological Journal* and *The Herpetological Bulletin* and as BHS President.

The following people gave their time and expertise reviewing manuscripts for *The Herpetological Bulletin* during 2018:- Kevin Arbuckle; Roger Avery; John Baker; Francois Brischoux; Adam Buxton; Xavier Bonnet; Daniele Canestrelli; Kimberley Carter; Dean Crowshaw; Sandeep Das; Thomas Doherty-Bone; Daniel Escoriza; Philippe Geniez; Chris Gleed-Owen; Stuart Graham; Richard Griffiths; Roland Griffin; Jihène Ben Hassine; Rick Hodges; Jim Labisko; John Iverson; Robert Jehle; Luca Luiselli; Inigo Martinez-Solano; Roger Meek; Christopher J Michaels; Kanto Nishikawa; Todd Lewis; Mark Paulissen; Sinlan Poo; Ben Tapely; Christine Tilley; Rob Ward; Romulus Whitaker; Marco Zuffi.

Roger Meek, Roger Avery, Rick Hodges  
Editors




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