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Front Cover: A salamander *Bolitoglossa* cf. *nympha* from Honduras using its prehensile tail to grasp vegetation, the subject of a Natural History Note on p.36. Photograph taken by Tom Brown.

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Range limitation of the Peaks of Otter salamander (*Plethodon hubrichti*) due to competition with the eastern red-backed salamander (*Plethodon cinereus*) in sympatry

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ABSTRACT - The Peaks of Otter salamander, *Plethodon hubrichti*, is a montane species found at altitudes above 442 m within a 117 km² area of the Blue Ridge Mountains in central Virginia, USA. In areas where this species is sympatric with the eastern red-backed salamander (*Plethodon cinereus*) it seemed likely that *P. hubrichti* populations were either depressed or eliminated. The habitability of areas beyond the current range boundaries for *P. hubrichti* is supported by several disjunct populations in areas sympatric with *P. cinereus*. From 2009 to 2012 we tested whether *P. hubrichti* was negatively impacted by competition with *P. cinereus* by removing *P. cinereus* from treatment plots at three sympatric field locations. The number of surface-active (SA) *P. hubrichti* increased significantly more on treatment plots than on corresponding reference plots, whereas the number of SA *P. cinereus* decreased significantly more on treatment plots than on reference plots. The removal of every one *P. cinereus* from the treatment plots led to an increase of 0.69 *P. hubrichti*. These results emphasise the importance of conserving mature hardwood forests along the perimeter of the *P. hubrichti* distribution, where it is sympatric with *P. cinereus*, so as to prevent future range contraction of this vulnerable species.

INTRODUCTION

he Peaks of Otter salamander (Plethodon hubrichti) is The Peaks of Otter salamander precises found in the state of Virginia, USA (Kramer et al., 1993; Petranka, 1998). It is a montane species with a very limited distribution, being found only in mature, deciduous forests at altitudes greater than 442 m within a 117 km² area of the Blue Ridge Mountains in central Virginia (Pague & Mitchell, 1990). We demonstrated previously that declines in altitude, which are associated with rises in temperature and falls in relative humidity, depressed the densities, survival rates, growth rates, eggs per female, and reproductive output of surfaceactive P. hubrichti (Reichenbach & Brophy, 2017). We also showed that P. hubrichti had greater dehydration rates and lower critical thermal maxima than the wider-ranging eastern red-backed salamander (Plethodon cinereus), reflecting its adaptation to montane environments (Reichenbach & Brophy, 2017). Based on these findings, we hypothesised that at low altitudes where P. cinereus, a potential competitor, is absent the range of P. hubrichti is limited primarily by abiotic factors; Arif et al. (2007) consider the entire distribution of P. hubrichti to be limited primarily by abiotic factors.

Interspecific competition is extremely common among salamanders in the genus *Plethodon* (Adams, 2007; Anthony et al., 1997; Hairston, 1980a, b; Jaeger, 1971, 1974; Marshall et al., 2004) and has frequently been cited as a limiting factor in the distribution of some of its species (Jaeger, 1970, 1971, 1980; Hairston, 1980b; Griffis & Jaeger, 1998). *Plethodon cinereus*, a wide-ranging and tolerant species (Petranka, 1998; Adams, 2007), has been implicated in limiting the distributions of several Virginia/West Virginia montane salamander endemics through interspecific competition (Highton, 1972; Jaeger, 1970, 1974; Pauley, 1991, 2005; Wicknick, 1995; Jaeger et al., 2002; Griffis & Jaeger, 1998; Kroschel, et al., 2014; Farallo & Miles, 2016). In fact, Wicknick (1995) found that *P. cinereus* and *P. hubrichti* showed evidence of interspecific competition with no clearly superior competitor, which led Jaeger et al. (2002) to suggest that their contact zone is static and, by inference, that competition is limiting the distribution of both species. A definitive way to demonstrate interspecific competition is by selective removal; for example Hairston (1980b) found that removal of the competitive species, *P. jordani*, led to significant increases in the number of *P. teyahalee* (formerly *P. glutinosus*) on experimental plots in North Carolina and Tennessee, USA.

There are many locations within the Peaks of Otter area of central Virginia where P. hubrichti is not found. Many of these locations, especially those NE and SW of its current species' distribution, have suitable habitat and are found at altitudes optimal (or near-optimal) for its survival and reproduction (Reichenbach & Brophy, 2017; see also Fig. 5A in Arif et al., 2007). We find no reason why P. hubrichti could not occupy such locations were it not for the potentially competitive presence of P. cinereus. Unlike our previous hypothesis regarding abiotic limitations in low altitude areas where P. cinereus is absent (Reichenbach & Brophy, 2017), we hypothesise that in areas where they are sympatric, P. hubrichti populations are depressed through competition with P. cinereus and that this competition is limiting the range of P. hubrichti in areas that would otherwise be habitable. To test this hypothesis experimentally we used removal studies at field locations where the two species were sympatric.



Figure 1. Peaks of Otter, Blue Ridge Mountains, VA. White dots represent allopatric *Plethodon hubrichti* sites, black dots are allopatric *P. cinereus* sites, and white & black dots are sympatric sites. ST – Sharp Top Mountain, FT – Flat Top Mountain, CM – Chestnut Mountain, HF – Headforemost Mountain, OM – Onion Mountain, SF – Sunset Fields, AO – Apple Orchard Mountain, WOR – White Oak Ridge; 1 – disjunct *P. hubrichti* population, Vright, 1988; 2 – disjunct *P. hubrichti* population, Pague & Mitchell, 1990 and F. Huber, Reichenbach, Brophy, and P.W. Sattler, unpublished data; 3 – disjunct P. hubrichti population, Pague et al., 1992 and Reichenbach, unpublished data. Other site information from Reichenbach & Sattler, 2007, Reichenbach & Brophy, 2017 and Brophy & Reichenbach, this study and unpublished data. Original image accessed on 11 September 2019 from https://www.google.com/earth.

MATERIALS AND METHODS

A pair of unfenced plots, each plot measuring 15x15 m, were selected at each of three different sites in the Peaks of Otter area (Blue Ridge Parkway National Park and George Washington National Forest) where P. hubrichti and P. cinereus were sympatric: Onion Mountain (OM), NE aspect, 1158 m asl; Sunset Fields (SF), SE aspect, 1189 m asl; and Apple Orchard Mountain (AO), N aspect, 1280 m asl (this site is within 8 m of the highest altitude in the P. hubrichti range, AO summit) (Fig. 1). The two plots at each site were assigned randomly to be either a reference plot (no P. cinereus removed) or a treatment plot (P. cinereus removed after baseline year data collected), and were within approximately 15 to 30 m of one another. Surface-active (SA) P. hubrichti and P. cinereus were counted at all three sites on the same day, with paired reference and treatment plots being sampled simultaneously by two field teams consisting of six to ten members each. All sites were sampled several times annually from 2009 through 2012 (5x in 2009, 2x in 2010, 4x in 2011, and 3x in 2012).

Removal of *P. cinereus* from the treatment plots began on the last trip of 2009 (baseline year) and continued on each of the sampling dates throughout the duration of the study. Each treatment plot also had a 1.0 m buffer from which *P. cinereus* were removed but not counted in the total for that plot. Our decision to use unfenced plots stems from the observation in an earlier study that the two species moved in a similar way and covered only short distances; median distances (and interquartile range) moved were 0.71 m (IQR = 0.62, n = 44) and 1.00 m (IQR = 1.00, n = 31) for *P. hubrichti* and *P. cinereus*, respectively (Kniowski & Reichenbach, 2009). There were no significant differences between linear distances moved (U = 634.0, n = 75, P = 0.60) for the two species. These data suggest that no one species would migrate into our study plots more than the other. The three year duration of our study was based on the findings of Hairston (1980b) who detected competition after three years in a similar removal study with *P. jordani* and *P. teyahalee* (formerly *P. glutinosus*) in North Carolina and Tennessee, USA.

SA salamanders were located by carefully turning over rocks and logs and sifting through leaf litter on cool days, following rain, so that all plots were moist and cool throughout the day. In all cases, salamanders were captured and handled in such a way as to reduce stress and discomfort. Salamanders were categorised into neonate or juvenile/adult groupings based on size. Neonates were only present in the autumn collections and were, therefore, not included in SA salamander counts used to assess interspecific competition. At treatment sites, removed P. cinereus were placed in ziplock bags with damp paper towels and then placed in coolers. We limited the number of salamanders placed in each bag so as to avoid overcrowding. Salamanders were then transported in enclosed vehicles and released later that same day in allopatric P. cinereus areas located within National Park boundaries, but distant from our study sites. We were also careful to release only a few P. cinereus by each natural cover object in the forest (i.e. rock or log) so they were evenly distributed among resident salamanders. These methods were employed to maximise survival of released animals. The experiment involved only the removal of P. cinereus and not P. hubrichti which has a very limited distribution and is currently considered a Tier I species ('Critical Conservation Need') on the Virginia Department of Game and Inland

Fisheries' list of "Species of Greatest Conservation Need" (Burkett, 2015). We wished to avoid favouring *P. cinereus* populations in any location within the range of *P. hubrichti*.

In this study, SA salamander counts were used as a proxy for population size because they correlated well with population estimates in previous mark-recapture studies, including one involving *P. hubrichti* (Smith & Petranka, 2000; Reichenbach & Sattler, 2007; Gifford & Kozak, 2012). Counts of SA salamanders should also be comparable between the two species since one of us found, during a mark-recapture study conducted in 2005 (Kniowski & Reichenbach, 2009), that detection probabilities were similar for *P. hubrichti* and *P. cinereus*, 0.309 (95 % CI = 0.233-0.397) and 0.251 (95 % CI = 0.175-0.347), respectively. Any potential reductions in SA *P. cinereus* or increases in SA *P. hubrichti* in treatment (i.e. removal) plots would, therefore, not simply be artefacts of one species spending more/less time at the surface.

Our study design was a replicated (3 replicates) pre and post-treatment difference in SA salamander counts for each species and each plot during baseline (2009) and subsequent years (2010 to 2012). Mean SA salamander counts from 2009 through 2012 were used to describe changes seen over time in the treatment and reference plots. Differences in SA salamander counts pre (2009) and post-treatment (2012) were compared using two-sample t-tests (one-tailed) to examine 1) declines in SA salamander counts for *P. cinereus* and 2) increases in SA salamander counts for *P. hubrichti*. Normality (Shapiro-Wilk test) and homogeneity of variances (F-test) were confirmed for data using SPSS software (SPSS Inc., Chicago, IL, USA) or EXCEL (Microsoft Corp., Redmond, WA, USA).

RESULTS

At the end of 2009, prior to removal of *P. cinereus* from the treatment plots, two of our sites (SF and AO) were generally dominated by *P. cinereus* and the other (OM) by *P. hubrichti* (Table 1). By the end of 2012, a total of 1020 *P. cinereus* had been removed from our three treatment plots (SF - 520, AO - 314, and OM - 186).

Table 1. Mean surface-active salamander counts for *P. hubrichti* (*Ph*) and *P. cinereus* (*Pc*) at both treatment (Trt) and reference (Ref) plots from 2009 to 2012. Pre and post-treatment differences were calculated by subtracting 2009 from 2012 values.

	Ph-Trt	Ph-Ref	Pc-Trt	Pc-Ref
Sunset Fields				
2009	8.8	7.8	28.8	38.8
2010	9.0	6.5	24.5	30.5
2011	18.8	12.3	31.3	49.0
2012	14.7	10.3	21.3	45.0
Pre & post-treatment difference	+5.9	+2.5	-7.5	+6.2
Apple Orchard Mountain				
2009	8.2	12.4	22.0	10.6
2010	8.0	6.5	13.5	7.5
2011	13.8	7.5	19.8	8.8
2012	22.3	13.0	17.3	16.3
Pre & post-treatment difference	+14.1	+0.6	-4.7	+5.7
Onion Mountain				
2009	30.2	37.8	18.4	27.2
2010	31.0	32.0	14.0	19.5
2011	45.8	43.5	10.3	29.3
2012	43.0	45.0	10.7	27.7
Pre & post-treatment difference	+12.8	+7.2	-7.7	+0.5

Overall, the mean number of SA *P. hubrichti* increased substantially on treatment plots (i.e. following *P. cinereus* removal) and rather less on corresponding reference plots. In 2009, mean SA *P. hubrichti* counts ranged from 8.2-30.2 in the treatment plots but by 2012 had increased to 14.7-43.0 (Table 1). This resulted in pre and post-treatment differences in the number of *P. hubrichti* ranging from +5.9 to +14.1 at treatment plots during this three-year period (Table 1). By comparison, the differences in references plots were increases of +0.6 to +7.2 (Table 1).

The overall trends for *P. cinereus* were much different at treatment (i.e. removal) plots. In 2009, mean SA *P. cinereus* counts ranged from 18.4-28.8 in the treatment plots but decreased to 10.7-21.3 by 2012 (Table 1). This resulted in pre and post-treatment differences of -4.7 to -7.7 during this three-year period (Table 1). On the other hand, trends for *P. cinereus* at reference plots mirrored those of their *P. hubrichti* counterparts on those same plots. In 2009, mean SA *P. cinereus* counts ranged from 10.6-38.8 in the reference plots and increased to 16.3-45.0 by 2012 (Table 1) resulting in pre and post-treatment differences for *P. cinereus* ranging from +0.5 to +6.2 (Table 1).

The number of SA *P. hubrichti* increased significantly more on treatment plots than on corresponding reference plots (t=2.32, *df*= 4, P=0.04; Fig. 2), whereas the number of SA *P. cinereus* decreased significantly more on treatment (i.e. removal) plots than on reference plots (t=-5.17, *df*=4, P=0.003; Fig. 2). Averaging across treatment plots, and adjusting for corresponding changes in reference plots, *P. cinereus* declined overall by 10.8 salamanders whereas *P. hubrichti* increased by 7.5 salamanders during this three year period (Fig. 2). Or stated another way, the removal of every one *P. cinereus* from the treatment plots led to an increase of 0.69 *P. hubrichti*.



Figure 2. Pre and post-treatment differences across sites in surfaceactive (SA) salamander counts (means ± 1 SE, N=3) for *P. hubrichti* (*Ph*) and *P. cinereus* (*Pc*) in both reference and treatment plots.

DISCUSSION

Plethodon hubrichti is restricted to mature hardwood forests at altitudes above 442 m in the Peaks of Otter area of central Virginia (Pague & Mitchell, 1990; Reichenbach & Brophy, 2017). Within the Peaks of Otter area, there are mature forests at altitudes well above 442 m that are occupied by *P. cinereus* but not *P. hubrichti* (Fig. 1). We hypothesised that, in these areas, *P. hubrichti* is restricted by

interspecific competition with *P. cinereus* instead of abiotic factors that limit its range at lower altitudes (Reichenbach & Brophy, 2017). The results of our removal study support this hypothesis (Table 1; Fig. 2). The rise in count of *P. hubrichti* in response to the removal of *P. cinereus* was less than the fall in count of *P. cinereus* (0.69:1). This was partially due to the fact that on average *P. hubrichti* is the larger of the two species (Kniowski & Reichenbach, 2009; Wicknick, 1995).

There is broad niche overlap between P. cinereus and P. hubrichti which are similar in terms of diet (Petranka, 1998), microhabitat use (Kniowski & Reichenbach, 2009), territoriality (Wicknick, 1995), foraging time and strategy (Kramer et al., 1993; Jaeger, 1978), and nesting sites (Kniowski & Reichenbach, 2009; Petranka, 1998). Interspecific competition is well documented in plethodontid salamanders (Jaeger, 1971, 1974; Jaeger et al., 2002; Hairston, 1980a, b, 1981) and in both lab and field studies on P. hubrichti and P. cinereus the two species showed evidence of interspecific competition without a clearly superior competitor (Wicknick, 1995). This led Jaeger et al. (2002) to suggest that the contact zone between the two species was static which has been supported by a reexamination of Wicknick's (1995) three sites in the National Park (one allopatric P. hubrichti, one allopatric P. cinereus, and one sympatric site), ten years later by Aasen & Reichenbach (2004). They showed that proportions of P. hubrichti relative to P. cinereus remained static in the undisturbed sympatric site.

Interspecific competition has long been suspected for plethodontid salamanders, but the specific effects of this type of competition are not well known. Character displacement has been noted in some interactions (Jaeger et al., 2002; Adams et al., 2007; Adams, 2010), range restrictions in other interactions (Jaeger, 1970, 1971; Hairston, 1980b), and population suppression in still others (Hairston, 1980b, 1981). For example, Jaeger (1971) determined that P. shenandoah would be able to survive beyond its typical talus slope locations, but was being competitively inhibited from doing so by P. cinereus. Additionally, Hairston (1980b) determined that a release from interspecific competition led to significant increases in P. teyahalee (formerly P. glutinosus) numbers on plots where P. jordani had been removed. Similarly, we demonstrate here that removing P. cinereus from treatment plots resulted in increased numbers of SA P. hubrichti (Table 1; Fig. 2). Since SA P. hubrichti counts correlate significantly with population estimates (Reichenbach & Sattler, 2007), we infer that P. cinereus depresses P. hubrichti populations and in some locations, depending upon the size of the P. cinereus population, might actually prevent coexistence of the two species. These inferences are also supported by the observation, from several of our previous studies, that P. hubrichti densities are considerably higher when found alone (1.6-3.3/m²) than they are in sympatry with *P. cinereus* (0.6/m²) (Kramer et al., 1993; Sattler & Reichenbach, 1998; Kniowski & Reichenbach, 2009).

In the NE corner of the *P. hubrichti* distribution, the transition from allopatric *P. hubrichti* areas to allopatric *P. cinereus* areas occurs in less than 1 km. This same transition, although documented for fewer sites, also occurs in the SW part of the *P. hubrichti* distribution midway on Flat Top Mountain (Fig. 1). The effects of *P. cinereus* on *P. hubrichti*, as demonstrated in this study, support the hypothesis that *P. cinereus* is competitively inhibiting *P. hubrichti* from expanding its range into areas with suitable altitudes and ecological conditions in the Peaks of Otter area, both to the NE and SW of its current range near the Blue Ridge Parkway

(Reichenbach & Brophy, 2017; see also Fig. 5A in Arif et al., 2007).

Disjunct populations of P. hubrichti in the SW and NE of the Peaks of Otter area also support the hypothesis that P. cinereus is competitively inhibiting P. hubrichti from expanding its range into areas with suitable altitudes and ecological conditions. In 1987, Wright (1988) found two specimens on a steep hemlock-birch slope, 744 m in altitude, 2.2 km SW from the nearest known location on the slopes of Flat Top Mountain (Fig. 1). In the NE part of the Peaks of Otter area, Pague & Mitchell (1990) found a P. hubrichti population just NE of White Oak Ridge. We confirmed that this population still exists and that it is likely disjunct (Fig. 1). Only P. cinereus was found along White Oak Ridge itself, and the nearest record for *P. hubrichti* is 1.5 km to the SW on Onion Mountain. This disjunct population ranges in altitude from 853 to 1097 m and is comprised, at its core, of large boulders on a steep slope. In two days of searching this area, we found 96 P. hubrichti (unpublished data; F. Huber, Reichenbach, Brophy, and P.W. Sattler). An additional disjunct population occurs even further to the NE at Thunder Ridge, 2.2 km from the White Oak Ridge population described above (Fig. 1). Two individuals were found at this location in 1991 (Pague et al., 1992) with six individuals discovered in October 2019 (unpublished data; Reichenbach). These disjunct populations suggest that P. hubrichti may have once had a wider distribution, at least along the NE/SW axis of its distribution, where altitudes are greater than 442 m. Plethodon hubrichti populations potentially persist in these areas because steep slopes and/or boulder fields limit timbering activities. In addition, boulder fields may have provided refuges for P. hubrichti from the effects of clear cutting and/or forest fires; a similar explanation has been proposed for multiple disjunct populations of *P. nettingi* in West Virginia (Pauley, 2008).

We propose that overall the distribution of *P. hubrichti* is limited by two main factors. In descending from mountains, relative humidities gradually fall as temperatures rise at comparable rates. In areas where *P. cinereus* is absent, these factors eventually create physiologically intolerable conditions for *P. hubrichti* and, therefore, place abiotic restrictions on its range (Reichenbach & Brophy, 2017; Arif et al., 2007). In areas with altitudes that are habitable by *P. hubrichti* (areas to the NE and SW of species distribution at altitudes greater than 442 m), we suggest that interspecific competition with *P. cinereus* is the primary factor restricting the range of *P. hubrichti* and that, in these areas, it is not restricted abiotically.

Understanding the reasons why the Peaks of Otter salamander has such a restricted distribution can contribute greatly to its conservation. In the core of its range, where altitudes are optimal and P. cinereus is absent, P. hubrichti dominates the salamander community (94.8 % of salamander species) and can be found at high population densities (mean = $2.7/m^2$; Reichenbach & Sattler, 2007). It is along the perimeter of this species' distribution, however, that extreme care must be exercised in forest management. The perimeter includes not only lower altitude areas but also areas sympatric with P. cinereus. These areas represent fragile salamander habitats where reduction of the forest canopy, due to timber removal or insect defoliation, would likely lead to increased temperatures and decreased relative humidities (Homyack et al., 2011). Such factors would subsequently lead to decreases in P. hubrichti densities in areas where it is allopatric with P. cinereus (Reichenbach & Brophy, 2017). In areas where P. hubrichti is sympatric with P. cinereus, we

predict that these same conditions would favour P. cinereus, the more widespread and, therefore, tolerant species which has virtually no altitudinal (e.g. found at highest altitudes at AO summit) or ecological restrictions (see Fig. 5B in Arif et al., 2007) within the range of P. hubrichti at present. Adams et al. (2007) suggested that P. cinereus may have morphological and behavioural flexibilities that allow it to adapt quickly to altered, local environmental conditions. This might give it a competitive advantage over P. hubrichti in becoming re-established in recovering forests. The net result of this situation would be to reduce the already limited distribution of P. hubrichti even more. Perhaps this is what occurred in areas surrounding the disjunct populations described above. Both private and public managers, such as those from the National Park and Forest services who manage the majority of land occupied by P. hubrichti, should focus conservation efforts on these perimeter areas so as to prevent future contraction of this vulnerable species' range.

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A communal nesting site of the Central American river turtle (*Dermatemys mawii*) along the New River, Belize

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ABSTRACT - The Central American river turtle, *Dermatemys mawii*, is a critically endangered species with declines in its population caused by human harvest. Previous knowledge of nesting behaviour is minimal, and communal nesting of *D. mawii* has not been described previously. Here we report the first observations of communal nesting of *D. mawii* and provide recommendations to researchers and conservationists on how to find *D. mawii* nests, based on observed communal nesting conditions, proximity to water, hydro-period, and vegetative cover.

INTRODUCTION

The Central American river turtle, *Dermatemys mawii* (also known as the hicatee in Belize), is a large, aquatic freshwater turtle that is found in the lowlands of southern Mexico, northern Guatemala, and Belize (García-Anleu et al., 2007; Briggs-Gonzalez et al., 2018, 2019). Due to heavy hunting pressure, *D. mawii* populations have been severely reduced in Mexico, are in decline in Belize, and are of unknown status in Guatemala (Moll, 1986; Polisar, 1994, 1995; Rainwater et al., 2012). *Dermatemys mawii* is classified as Critically Endangered by the International Union for Conservation of Nature and listed on Appendix II of the Convention on International Trade in Endangered Species (CITES, 2009; IUCN, 2009).

Most of the existing information on the nesting ecology of *D. mawii* has been obtained by examining reproductive tracts of animals harvested for consumption (Polisar, 1995, 1996; Vogt et al., 2011). In addition, a few studies have reported on nests found in the wild (Polisar, 1996). Based on these observations, most nesting in *D. mawii* across its range occurs at the peak of the wet season between September and December, with a secondary nesting period in the early dry season from January to February (Polisar, 1996; Vogt et al., 2011).

Female *D. mawii* nest in moist soil along the shoreline, generally within 3 m of water in riparian forests and savannas (Polisar, 1995; Vogt et al., 2011). As a result, nests often become inundated during the rainy season. Nests are normally solitary and placed at scattered locations, often in dense cover, along the shoreline that afford some protection from human harvest (Vogt et al., 2011). Polisar (1995) found that local harvesters rarely discovered *D. mawii* nests, and none had witnessed hatchling emergence. Here we present the first observation of communal nesting of *D. mawii* from the New River in northern Belize.

MATERIALS AND METHODS

The study site was a side channel of the New River at the mouth of the New River Lagoon (17° 47′5″ N, 88° 38′54″ W) in the Orange Walk District of northern Belize. The mean annual rainfall for this area is approximately 150 cm, with peak water levels from September to December and the lowest in April and May (Hartshorn et al., 1984; Polisar, 1996). The site was about 25 m long, 15 m wide and, at the time, slightly elevated at about 60 cm above the waterline of the New River. Both shorelines of the side channel have extensive *Cladium* spp. marshes with small patches of trees located on relatively elevated areas.

We were able to locate the study site from information provided by a local resident, who had found eggshells while searching for firewood. On 9 January 2019, we visited the nest site and found the eggshells of previously hatched turtles, confirming turtle nesting activity. The nest site was approximately 15 m from the water's edge through a dense canopy (75-100 %) of trees (Fig. 1A). The nest site itself was in a relatively open area with about 25 % canopy cover (Fig. 1B). Surrounding flora included bromeliads (*Aechmea bracteata*, *Tillandsia fasciculata*, *Billbergia viridiflora*), dog-tail cactus (*Selenicereus testudo*), bullet trees (*Bucida buceras*), logwood (*Haematoxylum campechianum*), and provision trees (*Pachira aquatica*). The ground was composed primarily of a fibrous peat soil (Fig.1B).

We deployed three Moultrie M-880 camera traps at this site on 17 January 2019 to record and observe nesting behaviour and to determine if the site was being used by *D. mawii*. The cameras were set on 10-second motion detect intervals, recording 10-second videos each time they were triggered. Infrared was used to capture videos during the night time. We checked cameras and searched for nests on 3 March 2019, 26 April 2019, and 24 June 2019.

RESULTS

On 24 June 2019, we found six nests at the study site within 10 m of each other. These nests were 14 m - 18 m from the water's edge. Akin to observations by Vogt et al. (2011), the nests were scattered and not easily identified. There were multiple eggshells near the openings of five nests, making them more apparent, and these nests were empty. The eggshells were brittle and all were similar in size, shape and texture. They also appeared fresh and naturally hatched rather than depredated, leading us to conclude the nests had been successful. One of these five nests was built beneath the exposed roots of a fallen bullet tree. The sixth nest was invaded by fire ants (Solenopsis invicta) and had no eggshells surrounding it. Nest appearance and dimensions were similar for all of the nests, with a circular entrance ranging from 7.2 cm to 9.6 cm (mean 8.8 cm) in diameter, and extending to a depth of 9.6 cm to 13.3 cm (mean 11.2 cm) (Fig. 1C). Upon gentle excavation of the sixth nest, we found eight hatchling D. mawii, six of which had already succumbed to fire ant predation (Fig. 1D). Hatchlings were identified as D. mawii based on characteristics recently described by Platt et al., (2019). The remaining two hatchlings were lethargic and exhibited extensive swelling from ant bites, which proved fatal within 24 hours. The eggshells found upon excavating the nest were the same size, shape and texture as those found scattered around the entrance of other nests. This observation confirmed that the site was being used by D. mawii for nesting.

We did not observe *D. mawii* or any other turtle species on the camera traps. However, we did capture videos of other animals at the nesting site, including raccoons (*Procyon lotor*), gray four-eyed opossums (*Philander opossum*), lowland pacas (*Cuniculus paca*), tapir (*Tapirus bairdii*), neotropical otters (*Lontra longicaudis*), northern tamanduas (*Tamandua* *mexicana*), white-nosed coatis (*Nasua narica*), green iguanas (*Iguana iguana*), great curassows (*Crax rubra*), and rufousnaped wood rails (*Aramides albiventris*). On eight occasions, video images captured coatis actively smelling around the location of the turtle nests, but no digging was observed.

DISCUSSION

In this study, we report an observation of communal nesting by D. mawii in the wild. Previous studies have reported solitary nests of D. mawii (Polisar, 1996; Vogt et al., 2011) but the possibility of communal nesting was not excluded. For example, Polisar (1996) found four D. mawii nests concentrated within 6 m of each other on an old logging road. Although in the current study, turtle identity was based on the observation of hatchlings in only one of the nests, there is strong evidence that the other five nests were of the same species, as egg shell remains were consistent with D. mawii. Other freshwater turtles, including the Mesoamerican slider (Trachemys venusta), overlap in range with D. mawii, but the two species have dissimilar preferences in nesting habitat (Moll & Moll, 2004; Vogt & Flores-Villela, 1992). The nesting behaviour and reproductive biology of T. venusta is similar to that of other slider populations except they have larger egg size, clutch size, and nest dimensions (Moll, 1994). Although T. venusta hatchlings emerge similarly to D. mawii, around May and June, their nests are typically constructed on upper parts of beach berms, usually under cocoplum vegetation (Moll, 1994). Open beach and forested areas are not used for T. venusta nesting (Moll, 1994; Moll & Moll, 2004). Eggs of *T. venusta* are usually oblong and leathery and have a mean size of 38.1 mm x 22.6 mm (Moll & Moll, 1990; Vogt, 1990), while eggs of D. mawii are usually large and brittle-shelled with an ellipsoidal shape and a mean size of 61.6 mm x 35.8 mm (Polisar, 1996).



Figure 1. A. The hicatee (*D. mawii*) communal nesting site entrance from the New River, B. View of the site upon entering on foot, C. The nests were small and well hidden (Leatherman multi-tool ~10 cm long, included for scale), D. Eight hatchlings in a nest with fire ants (*S. invicta*) present inside, six of which had succumbed to their predation

The six nests we discovered were congregated in a small clearing, separated from the surrounding moderately dense vegetation. The site's elevation and open canopy provides suitable conditions for egg incubation. We hypothesise that communal nesting of *D. mawii* may have been influenced by lack of similar suitable habitat in surrounding areas due to low elevation and wet conditions. The distance of nests observed in this study from the water was greater than in previous observations (Polisar, 1996; Vogt et al., 2011). This may be explained by the combination of elevation and open canopy at the former. It is also probable that water levels were higher and closer to the nest site when eggs were deposited, since water levels fluctuate during nesting season (Polisar, 1996).

We infer that these nests were laid prior to our camera trap deployment in January 2019, as most nesting occurs between September and December (Polisar, 1996; Vogt et al., 2011). In addition, peak water levels occur during this period in the New River, (Hartshorn et al., 1984) so the nest soil was likely saturated or inundated when the eggs were deposited. This is supported by the absence of *D. mawii* at the nesting area based on our camera trap data. It is also possible, that even though we used infrared detection for night time observations, turtles or other reptiles may have visited the site undetected if their body temperatures were the same or very similar to ambient (Richardson et al., 2018). For example, photos of *I. iguana* were captured visiting the *D. mawii* nesting site during the day, but they were not observed at night.

Eggs of *D. mawii* can withstand inundation for more than 30 days (Polisar & Horwich, 1994; Polisar, 1996), suggesting that they can undergo embryonic diapause (Vogt et al., 2011). Development resumes after water levels recede and the nest is no longer saturated, which at this site would likely occur in January. Hatching occurs when moisture in the nest increases, typically with the summer rains in June and July (Polisar, 1992, 1995). The timing of *D. mawii* nesting at this site on the New River follows this pattern.

Based on our observations of D. mawii nesting, we make the following recommendations to researchers and conservationists searching for *D. mawii* nests. In areas that are likely to be inundated for periods exceeding 300 days, seek places with vegetation indicating higher elevation and, hence, periods of shorter inundation (< 120 days). In these locations, be watchful for sites with relatively open canopy, and keep in mind that D. mawii will nest in areas that are inundated or saturated during the rainy season. Also, be aware that this species can nest communally, especially in areas where nesting habitat is limited. To be thorough, we suggest that nest searches extend inland from the shoreline for at least 25 m or the width of the elevated area. Improving the ability to identify nesting areas and locate nests will enhance our knowledge of D. mawii nesting ecology and therefore our ability to conserve this critically endangered species.

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Husbandry and reproduction of the Saharan spiny-tailed lizard, Uromastyx geyri

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INTRODUCTION

The Saharan or Geyr's spiny-tailed lizard, *Uromastyx geyri* (Muller, 1922) is a relatively small lizard (Schleich et al., 1996) with a maximum total length of 35.5 cm of which 45 % is tail (Wilms et al., 2009). It is endemic to the Hoggar and Air mountains of north-east Mali and southern Algeria where it inhabits rocky outcrops. Colour can range from magnificent cinnabar red or bright yellow with blackish brown squiggles (Wilms, 2005). The species is assessed as Near Threatened and populations are in decline (Wilms et al., 2018). All species within the genus *Uromastyx* are hunted for food, souvenirs and traditional medicine (Wilms, 2007). *Uromastyx geyri* is one of nine species of *Uromastyx* to be affected by the international pet trade and due to their commercial value are listed in Appendix II of CITES (2020).

Little information is available on the ecology, captive maintenance and breeding of *U.geyri* (Wilms, 2005). This article presents the first experiences of the Birmingham Wildlife Conservation Park (BWCP) in maintaining and breeding the species.

METHODS & MATERIALS

Livestock

In 2015, four male and one female sub-adult wild caught *U. geyri* were acquired by BWCP following a confiscation. Two of the males were high orange/red and two males were high yellow (Fig. 1). The female had light orange markings on the dorsum. The ventral surface of female *U. geyri* is pale and uniform in colour unlike the males in which the dorsal coloration and markings continue onto the ventral surface. The males weighed between 220–240 g and the female weighed 182 g at the beginning of February 2016.

Enclosure and heating

The lizards were housed together in a 120 x 60 x 60 cm plastic moulded Vision Cage (Fig. 2). Three Arcadia 160 W basking lamps were used as sources of heat, visible light and UVB radiation. The lamps were installed to heat the enclosure efficiently and spaced away from each other to prevent antagonistic behaviour between male lizards. The substrate was a mixture of bird sand and coco-fibre (ratio 4:1) which was approximately 6–8 cm deep. Various sized sandstone rocks were positioned under the basking areas at

various heights (Fig. 1) to provide gradients of heat and UVB. Two pieces of driftwood were positioned higher than the rocks but away from the basking areas to provide climbing opportunities and to increase usable enclosure space. Pieces of curved tree bark was placed on the substrate to act as refuges. A small ceramic bowl contained water.



Figure 1. Red male (left) and yellow male (right) U. geyri



Figure 2. Basking area of the enclosure holding an adult group of four male and one female *U. geyri*. The enclosure was also furnished with drift wood and pieces of curved bark to the right but out of view in this image.

The enclosure was maintained on a 12/12 light/dark cycle throughout the year. Temperatures and humidities in the enclosure were measured using a max-min thermohygrometer, spot temperatures recorded using an infrared temperature gun (IR Laser Point Version 320-EN-00), and UV index assessed using a Solarmeter 6.5 UVI radiometer. Ambient daytime temperature ranged from 28-35 °C, basking temperature was 45–50 °C with the surface temperature of the rocks occasionally reaching 55 °C, and the UV Index was 6-8 at a position equivalent to the dorsum of a basking lizard. Night temperatures were between 20–22 °C. Humidity was maintained at 20–40 % RH but occasionally reached 70 % after weekly substrate sprays. Specimens hid between the rocks during spraying but would soon come out to resume basking and were occasionally observed drinking. From November to January, night time temperatures were gradually reduced to 14–16 °C, the rest of the temperature profile remained unchanged. During these months, the group of U. geyri were active for shorter periods during the day and they didn't begin basking until midday. Night time temperatures were gradually increased over a period of two weeks in February; at this point all specimens began basking from 09.00h.

Food and feeding adjustments

From spring to autumn (March - October), the lizards were fed dandelion leaves and flowers, broad and narrow leaf plantain, sow thistle and clover. These were picked fresh from the zoo grounds and were presented 4-5 times per week. When fresh weeds were not available during the winter months, they were substituted by a mixture of endive lettuce, coriander, water and mustard cress, grated carrot and chicory (3-4 times per week). A dietary supplement Nutrobal (Vetark) was sprinkled over the food once per week. This is a multi-vitamin supplement containing calcium and the following vitamins A, C, E, D3, K3, and the B group. Live invertebrates were readily accepted with locust, Locusta migratoria (small and large sized) being the most favoured. All live food was dusted with Nutrobal and fed to the group once every two weeks. Tidymix Pulse & Rice Soaking Parrot Food (Tidy Mix Diets) that had been soaked for 48 h were fed to the lizards once, every 3-4 weeks. The product contains the following pulses - soya, adzuki, mung, pinto, blackeye, haricot beans, chickpea, green and yellow peas, puy & red lentils, as well as the cereals wheat and brown rice.

From November to January, when night time temperatures were reduced, food portions were reduced by about 50 % and as the night time temperatures were gradually increased in February, the amount of food offered was also increased back to normal.

Incubation of eggs

An opaque plastic box $(30 \times 20 \times 20 \text{ cm})$ was provided for egg laying. A hole was cut into the lid to allow access for the female and the box was filled to a depth of about 15 cm with damp coco-fibre. Rocks were built up around the box to allow ease of access and the area by the hole was covered with bark for seclusion. After oviposition, eggs were transferred to a clear air-tight plastic box that fitted into a large neonatal incubator (Vickers Medical Model 79 Servo). Fine grade vermiculite mixed with water (ratio 3:1) was used as the incubating medium. The eggs were very soft and care was taken during the transfer. Eggs were half buried in the medium and damp sphagnum moss was added to all corners of the box for extra humidity. Eggs were incubated at 30.5 °C and relative humidity ranged from 90–95 %, measured using a max-min thermohygrometer with a probe going directly into the box. After 14 days of incubation, eggs were candled using a torch to observe vascularity as evidence of viability. The eggs were aired every three to four days by lifting the lid of the box.

Rearing hatchlings

Once hatchlings were free from the eggs, they were transferred to a plastic container supplied with damp kitchen towel and left for approximately two days post emergence. This was enough time for the umbilicus to dry up. They were then transferred to a rearing tank (Exo-Terra Turtle Terrarium, 90 x 45 x 45 cm). All environmental parameters were the same as for the adults. At 5–7 days post emergence, the hatchlings were fed daily on the same mix of food as the adults. Small locusts were taken readily and nuts and pulses were soaked for 48 h and then crushed before offering. Food was scattered all over the rocks to ensure all individuals could feed. A small pinch (not measured) of Nutrobal was added to the food and occasionally sprinkled over the rocks once every 7–10 days. The rocks were sprayed with water daily and the hatchlings were often observed running over to the wet surface to drink. This behaviour lasted for about two months after which the hatchlings retreated whenever the enclosure was sprayed.

OBSERVATIONS AND DISCUSSION

Reproductive behaviour

From November 2015 to January 2016, males were noticeably active and displayed dominant gestures such as head bobbing, hissing, body arching and circling on the spot. These displays would occasionally escalate and the dominant male would climb onto the other males and circle on top of them. The submissive male(s) would either retreat or remain motionless. Occasionally biting was observed and this resulted in superficial marks to the neck and flanks. Submissive males continued to eat and bask. Copulation between the dominant male and the female was only observed on 21st February. On the 3rd April, the female was observed basking earlier than usual and for extended periods during the day. This raised her body temperature to 45-48 °C. Once these temperatures had been reached the female would move in and out of the basking zones to maintain this temperature. This behaviour was observed until oviposition. During gestation, eggs were visible laterally when the female was basking. On the 1st April, the egg laying box was placed in the enclosure and on 15th April the female entered the box and was observed digging and moving substrate. This happened for 3-5 minutes and then the female would retreat. This behaviour continued and was rather sporadic for the next few days. The temperature in the box ranged from

28–32 °C. On 20th April, the female was seen egg-laying in the box for about 90 minutes and laid eleven eggs. Three eggs were discolored and waxy in appearance and were deemed infertile and immediately discarded. Following oviposition, the female looked emaciated but was still active and alert.

Incubation of eggs and hatching

On day 8 of incubation, two of the eggs began to discolour and sweat. They were left for a further two days, after which they had completely desiccated. Both eggs were removed. Fifty-two days into incubation another egg began to sweat and discolour. The shape of the egg was still spherical, so it was decided to wipe away the excess water and wrap the egg in kitchen towel. Four days later the egg was emitting a foul smell, so it was decided to cut open the egg. On doing this, a dead and premature embryo was revealed. It was then decided to place a plastic egg crate between the remaining five eggs and vermiculite (Fig. 3). The incubation substrate could then be flooded with water to increase humidity without the eggs coming into direct contact with water/excess moisture (Basford, L. pers.comm). On day 91 of incubation, one of the eggs pipped at around 11:00h (Fig. 3A). The specimen was only halfway out of the egg by 17.00h and looked exhausted. On day 92, the first hatchling was completely out of the egg and two more eggs had pipped. On day 93, three hatchlings were completely out of the egg. On day 94, a noticeably smaller hatchling had emerged from one of the eggs. Under closer inspection, it was a twin. The other specimen had died and was fully formed (Fig. 3B). On day 96, five days after the first specimen hatched, it was decided to cut open the final egg using a scalpel. A blunt snake probe was used to gently touch the specimen to determine life and movement inside the egg was observed. It was decided to totally remove the fully-formed live foetus from the egg and place it in a separate container with some damp kitchen towel. After 24 h the hatchling had separated from its umbilicus and was introduced to the others.



Figure 3. Eggs of *U. geyri* in the incubation box with an egg crate separating the eggs from the incubation medium – **A.** The first egg to pip, and **B.** An egg containing twins

Rearing the hatchlings

Hatchlings were too small to sex accurately before they were moved to other zoological collections. They weighed 5.6–6.5 g at birth with the live twin weighing 2.9 g and the dead twin weighing 2.3 g. After 28 days, the other twin died but all the other hatchlings survived to the time that they were dispatched to other collections and at the time of writing (after about four years) they are all apparently still alive.

Lesson learnt

Incubation of eggs: To incubate a clutch of 22 *U. geyri* eggs, Turnbull (2012) used a particularly high proportion of water in the incubation medium, vermiculite to water (1:5). Half of the eggs succumbed to mould and splitting and only four hatched successfully. In our subsequent breeding programmes, we have continued to use a vermiculite to water ratio of 3:1 and continue to separate the eggs and incubation medium with plastic egg crates. This has improved our hatch rate. In 2017, we hatched a total of six individuals from seven fertile eggs and in 2018 we hatched a total of seven from eight fertile eggs.

Feeding: At the time the *U. geyri* were acquired there was no published recommendation for a suitable feeding regime. When we compared photographs of wild animals with captive animals we suspected that many captive *Uromastyx* species were overweight. We therefore adopted an approach of increasing the intervals between feeds and of reducing portion sizes which seemed to produce healthier and more active animals. After oviposition, it is important to feed the females daily as they are emaciated and need good nutrition to recover and be healthy enough to reproduce annually. At this time, males were usually removed from the enclosure to prevent them eating too much and to accurately monitor the females' food consumption.

General husbandry: Copulation has only been observed at one time of the year and so leaving the female with the males in the group after oviposition did not present a problem.

During the winter months, it was decided to only drop night temperatures to 14–16 °C. At these temperatures activity was substantially decreased but we still observed occasional basking and feeding continued albeit at a lower rate. Had we adopted much lower temperatures then the lizards would have been forced to brumate but according to Wilms (2005) *U. geyri* do not require an extended winter brumation (Wilms, 2005).

Many species of Uromastyx have been kept at BWCP over the last 20 years including U. benti, U. nigriventris, U. ocellata and U. dispar maliensis with little reproductive success. A clutch of 18 fertile eggs from U. benti hatched from wild caught adults but the breeding success was never repeated. A clutch of 12 eggs were incubated from U. d. maliensis but the individuals died as they were hatching. Historically, Uromastyx at BWCP were kept under UVB lighting but the amount of UVB they were exposed to was never tested and basking temperatures were <40 °C. The U. d. maliensis were kept in a large display (approx. 3 x 1.5 x 1.5 m) so that maintaining temperature >40 °C was problematic. Specimens were observed basking for most of the day and only left the basking site to feed. It is likely that our changes to husbandry including higher basking temperatures, better visible light, appropriate UVB provision, diet and incubation techniques have enabled U. geyri to reproduce in captivity annually.

Our method of husbandry has continued without change



Figure 4. Radiograph of an 18 month U. geyri

since the breeding success described here. In 2019, as a check on our husbandry methods it was decided to use radiographs to make direct observations on skeletal development. Radiographs were taken of the dorsum of four 18 month old *U. geyri*. All specimens were conscious during the procedure and placed in a small clear container to restrict movements. The skeletons of all four specimens appeared healthy (Fig. 4). This suggests that the current husbandry of the juveniles was satisfactory and should be continued (Archer, K. pers. comm.)

According to Species 360 (2020), there are 41 zoological institutions around the world keeping *U. geyri* with a total of 79 individuals. BWCP is currently the only institution regularly breeding this species. We hope that if other institutions use the husbandry methods describe here they will be able to breed this species and so maintain viable captive populations.

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Breeding behaviour of Dubois's leaping frog *Indirana duboisi* (Anura: Ranixalidae)

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here is still much to learn about the reproductive behaviour of amphibians that lay their eggs terrestrially. Some species show elaborate forms of parental care, in particular oviposition site fidelity and the construction of nests that act as oviposition sites (Wells, 2007). In this context, a nest is defined as any modifications or clearing of habitat or vegetation for egg laying (Peter & Reid 2010). Frogs of the genus Indirana are endemic to India (Modak et al., 2018) and may either attach their eggs to tree bark, place them within rocky crevices (Gaitonde & Giri, 2014) or lay them in nests prepared by males. These frogs show inguinal amplexus (male grasping the waist of the female) and at least in the case of Indirana leithii have semi-aquatic tadpoles that live on rocks and feed on algae and diatoms using their oral apparatus that develops immediately after hatching (Modak et al., 2018). There is also a report of possible egg clutch guarding in Indirana semipalmata (Tapley et al., 2011).

Dubois's leaping frog *Indirana duboisi* Dahanukar, Modak, Krutha, Nameer, Padhye, and Molur, 2016 was erected as a new species following taxonomic revisions of the Ranixalidae (Dahanukar et al., 2016; Garg & Biju, 2016). In 2019, the species was recorded for the first time from the laterite plateaus of Manipal (Karnataka State) that adjoin the Western Ghats (Mudke et al., 2020). The site where it was observed (13°20' 27.24" N 74°47' 13.56" E) is at an altitude of 80 m a.s.l. and has an average rainfall of 3700 mm during the south-west monsoon (Seshadri et al., 2016). This site is close (8.7 km) to the sea and at a lower altitude than the earlier records of *Indirana* spp. (Dahanurkar et al., 2016; Garg & Biju, 2016). This new record lies outside the popular Western Ghats boundary on a plateau that is considered as a 'wasteland'.

This new locality record of *I. duboisi* offered an opportunity to observe the habitat in which breeding occurs and the capacity of the species to modify its micro-habitat for the purpose of breeding. In July to August 2019, the peak months of the monsoon, a total of 16 surveys (2 surveys per week) were made as a part of our larger project on impacts of urbanisation to observe the frogs in their breeding habitat. The surveys were at night between 19.00 h and 21.00 h and in that period the frogs were observed for a total of about 60 minutes. Temperature and humidity was measured using a portable thermohygrometer (Hanna Instruments, HI9564). The dimensions of a single nesting site was measured using a

flexible metal tape measure and to avoid disturbing the frog tending the nest, measurements were only done immediately after the end of monsoons when the male had dispersed.

Confirmation of identity

Ranixalid frog species tend to be cryptic which in the past has resulted in misidentifications (Garg & Biju, 2016). We determined the species identity based on DNA extraction from 2 tadpole tail tips taken from the study site where nest building was observed. During the field surveys, wet rocks were scanned under torch light to find semi-aquatic tadpoles. A clean pair of powder free, nitrile gloves was worn for tadpole handling. Each tadpole was placed on a sterile, wet Petri dish and approximately 2.5 mm tail-tips were removed using a sterile scalpel and separate blades for each individual (Clarke et al., 2019). At the end of the process we observed the tadpoles for 10 minutes and recorded no mortality or distress. Tail tips were stored in 90 % ethanol and transported to the molecular genetics lab in Ashoka Trust for Research in Ecology and the Environment (ATREE). Total genomic DNA was extracted using Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol. The mitochondrial 16S rRNA gene was chosen for species identification based on earlier studies (Biju et al., 2014). PCR amplification was done using two primers 16sF (5' CGCCTGTTTATCAAAAACAT 3') and 16SR (5' CCGGTCTGAACTCAGATCACGT 3') (Palumbi, 1991). The PCR was performed following the protocol laid out in Biju et al., (2014) and Gururaja et al., (2014). The amplified PCR products were purified and sequenced at Barcode Biosciences, Pvt. Ltd. (Bangalore, India) using ABI 3730xl DNA sequencer. Each DNA sequence was trimmed to clear the initial and concluding low quality base pairs and aligned in MEGA 10.1.6. Multiple forward sequences thus obtained were used to query the NCBI BLAST database. Our sequences showed 100 % identity with I. duboisi accession KX641817 collected from Karnataka, Kerekatte (13° 19'21.9" N, 75° 8'45.6" E) (Dahanukar et al., 2016). We deposited the study sequences in GenBank under accession numbers MN952996 and MN952997.

Breeding habitat

Adult males were visibly smaller than females and were seen and heard vocalising during the field surveys (Fig. 1). We observed that *I. duboisi* tadpoles and adults were associated with moist, laterite rocks and the ephemeral vegetation that grows on the rocks during peak monsoon months of July to August (Figs. 2A&B & 3A). On almost all laterite rocks the semi-aquatic tadpoles were seen feeding on the algae that grew on them, in a similar manner to *I. leithii* (Modak et al., 2018). During the survey period (19.00h to 21.00h) at the study site the mean temperature was 26.6 °C and relative humidity 90 %.



Figure 1. Adult *I. duboisi* - A. Male (SVL = 32 mm), and B. Female (SVL = 45 mm)



Figure 2. Tadpoles of *I. duboisi* - **A.** Three tadpoles at different developmental stages (indicated by white ellipses) on moist rocks interspersed with ephemeral vegetation, **B.** Nearly fully metamorphosed froglet on a moist rock

Nest building and nest attendance

A single male was observed creating a nest in an existing cavity within the laterite rock (Fig. 3B). It used its hind limbs to clear fallen leaves, saturated mud, ephemeral vegetation and other debris from the cavity. The nest cavity, which can be seen in a video (YouTube, 2020), had an opening measuring roughly 7.5 cm by 4.5 cm and a depth of 3.5 cm. There was a stream of water dripping into the cavity, creating a puddle of about 4 cm diameter, and an outflow (Fig. 3C). These characteristics appear to help maintain the humidity, temperature and availability of water at the oviposition site. The male continued to attend this cavity in the monsoon months and made advertisement calls that can be heard in the video. Within the nest there were impressions in the mud, visible in the video and the photographs, from what may have been a previous egg clutch (Fig. 3C). It seems possible that the nest was being used for successive egg clutches. We did not actually observe any eggs in the nest despite the presence of the male frog, suggesting these may have been predated, that the male was never favoured by a female, or that egg laying and hatching occurred in an interval between our surveys. The last conjecture is suggested as a possibility since Tapley et al. (2011) observed that the eggs of *I. semipalmata* hatched within only four to five days. In the last week of August 2019, approaching the end of monsoon season, the nest site was abandoned by the male and debris, leaves and vegetation had accumulated within it (Fig. 3D).

The lateritic plateaus are currently categorised as 'wastelands' by the Ministry of Rural Development and National Remote Sensing Agency, (2010). Yet they are home to unique and interesting endemic frog species. The



Figure 3. Habitat for nest construction by *I. duboisi* - **A.** Habitat containing a nest consisting of wet rocks and ephemeral vegetation (nest position indicated by white ellipse), **B.** Close up of the nest attended by a male (in white ellipse), **C.** The nest with the male (white ellipse), potential egg clutch impressions (white arrow) and puddle of water (blue ellipse), **D.** The same cavity showing accumulation of debris and vegetation after the male had abandoned it

observations reported here show that there are still important secrets to be uncovered in the biology and behavior of India's native species and that the plateaus can serve as effective ecological study sites and conservation areas if managed with biodiversity concerns in mind.

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Substrate preference in the fossorial caecilian *Microcaecila unicolor* (Amphibia: Gymnophiona, Siphonopidae)

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INTRODUCTION

Amphibians are the most threatened major group of Vertebrates; 42 % of assessed species are threatened with extinction (IUCN et al., 2019). When the threats cannot be reversed or controlled in the short term, conservation breeding programmes are often considered essential (Gascon et al., 2007; Griffiths & Pavajeau, 2008). However, amphibians are not always easy to maintain and breed in captivity (Tapley et al., 2015). Although natural conditions in which animals may be encountered in the wild may not always be optimal, it is considered best practice to incorporate field data into captive management practices (Michaels et al., 2014a) and where these are lacking, an evidence-based approach to husbandry practices should be pursued as far as possible (Arbuckle, 2013).

Caecilians epitomise the complexities of maintaining poorly known amphibian taxa in captivity. More than 55 % of assessed caecilian species are listed as Data Deficient by IUCN (IUCN, 2019) and only six species are currently maintained by zoos and aquaria globally (Species 360, 2020) but failure to thrive or breed is not uncommon (Flach et al., 2020). Consequently, advances in husbandry are difficult to achieve. There are at least eleven published accounts of caecilian ex-situ management, but these are biased towards aquatic typhlonectid caecilians. Following Amphibian Ark's Conservation Needs Assessment process (Johnson et al., 2018), several species of caecilian have now been assessed as requiring ex-situ management for conservation purposes. These include six of the eight species of caecilian that are endemic to the Seychelles (e.g. Maddock, 2018). If these and other similar programmes are to be realised, it is imperative that advances are made in caecilian captive husbandry.

Empirical data on even the most basic husbandry parameters are lacking for most species of caecilian, including the substrate used to maintain them. We used a simple choice chamber to compare two commonly used substrate types. All methods used in this study were non-invasive and did not require a UK Home Office Licence and were compliant with the BHS Ethics Policy (British Herpetological Society, 2017). No adverse effects of this husbandry intervention were foreseeable.

METHODS

Five Microcaecila unciolor of unknown sex and age were collected from the Kaw mountains at Camp Patawa between 2008 and 2010 by two of the authors (DG & MW). These were transferred to ZSL London Zoo in 2013 as part of a collaborative project with the Natural History Museum's Herpetology Research Group aimed at refining methods for caecilian husbandry, developing and validating field methods, and discovering aspects of life history and behaviour. Prior to our study, specimens were housed individually in a climatecontrolled facility. Room temperature ranged from 24-27 °C (night minimum-day maximum). All enclosures (56 x 56 x 35 cm) were glass and custom-made with slanted bottoms to create a humidity gradient. Ten percent of the glass lid consisted of a fine mesh for ventilation. Specimens were provided with a 15 cm deep layer of Megazorb (Northern Crop Driers (UK) Ltd.) substrate, a waste product from the paper making industry containing unbleached, wood derived cellulosic fibre and inorganic pigment (Kaolin and calcium carbonate), which is sold for equine husbandry (Tapley et al., 2104). Megazorb was soaked in reverse osmosis water mixed with tap water to an alkalinity of 15-20 mg/L and a pH of 7.5 and dosed with tap water conditioner (Tetra products), for 24 h until saturated and then drained of excess water in a cotton pillowcase.

On the 27 March 2017, five M. unicolor were weighed and moved into five individual choice chambers constructed using 360 mm x 200 mm x 200 mm faunariums (Exoterra, Rolf C. Hagen (UK) Ltd., Castleford, UK). A solid 150 mm acrylic sheet secured with aquatic grade silicone, incompletely divided each enclosure equally such that caecilians could only move between substrates by moving over the surface of the substrate. On one side of the chamber we used Megazorb (as described above) and on the other we added moistened topsoil that had been steam treated by the manufacturer and came from a single batch. Substrates were sprinkled into the choice chambers to a depth of 15 cm by hand and were not compacted. A pH test of each substrate (K181 pH Soil Testing Kit, Bosmere © UK) showed that both were pH 7.5. An identical choice chamber (without caecilians) included a humidity and temperature data logger (Lascar (UK) EL-USB-2-LCD) in each of the substrate types, recording at five minute

intervals for the duration of the 40 day study. To control for potential positional effects, each choice chamber was rotated 180° every three days. During the study, ambient temperature ranged between 23-26 °C (night minimum-day maximum) and photoperiod was 10L:14D. Caecilians were offered food three times a week; two live Eisenia earthworms were placed in each side of the choice chamber at each feeding event. We recorded the position of each caecilian once, daily between 09:00 and 16:00 h; the choice chambers were gently lifted, and the location of the caecilian determined as part of the body was visible through the side or base of the container. The experiment ended after 40 days, on the 05 May 2017, the *M. unicolor* were weighed again at the end of the study. We used Social Science Statistics, (2020) for all statistical analyses. The presence of each individual caecilian was scored daily with a 0 (present in topsoil) or 1 (present in Megazorb), following methods used for Agalychnis frogs by Michaels et al. (2014b). Mass changes in each caecilian were calculated as the difference between initial and final masses. Mean choice scores over the 40 day period were calculated for each individual caecilian and these were analysed using a one-sample t-test (two-tailed) against a test mean of 0.5 (i.e. the mean expected if there is no substrate preference), following methods used by Michaels et al. (2014b).

RESULTS AND DISCUSSION

All five *M. unicolor* were recorded much more frequently in the Megazorb substrate (93 % of the 200 daily observations across all individuals). The mean (± standard deviation) preference score was 0.945 ± 0.051 , which was significantly different from a mean of 0.5 (t = 17.454, df = 4, *p*=0.000032 two-tailed). The mean temperature of topsoil (25.4±0.437 °C) was not significantly different from the mean temperature of Megazorb (25.2 ± 0.753 °C) (t =-0.987485, df = 4, *p*=0.37931 two-tailed). Humidity of the two substrates was also not significantly different. The air in both substrate types was supersaturated with water vapour, the mean relative humidity exceeding 100 % 105.9 ± 1.85 % and 104.0 ± 1.26 % in topsoil and Megazorb respectively (t =1.533475, df = 4, p=0.19994 two-tailed). We did not record any null observations and caecilian burrows were observed in both substrate types in every enclosure even though the caecilian may not have been observed in one or other of the substrates during daily checks. Caecilians were generally secretive and never observed feeding. All individuals decreased in mass over the course of the study (mean percentage mass decrease 16.4 % [12.2–21.3 %]), however we were unable to quantify how well specimens were feeding during the study because this would have resulted in unnecessary disturbance and a significant deviation from our standard husbandry practices with this species (Table 1).

Our results show that *M. unicolor* has a statistically significant preference for Megazorb as a diurnal resting site. Although substrate preference might differ among caecilian species, our findings are congruent with previous research showing that another burrowing caecilian (*Geotrypetes seraphini*) exhibited a strong preference for the Megazorb substrate over another substrate (coir) under almost identical experimental conditions (Tapley et al., 2014). To

Table 1. The mass of five *M. unicolor* at the beginning and the end of the substrate choice test and mean daily substrate choice score based on scoring 0 (present in topsoil) or 1 (present in Megazorb)

Choice chamber #	Mass at start of test (g)	Mass at end of test (g)	Mass change during test (g)	Mean choice score
1	29.6	23.8	-5.8	0.975
2	35.4	30.4	-5	1
3	32.7	28.7	-4	0.95
4	29.1	22.9	-6.2	0.85
5	25.7	21.8	-3.9 15.2	0.95

ensure data from the current study were comparable with the previous study, observations were not made between 16:00 and 09:00 h. Coir is a relatively powdery substrate and does not retain burrow structures; Tapley et al. (2014) suggested that this could have explained the preference of Megazorb over coir because burrows are energetically costly to construct (Ducey et al., 1993). In the current study with M. unicolor, topsoil did retain burrow structures and none of the recorded environmental factors differed between topsoil and Megazorb, so it is unclear why there should be a preference for Megazorb over topsoil. However, given that immediately before the start of the test all the caecilians had been housed in Megazorb, the preference could have resulted from learned behaviour and/or neophobia. All the caecilians lost mass during the test and we suspect this results from increased activity associated with being moved to a new enclosure rather than both, or one of, the substrates being inappropriate.

Despite the limited number of substrates that have been tested to date, the current results and those of Tapley et al. (2014), which together involve two species in two families of caecilians, suggest that Megazorb is a justified choice of substrate for the maintenance of terrestrial caecilians. However, it would be preferable for husbandry practitioners to have access to microhabitat data from the wild prior to the implementation of any conservation breeding programme for caecilians and for these substrate conditions to be replicated in captivity. The provision of multiple substrate types in zones within an enclosure may also be considered in order to provide the animals with a choice.

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Trachemys scripta elegans (Testudines: Emydidae) threatens a wetland of international significance in Iran

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The freshwater turtle *Trachemys scripta elegans* (Wied, 1838), commonly called the red eared slider, is native to the Mississippi Valley, USA (Ernst & Lovich, 2009). Due to the vividly coloured shell of hatchlings the species has been favoured by the pet trade since the 1950s and is now considered to be among the most commonly-traded reptile pets worldwide (Lowe et al., 2000). Consequently, the species may now be encountered as an exotic on five continents (Newberry, 1984; Gasperetti et al., 1993; Chen & Lue, 1998; Feldman, 2007; Natividad et al., 2008) and in some countries breeding has been confirmed.

The red eared slider has been observed by ourselves and other researchers in six water bodies in northern Iran, close to the Caspian Sea (Table 1, Fig. 1). All our own observations of the species were made by naked eye or with 6×30 vixen binoculars. The first sighting was of a sub-adult male in 2002 in Amir-kabir reservoir, followed in 2005 by a specimen sunning itself on the bank of Sefidrud reservoir. In 2012, a group of 20 individuals (12 males and eight females) were observed basking on the shoreline of Shoormast Lake (Moradi & Rastegar-Pouyani, 2015). Safaei et al. (2015) reported a single specimen in the Mamloo reservoir and in 2017 we observed a group of more than 40 individuals in Eynak wetland. Then in 2018, we observed three specimens in the Anzali wetland (a Ramsar site - wetland of international importance), two of which were basking alongside two native pond turtle species (Fig. 2).



Figure 1. Map of all known sites of T. scripta elegens in Iran



Figure 2. Turtles basking in the Anzali wetland **A.** Red-eared slider (*T. s. elegens*), **B.** Caspian turtle (*M. caspica*), and **C.** European pond turtle (*E. orbicularis*)

During the 1990s, modern aquarium keeping became very popular in Iran and relied upon the importation of aquarium fish but also included red eared sliders. Many fish keepers bought these reptiles and kept them with their fish, most did not survive except for a few that grew large and started to consume the fish. Consequently, these turtles were released into the wild alongside two native pond turtle species: the Caspian turtle (*Mauremys caspica*) and European pond turtle (*Emys orbicularis*) (Mozaffari et al., 2016). The red eared slider is a generalist species occupying a broad ecological niche (Ernst & Lovich, 2009) and is a potential competitor with the two native species for food and basking sites (Luiselli

Locality	Grid reference	Description	First report	Authority
Amir-Kabir reservoir	35° 58.2′ N, 51° 06' E	Man-made reservoir for drinking water and hydroelectric power	2002	Present authors
Sefidrud reservoir (Manjil dam)	36° 44.7′ N, 49° 22' E	Man-made reservoir for irrigation water and hydroelectric power	2005	Present authors
Shoormast Lake	36° 05.27' N, 53° 02.8' E	Natural lake	2012	Moradi & Rastegar-Pouyani, 2015
Mamloo reservoir	35° 36' N, 51° 47.3' E	Man-made reservoir - irrigation and drinking water	2015	Safaei et al., 2015
Eynak wetland	37° 16.38′ N, 49° 32.5′ E	Four natural lagoons surrounded by urban development	2017	Present authors
Anzali wetland	37° 22' to 37° 32' N, 49°14' to 49º37' E	Natural lake – shoreline agriculture	2018	Present authors

Table 1. Water bodies where T. s. elegans has been detected in Iran

et al., 1997; Cadi & Joly, 2004). How significant this might be for native turtle conservation in Iran is not clear, not least because in some locations the species may not be able to reproduce due to low temperatures or limited precipitation (Ficetola et al., 2009). This limitation could apply to Amir-Kabir, Mamloo and Sefidrud reservoirs that are located to the south of the Alborz mountains where there are long, cold winters. The very low numbers of *T. s. elegans* observed at these locations suggest that the species is not reproducing. Furthermore, although the Sefidrud reservoir connects to the Caspian sea via the Sefidrud river that crosses Boojagh national park, due to the length, water speed, and depth of reservoir it seems that there is little chance of turtles reaching Boojagh national park, alive.

Shoormast Lake, Eynak and Anzali wetlands are all located to the north of the Alborz mountains where winters are shorter and warmer. Although there is no record of successful reproduction in these habitats it does seem likely to occur because of both the large number of red eared sliders observed and the environmental similarity to sites with confirmed reproduction in Turkey (Çiçek & Ayaz, 2015). But in the case of Shoormast Lake and Eynak wetland the threat to native species may be limited as they are effectively small isolated habitats not connected to river systems. They are also fully accessible so that it may be possible to eradicate the red eared sliders. In the Anzali wetland the situation is quite different. This is a vast area fed by rivers and canals and surrounded by agricultural land, especially rice farms, creating a significant dispersal corridor to other natural habitats along the southern side of the Caspian Sea. There are many places in and around the wetland that are inaccessible to people so that there is little prospect of eradication. However, we would urge all governmental and non-governmental organisations to take whatever action they can to limit the spread of T. scripta elegans in this wetland. There is also a need to address the fact that red eared sliders are still being imported into Iran. We think that banning such importation would be counterproductive since it would push the trade underground and make it even more difficult to control. Instead, we would recommend that current restrictions on keeping, selling, buying and captive breeding of the pond turtle *Emys obicualris* should be lifted. This species is plentiful in Iran and would be an ideal local

substitute for *T. s. elegans*. This action combined with a comprehensive educational programme for pet keepers has the potential to limit future releases or escapes of red eared sliders.

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Rediscovery and updated distribution of *Lycodon septentrionalis* from Mizoram state, north-east India

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The white-banded wolf snake *Lycodon septentrionalis* (Günther, 1875) is a nocturnal, terrestrial, colubrid snake known to feed on small vertebrates including snakes. It can reach up to 1,800 mm in total length and inhabits the midhills of evergreen forests at elevations ranging between 220 - 2,100 m a.s.l. (Das & Das, 2017). The species is considered rare and there is little information on its natural history (Murthy et al., 1993). The known range of this species includes India (northern West Bengal, Assam, Arunachal Pradesh, Nagaland and Mizoram), Bhutan, Myanmar, Laos, Vietnam, Thailand, Cambodia, and China (Uetz et al., 2020). A total of only nine *L. septentrionalis* specimens have hitherto been reported from India (Das & Vasudevan, 2015).

In this study, we document 10 new location records for *L. septentrionalis* in Mizoram state represented by four specimens we collected as well as information obtained from private collections and photographs (Fig. 1). The four collected specimens have been deposited in the Departmental Museum of Zoology, Mizoram University (MZMU). The locations for the ten new records are plotted in Figure 2 and the collection details are given in the Supplementary Material on the British Herpetological Society website (see note at the end of this article).

Lycodon septentrionalis can be diagnosed and distinguished from its sympatric congeners in the region by having a purplish-black dorsum with narrow transverse white bands and a white venter that is sometimes spotted or barred with black. This differs from other species - Lycodon zawi Slowinski, Pawar, Win, Thin, Gyi, Oo & Tun, 2001 has poorly developed white cross-bands on a brownish black dorsum; Lycodon laoensis Günther, 1864 has yellow cross-bands on a brownish black dorsum; Lycodon fasciatus (Anderson, 1879) has yellowish cross-bars of irregular outlines on a black or purplish-black dorsum; Lycodon jara (Shaw, 1802) is striped all over with a yellow pattern being formed by small spots or short longitudinal lines on a brownish or purplish black dorsum; and Lycodon aulicus (Linnaeus, 1758) has white or yellowish cross-bars on a brownish or greyish dorsum. Moreover, we diagnosed the species by using an adult male specimen that had a maxillary bone extending beyond the palatine, bent inwards but not arched, with 7 anterior teeth increasing in size, fang-like, and a diastema separated the 7 anterior teeth from the other 5 teeth, the last three of them are larger than the others. In the case of other Lycodon species the maxillary bone is strongly arched and bent



Figure 1. Lycodon septentrionalis from different localities in Mizoram, India; A. Juvenile collected from Sawleng; B. Juvenile found at Durlang; C. Juvenile from Chandmari West; D. Road-killed adult male from Mamit Jail Road, Mamit; E. Juvenile from Hunthar; F. Juvenile from Khawbung; G. Juvenile from PTS Thenzawl; H. Unsexed adult killed by local people at Khawrihnim

inwards anteriorly, with 3–6 anterior teeth increasing in size, fang-like, and separated by a toothless space from the rest 7–15 in number, the last two of which are larger than the others (Smith, 1943). Detailed morphometry and scalation data of four specimens were recorded (see Supplementary Material).

The first specimen of *L. septentrionalis* from Mizoram state was collected by T. G. Vazirani on 23 October 1960 (Z.S.I. Reg. No. 21904; snout-vent length=305 mm; tail length=85 mm) from Bhumtilong, ca. 16 km from Aizawl in the northern part of Mizoram (Talukdar & Sanyal, 1978). Today, it is very difficult to be certain of this site as the name of the locality provided is nowhere to be found in the state. Moreover, Das & Vasudevan (2015) plotted Bhumtilong in the location of Ratu village, in the north-eastern part of Mizoram



Figure 2. Map showing the distribution of *L. septentrionalis* in north-east India with emphasis on Mizoram state. The records 1 to 10 are new and from the following localities in Mizoram - 1. Sawleng, 2. Durtlang, 3. Chandmari West, 4. Mamit Jail road, 5. Hunthar, 6. Khawbung, 7. Thenzawl, 8. Pangzawl, 9. Khawrihnim, 10. Dampu; 11 is the only previously published record from Mizoram(Bungtlang/Bhumtilong); 12 to 18 are published records from localities in other states (see Das & Vasudevan, 2015) as follows- 12. Kohima, 13. Namsang, 14. Kahare, 15. Ziro, 16. Zimithang, 17. Darjeeling, 18. Phubsering

which is ca. 143 km from Aizawl. We suggest the locality is Bungbangla (Bungalow) formerly known as Bungtlang located at ca. 16 km from Aizawl (23° 43'47" N, 92° 46'46" E) in the northern part of Mizoram state where the state government constructed an inspection bungalow and later a guest house (C. Vanlallawma, pers. comm.).

Lycodon septentrionalis has not been reported from Mizoram for 60 years after it was first collected. Our new records were made between 2013 and 2019 and constitute the southernmost localities in India at altitudes ranging from 703 m to 1310 m a.s.l. (Fig. 2) and represent a rediscovery of this species within the state, with confirmation of its occurrence in five Mizoram districts. It may be inferred that *L. septentrionalis* is not an uncommon species in Mizoram which is not surprising as until recently the ophidian fauna of Mizoram had remained very poorly surveyed.

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Death-feigning as defensive behaviour of eight lizard species of the Amazon rainforest

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Death-feigning or thanatosis is a defensive behaviour in which an animal adopts a posture resembling a dead individual when it is physically threatened by a potential predator (Hamphreys & Ruxton, 2018). This can be an effective mechanism against predators that will not feed on dead animals or need to detect prey by its movement (Toledo et al., 2011). The evolution of anti-predatory behaviours is an important component of the natural history of lizards (Downes & Hoefer, 2004), although until now thanatosis had only been reported for Amazonian lizard in the families Gymnophthalmidae and Tropiduridae. In this study we report the death-feigning behaviour displayed by eight lizards species from the Brazilian Amazon rainforest (Table 1) and add new records for the families Dactyloidae, Alopoglossidae, Gymnophthalmidae and Sphaerodactylidae.

We undertook fieldwork in a terra firme forest at the Reserva Extrativista Beija-Flor Brilho de Fogo, municipality of Pedra Branca do Amapari, Amapá state, Brazil (0°47'30.6"N, 51°58'42.1"W). The area consists of dense forest with large trees and sandy and clay ground being the watershed of the Amapari River (Drummond et al., 2008). The local climate is classed as Equatorial (Am) according to the Köppen-Geiger classification with two well-defined seasons: a dry season occurring between July and September, and a rainy season between December and June (Alvares et al., 2013).

The data presented in this study were collected between July and October 2019. During field surveys of the herpetofauna, we captured eighteen species of lizards during active visual searches (Heyer et al., 1994) during the morning. All lizards were handled carefully during measurements (body size and mass). Of the eighteen species of lizard captured, eight species exhibited thanatosis during handling.



Figure 1. Death-feigning behaviour of Amazonian lizards *N. chrysolepis, A. angulatus, N. bicarinatus* and *A. reticulata*. (top to bottom)

 Table 1. Death-feigning behaviour observed in eight species of Amazonian lizards

Family	Species	Date in 2019	Time	Duration of thanatosis
Dactyloidae	Norops chrysolepis	13 July	13.01 h	40 s
Alopoglossidae	Alopoglossus angulatus	23 October	08.09 h	25 s
Gymnophthalmidae	Neusticurus bicarinatus	23 October	08.13 h	50 s
	Arthrosaura reticulata	24 October	09.03 h	120 s
	A. kockii (male)	24 October	09.48 h	100 s
	Cercosaura aff. argulus	25 October	10.16 h	30 s
Sphaerodactylidae	Gonatodes humeralis (female)	26 October	09.56 h	30 s
Tropiduridae	Plica plica	26 October	10.20 h	60 s



Figure 2. Death-feigning behaviour of Amazonian lizards A. kockii, C. aff. argulus, G. humeralis and P. plica. (top to bottom)

The lizards put themselves in a death-feigning posture by turning their ventral surface upward exposing the cloaca area, contracting the limbs, closing their eyes, and curving their tails laterally (Fig. 1, Fig. 2). This is the first report of death-feigning behaviour of the lizards *Norops chrysolepis*, *Alopoglossus angulatus*, *Neusticurus bicarinatus*, *Arthrosaura reticulata*, *Arthrosaura kockii*, *Cercosaura* aff. *argulus* and *Gonatodes humeralis*, and the second record for *Plica plica*. The specimens collected were deposited in the Herpetological Collection of the Universidade Federal do Amapá, municipality of Macapá, Amapá State, Brazil (CECC).

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A caution on handling *Trachycephalus venulosus* (Anura: Hylidae); toxic effects of skin secretion on human eyes

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The milky or veined treefrog *Trachycephalus venulosus* (Laurenti, 1768), is a large, robust, arboreal species distributed widely across the neotropics (Fig. 1). It is the only member of its genus (totalling 12 species) to occur in Central America (Frost, 2020) and inhabits a range of primary and disturbed habitat types. It can be identified using the guide to Central American amphibians by Kohler (2011).

Numerous guidebooks heed caution when handling T. venulosus, referring to its ability to secrete a large amount of sticky, white, volatile, poisonous, alkaline, water insoluble mucus that is highly irritating to mucous membranes (Savage, 2002; Kohler, 2011). These collagenous proteinbased secretions have been demonstrated to discourage potential predators (e.g. snakes - Yeager et al., 2019), as well as reduce cutaneous water loss (McDiarmid, 1968). The exact chemical composition of T. venulosus secretions remain undetermined; however, various literature documenting the effects of Trachycephalus secretions suggest they can cause harm to humans (Smith, 1941; Duellman 1956; Tanecs & Littlefair, 2014). According to those studies, human reactions to T. venulosus secretions can be extremely painful, and include symptoms such as sneezing, skin irritation, rashes, swelling, and even temporary blindness. In support, the following note details my own experiences with the skin secretions of this frog.

On the 25 September 2015, I was in the region of La Democracia, Belize District, Belize (17°21'27.9"N 88°32'32.9"W). At 21.15h, I encountered an adult hylid frog on a high branch in vegetation next to a water pool. At that time, I was not familiar with T. venulosus and in order to make an identification I carefully captured the frog (sex unknown) with moist (insect-repellent-free) hands and promptly released it on a lower perch so I could photograph and observe its defining characteristics more closely. The individual was held in-hand for less than 20 seconds, not subject to excessive stress, nor did it visibly secrete any white mucus in response to capture. However, within five minutes of capturing the individual, I began to develop a rash-like burning sensation on my forehead, after unintentionally wiping sweat from it using my hand. Before I understood what was causing the irritation, I proceeded to further touch and wipe my forehead, which combined with running sweat, then transferred the irritant into my eyes. Immediately, I developed intense localised pain in both eyes, visual impairment and uncontrollable eye watering as a response. Understanding the possible seriousness of my situation, I quickly made my way to a nearby tap and mirror and proceeded to wash my hands and face with soap, and flush my eyes with fresh water. For about 30 minutes after, I could barely keep both eyes open owing to sharp burning pains, despite vigorously



Figure 1. Trachycephalus venulosus at the reported locality in Belize

irrigating them with water during that time. In total, it took approximately 90 minutes for normal vision to return and the pain to subside, with no side effects other than bloodshot eyes and slight inflammation of the facial skin around them. Now recovered, I successfully identified the hylid species as *T. venulosus*, and finally understood the error of my ways. I am a white Caucasian male with no known allergies and at the time was 22 years old.

In addition to documenting first-hand the effects of *T. venulosus* secretions, this case serves as a reminder to myself, and others, for the need to familiarise ourselves with the species present at study locations, follow correct fieldwork and handling protocols, always consider the potential hazards posed by native wildlife, and to avoid lone fieldwork where possible. Concerning *T. venulosus* and ideally all wild amphibians, capture and any necessary handling should always be performed wearing appropriate sterile gloves (Greer et al., 2009), to minimise any risk to the animal and provide a protective barrier between yourself and the amphibian.

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First record of the aesculapian snake (*Zamenis longissimus*) in South Wales

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The aesculapian snake (*Zamenis longissimus*) is a large rat snake found throughout Europe, Turkey, Iran and the Causcaus (Arnold & Ovenden, 2002; Gasc et al., 1997) and in Great Britain is an exotic species with a very limited distribution. Adults can grow to 2 m in length and are uniform olive-brown or grey in colour (Arnold & Ovenden, 2002) while juveniles and sub-adults are mottled/blotched and with a yellow or cream crescent-shaped blotch at the sides of the neck, not dissimilar from a grass snake (*Natrix helvetica*). The species is capable of scaling trees and bushes and feeds primarily on small mammals (Arnold & Ovenden, 2002).

Currently two populations are recognised in Britain, one along the banks of the Regent's Canal in London and the other on the site of the Welsh Mountain Zoo in Colwyn Bay, North Wales (Beebee & Griffiths, 2000; Langton et al., 2011). The population within and around the grounds of the Welsh Mountain Zoo has been present since the 1960s (Lever, 2003) whereas the population along the Regent's Canal has only been there since the mid-1980s (Langton et al., 2011). It is probably no coincidence that both populations exist within or near the grounds of zoos. It is likely that the local abundance of prey and egg laying sites, such as compost heaps, allows the snakes to persist. However, we present evidence showing that there is a third viable population in Britain near Bridgend, South Wales (Fig. 1).



Figure 1. A map of the southern England and Wales highlighting both the two known populations of *Z. longissimus* (green triangles) and the population described here (blue diamond)

On the 19th September 2016, a family member of a local resident contacted us asking for help with the identification of a snake in his garden. The person in question suspected the snake to be *Z. longissimus* and we were able to confirm the identification from the photos that were submitted (Fig. 2). Following further investigation, it is also evident that the species was also present at nearby allotments. Close by there are several linear features, including a railway line, that may act as a route for dispersal. The species is thought to have been present for 15-20 years (based on information provided to us by residents) although it is unknown how far they have dispersed in that time.



Figure 2. Some of the photos sent to us confirming the presence of *Z. longissimus* in Bridgend, South Wales

In Bridgend only a small number of snakes have been seen at a time. Most of these have been sub-adults or adults which suggests successful reproduction even though hatchlings have yet to be seen. As far as we are aware, no structured surveys have been completed to establish the extent of the Bridgend population. So far the snakes seem to have gone largely unnoticed by the local residents of Bridgend, as suggested by the lack of records for the species in the area.

A number of isolated populations exist throughout northern Europe as relics of a past warmer climate and likely due to the activities of humankind (Musilová et al., 2007). *Zamenis longissimus* remains have been found in eastern England providing evidence that they were once present during the Pleistocene (Holman, 1990; Holman et al., 1994). They became extinct and are now found in the UK as introduced isolated populations such as the one at Bridgend. *Zamenis longissimus* is a fairly popular pet and can be purchased from breeders or dealers. Consequently, the founders of the Bridgend population are likely to have been escaped or released pets. Their origins could be investigated further by comparing DNA samples with those from other populations elsewhere in the species range, including those from the other two UK populations. Over the coming years, surveys are planned in Bridgend to document the species range, size and population structure.

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Coronella austriaca (smooth snake) - mortality after prey ingestion

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'he smooth snake is a medium sized European species that feeds mainly on vertebrates. Reptiles and particularly lacertid lizards are considered as the main fraction of its diet (Reading & Jofré, 2020). Legless reptiles are also preyed upon by the smooth snake, but such cases seem to be reported less frequently (Völkl et al., 2017). On 24 May 2019 a dead specimen of Coronella austriaca was found by the roadside in Gogolin (opolskie vovoideship, south-west Poland) with the tail of a slow-worm Anguis fragilis sticking out of its mouth. The snake had no external signs of being run over by car or bicycle, had no wounds and also did not look starved or weakened. X-ray examination was performed with the use of computed radiography by Konika Minolta (Regius Model 110s) and Siemens Polydoros LX 30 lamp (Fig. 1A). Computed tomography was performed with the use of the Siemens 16 slice CT scanner (Fig. 1B). The body length and diameter of both the snake and slow worm were measured and both were weighed.



Figure 1. A. Total body radiographic image in latero-lateral projection of a dead *C. austriaca* and its prey, and **B.** CT image of the same animal, 3D image reconstruction (60mAs i 130 kV, 0.6mm SD)

Slow worms are not unusual prey items for smooth snakes, so mortality following ingestion of a slow worm seems unlikely and has never been reported previously. Greene (1983), who explored the maximum limits of the ratio prey mass/predator mass (WR), showed that the limiting ratio for non-venomous snakes is WR = 0.6. Based on body measurements of the snake we collected (SVL = 340.0 cm; tail length = 76.0 cm; head diameter = 12 mm; weight = 14 g) and the slow worm (SVL = 86.0 cm; tail length = 97.0 cm; body diameter = 8 mm; weight = 3 g) giving a WR of approximately 0.2 which is far below the threshold suggested by Greene (1983). Thus the smooth snake which we collected should have been fully capable of swallowing prey of the size here recorded.

Indeed, larger prey items have been recorded as swallowed and digested by smooth snakes (Juszczyk, 1987). However, the skin of *Anguis fragilis* has osteoderms (Zylberberg & Castanet, 1985); these reduce skin flexibility. This could constrain the capacity of the snake to both swallow such prey and to regurgitate it and could eventually lead to suffocation, which is the proposed cause of death here. Constraints of this kind might also result in lower size limits for swallowing prey such as slow worms compared to other prey types and explain why juvenile slow worms are more commonly ingested by smooth snakes than adults (Zimmermann, 1988).

Our observation suggests that ingestion of legless prey could carry the high cost of increased risk of mortality. This, in turn could provide an additional explanation of why ophiophagy, including cannibalism, is relatively rarely reported in snakes (Jackson et al., 2004) and why slow worms seem not to avoid shelters already inhabited by potential predators, i.e. smooth snakes (Kolanek et al., 2019) or *Vipera berus* (R.J. Hodges, personal communication).

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A malformed oriental garden lizard *Calotes versicolor* from north-east Bangladesh

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The oriental garden lizard, Calotes versicolor (Daudin, 1802) (Fig. 1A) is a widely-distributed agamid species found across the Indian subcontinent, Afghanistan, Iran, Thailand, Vietnam, Cambodia, Laos, Indonesia, Singapore and southern China (Das, 2010; Hasan et al., 2014; Uetz et al., 2019). In Bangladesh, C. versicolor inhabits a wide range of habitats, including dry and moist forest, near human habitation, parks, forest edges and roadside vegetation (Khan, 2008; Hasan et al., 2014; Khan, 2015; IUCN Bangladesh, 2015).



Figure 1. The oriental garden lizard (*C. versicolor*) from north-eastern Bangladesh **A.** Normal adult male, **B.** Deformed female from the same area

This note describes a single malformed specimen of C. versicolor that was sighted and photographed on the 28 March 2016 at 11:05 h at Madhabkunda Eco-park (24.6403°N, 92.2280°E; WGS 84) in Bangladesh. The habitat of the park is composed of mixed-evergreen tree species with several streams. The observed C. versicolor was seen basking on the ground. Photographs were taken promptly and the lizard was then caught with the aid of a butterfly sweep net without apparently causing any injuries. The specimen was 345 mm (snout-vent length: 70 mm, tail length: 275 mm) and showed clear signs of physical malformations (Fig. 1B). The trunk was highly deformed with two humps at the beginning of the thorax and the end of the pelvic region. Both forelimbs and hindlimbs seemed to be without any deformities. The tail also showed obvious malformations; it was stiff and spirally twisted along its entire length. After taking measurements, the specimen was released at its original location and it later

left the area - showing no difficulties in locomotion. On the same day, we found four more specimens of *C. versicolor* that were all of normal physical appearance.

We could not collect or preserve the specimen for further investigations as we had no legal permits to collect specimens at that time, which makes it impossible to postulate a definite cause of the malformations. Ideally, the animal should have been radiographed in order to determine the exact nature of its vertebral developmental abnormality. The gross appearance strongly suggests that this was kyphosis (an abnormally excessive convex curvature of the vertebral column) but, without radiographs or dissection, concurrent scoliosis (the vertebral column twists and curves laterally) and/or lordosis (an abnormally excessive inward curvature of the vertebral column) cannot be excluded.

For many decades, developmental abnormalities have been recognised in captive and to a lesser extent freeliving reptiles. A seminal work, which helped define and categorise such abnormalities including those involving the vertebral column, was provided by Bellairs (1981). Recent descriptions of anomalies in free-living lizards include the report by Gehring (2009) of an apparently similarly deformed chameleon, *Furcifer pardalis* from north-eastern Madagascar. Arrivillaga & Brown (2019) reported kyphosis in a free-living Skink *Marisora brachypoda* from Utila Island, Honduras. Hindlimb deformities were also observed in a Brown anole, *Anolis sagrei* from Taiwan (Norval et al., 2009).

Bellairs (1981) stated that developmental abnormalities of reptiles are essentially of either genetic or environmental origin. The latter can be particularly significant, especially incubation of reptile eggs at excessively low or high temperatures and low relative humidity. Biochemical substances specifically, toxins and pollutants might be responsible for some of the malformations observed in reptiles in the wild (Bellairs, 1981). Other causes that might trigger malformation in reptiles include inbreeding of reptile populations with low genetic diversity (Madsen et al., 1992; Olsson et al., 1996; Norval et al., 2009).

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Salamander using its prehensile tail - *Bolitoglossa* cf. *nympha* (Plethodontidae; sub-genus Nanotriton), Honduras

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 $B^{\it olitoglossa\ nympha}$ Campbell, Smith, Streicher, Acevedo, & Brodie 2010 is a diminutive salamander; snout-ventlength <43 mm, tail-length/SVL ratio 0.70-0.95 (Kohler, 2011). It occurs at low-to-mid elevations (275 to 1165 m asl) from Huehuetenango and Baja Verapaz provinces in Guatemala, through to Copán, Cortés, and Yoro in Honduras (Frost, 2020). The species belongs to a complex of four closely related bolitoglossine salamanders in the Bolitoglossa rufescens group (subgenus Nanotriton; Parra-Olea et al., 2004). In north-western Honduras, in-situ identification of Bolitoglossa (Nanotriton) is tentative given the occurrence of at least two cryptic taxa (B. nympha & B. rufescens) in microsympatry; both these species being morphologically indistinguishable in the field (Hess et al., 2017). Species of Bolitoglossa (Nanotriton) primarily inhabit lowland tropical rainforest habitats, but are also associated with agricultural habitats such as banana plantations (Rovito et al., 2012 a&b). They are primarily nocturnal and semi-arboreal, frequently climbing moist vegetation to use leaf-axil refugia and bromeliads (Rovito et al., 2012b). Unlike other Bolitoglossa, species of the subgenus Nanotriton are unique in having small robust bodies, with a tail length usually shorter than their SVL, and underdeveloped feet with extensive webbing (Parra-Olea et al., 2004; Kohler, 2011).

Previously, the tail of *Bolitoglossa* (Nanotriton) spp. has been linked to defensive strategies such as raising, posturing and autotomy (Arrivillaga & Brown, 2018). However, the following note details B. cf. nympha using its tail to grasp and assist climbing following a flip and fall defensive manoeuvre. On 14 July 2016, c. 19.40 h, I encountered an adult Bolitoglossa (Nanotriton sp. - assumed to be B. nympha) active on streamside vegetation bordering agricultural land, in the lowland buffer region of Parque Nacional El Cusuco (PNC), Santo Tomas, Cortés, Honduras. The individual was climbing c. 2m above ground, but when approached, flipped and dropped from its perch, then caught itself using the tail like a hook to grasp a stem c. 1 m below (Fig 1A). After dangling motionless for c. 1 minute, the tail was used to lever the body upwards and prevent the salamander from falling (Fig 1B). Such a mechanism might not only protect the salamander from an injurious fall, but could also fool a predator as to its whereabouts, assuming it to have fallen completely to the ground.

Long prehensile tails are a widespread adaptation among arboreal plethodontid salamanders (Duellman & Trueb, 1986), but evidence of their use in assisted climbing, escape



Figure 1. *Nanotriton (B. cf. nympha)* exhibiting caudal prehensility following a flip and fall escape, **A.** The salamander hanging momentarily after having caught itself using the tail to grasp a stem, **B.** The salamander levering itself back up onto the stem

and/or defence remains rare. Phillips & Nicholson (2013) coined the term 'caudal prehensility' to define this behaviour in salamanders, reporting an observation of *Bolitoglossa lignicolor* using its tail in a hook-like fashion to catch itself following a flipping escape. Until now, *B. lignicolor* remained the only *Bolitoglossa* species in which this behaviour was reported definitively. The present observation demonstrates prehensility in *B.* cf. *nympha*, suggesting that even short tails like those of sub-genus *Nanotriton* are practical for locomotion and defence in an arboreal setting. Our collective studies call for more field observations in order to determine whether prehensility is widespread in bolitoglossine species.

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Aberrant body markings in the Cuban banded dwarf boa, Tropidophis feicki (Squamata: Tropidophiidae)

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The genus *Tropidophis* contains 32 species distributed in South America, The Bahamas, and the Greater Antilles (Uetz & Hošek, 2018), with a major radiation in Cuba (Hedges, 2002). *Tropidophis feicki* Schwartz 1957 is a small snake (411 mm snout-vent length in males, and 448 mm in females) endemic to forested karstic areas of western and central Cuba (Rodriguez-Cabrera et al., 2020). It is typically a nocturnal species but in caves it may be active during daytime (Schwartz & Henderson, 1991). Here we report an observation from a cave of a specimen of *T. feicki* with aberrant body markings.

The normal body markings of *T. feicki* consist of two dorso-lateral rows of black blotches, sometimes fusing into bands across the body (Fig. 1A & B), with a pale immaculate venter (Schwartz, 1957). This species shows colour change between night and day. During nighttime, they adopt a light coloration (light phase) (Fig. 1A) that consists of a milky white or light grey background with the blotches clearly distinguished from it. During daytime they change to a dark phase (Fig. 1B) that is characterised by a brown or dark grey background with the blotches much less contrasting with the background (Henderson & Powell, 2009). The background colour change is mediated by an expansion or contraction of the melanophores in the skin (Hedges et al., 1989) and may be triggered by low temperatures irrespective of time of the day (Rehak, 1987).

In November 2018, during a herpetological survey of the Santo Tomás Great Cavern (STGC), Moncada, Viñales, Pinar del Río (22°54′44′′ N, 83°84′68′′ W, WGS 84, 230 m a.s.l.), we captured an adult T. feicki that measured 300 mm SVL (Fig. 1C & D). The snake was in the "Lechuza" gallery that is part of a daytime speleotourism trail for dozens of tourists daily. The snake was found at 21:00 h climbing a rock, 20 m inside the Lechuza gallery and was in the light colour phase. It had aberrant body markings where many of the black blotches were completely or partially absent, revealing areas of extended background colour (Fig. 1C & D). We released the individual at the site of capture immediately after taking measurements and observed it for 10 minutes after release. No abnormal behaviour was detected during the observations. A photo voucher was deposited in the collection of Museo de Historia Natural "Tranquilino Sandalio de Noda" from Pinar del Río, Cuba under the label "Moncada 20181126 215617."



Figure 1. The body markings and coloration of *T. feicki* photographed in caves – A. Normal specimens in light phase, B. dark phase, and C. & D. aberrant specimen

One possible explanation for the aberrant marking of this snake is that it is suffering from piebaldism, an abnormal body coloration in which melanocytes are absent from various parts of the body surface but not the pupil (Davis, 2007). A simple way to confirm or reject 'piebaldism' in this case would be to make observations on the snake's ability to change from the light to the dark phase. If some of the white areas on the body were not capable of darkening in the normal way then this would suggest the absence of melaocytes and confirm the aberration as piebaldism. We will endeavour to make such observations in the future.

Factors associated with piebaldism, aside from genetics, have been thought to be diet, senility, shock, disease or injury (Sage, 1962). In the current case, we would reject senility or injury because, according to its size (300 mm SVL), the individual was a young adult, and no sign of injury was detected. It is of interest to note that at the same locality piebaldism has recently been reported in the frog *Eleutherodactylus zeus* (García-Padrón & Alonso, 2019).

Depigmentation could be a disadvantage to animals in the wild as it may render camouflage ineffective, disrupt mimicry, etc. (Uieda, 2000; Sandoval-Castillo et al., 2006). However, in low light conditions, such as those inside caves or during the night, such disadvantages may not apply.

We strongly suggest that there should be a longterm survey of the population of *T. feicki* in the area to determine whether the aberrant markings were provoked by environmental stresses, perhaps due to human activity, or whether the cause is hereditary. If it results from inbreeding, which might be expected in small isolated populations (Coyne & Orr, 2004), then it would be of concern to conservationists.

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First evidence of Fogo Island skink (*Chioninia fogoensis*) introduction to the island of Madeira

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he Madeiran wall lizard (Teira dugesii) is the only native reptile species found on Madeira, although other species such as the Tenerife lizard (Gallotia galloti) and moorish gecko (Tarentola mauritanica) have been introduced to the island and become established (Arnold & Burton, 2002; Jesus et al., 2013). Madeira lacks any native snakes however the flower pot snake (Indotyphlops braminus) has been accidentally introduced to the island, likely by the movement of horticulture products from the Canary Islands (Jesus et al., 2013). There are a number of other reptile (and amphibian) species recorded from Madeira, however these likely represent single individuals that may have escaped captivity and therefore do not represent self-sustaining breeding populations (Malkmus, 2004). Madeira's depauperate herpetofauna assemblage is probably due to its relative isolation in the Atlantic Ocean, being 700 km off the west coast of Africa and about 900 km from the Iberian Peninsula (Jesus et al., 2013).

In mid-December 2019, an elderly couple returned to Britain from a holiday in Madeira to find a skink in their suitcase. Upon discovering the emaciated lizard they contacted the RSPCA (Royal Society for the Prevention of Cruelty to Animals) who rehomed the skink with an exotic pet dealer. From here the lizard found a permanent home with DJC (in mid-January 2020) and it has been in his care since (Fig. 1). Due to the emaciated nature of the skink, a robust identification was not possible until fairly recently. The individual was easily identified as a skink due to the lack of femoral pores. After consulting with other herpetologists the skink was successfully identified as the Fogo Island skink (Chioninia fogoensis) following information in Miralles et al. (2010). Whilst we do not know where in Madeira the holidaymakers were staying we do know that this island was their only holiday destination. There is no evidence to suggest that the aircraft in which the couple travelled landed anywhere prior to arrival back in London. Consequently, the simplest explanation is that the skink entered their luggage whilst holidaying in Madeira.

The Fogo Island skink is endemic to the island of Santo Antão, Cape Verde although before a recent taxonomic split, subspecies were thought to be more widespread among neighbouring islands (Miralles et al., 2010). These subspecies have since been elevated to species level, reducing the potential source of *C. fogoensis* to a single island. Tropical house geckos (*Hemidactylus mabouia*) also originating from



Figure 1. Fogo Island skink (*C. fogoensis*) in a temporary enclosure in Britain some weeks following air travel from Maderia in a suitcase

Cape Verde have been introduced to Madeira in recent times (Jesus et al., 2002). Madeira and the Cape Verde archipelago are over 1900 km apart separated by the Atlantic Ocean which rules out natural dispersal of either species. As both territories are former colonies of Portugal, it is likely that there are established trade routes by which the introduction of such reptiles could occur.

The native fauna of Madeira has also been a source of colonists; the endemic *T. dugesii* has been introduced to the Azores, Canary Islands and mainland Portugal (Silva-Rocha et al., 2016). Such movements risk the introduction of their diseases or parasites, which could potentially threaten native species (Burridge, 2001). Research suggests that *T. dugesii* is a potential host for tick-borne pathogens (De Sousa et al., 2012) that may be novel to lizards, people and livestock in the extended range.

Up until now, C. fogoensis has likely gone undetected on

Madeira as it is similar in appearance to native lizards. No records of *C. fogoensis* occurring outside its natural range (including Madeira) were found through HerpMapper or iNaturalist. However, this isn't the first time a skink from the genus *Chioninia* has entered Britain as a stowaway. In 2012, a *Chioninia* sp. stowed away in the baggage of a holiday maker returning from Cape Verde (Morris, 2012). This specimen not only survived the flight back to the UK but also being washed with clothes in the washing machine on a 30 min 'delicates' cycle. The species is clearly very robust.

Based on the evidence available *C. fogoensis* is likely to have colonised Madeira recently and one or more breeding populations may be present. Further surveys are needed to confirm this.

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Piebald Common Frogs Rana temporaria

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The coloration and markings of the common frog *Rana temporaria* are highly variable. Specimens with unusual coloration have long attracted attention with observations going back to as early as 1891 (Webb, 1975). Here we report on several partially amelanistic or piebald frogs (Figs. 1 & 2) observed in a garden pond in Trimley St. Martin, Suffolk, England. Piebaldism is a partial loss of pigmentation resulting in areas or patches of white skin within otherwise normal coloration. One of the frogs, photographed in 2006, also had yellow blotches (Fig. 2). Curiously, this frog also had one normally coloured eye and one pink eye. It is impossible to be sure whether the latter was due to damage from injury, such as attack by a predator, or whether it was a result of the piebald condition.



Figure 1. Piebald common frog, March 2020



Figure 2. Piebald common frog, March 2006

Piebald frogs have been seen in this pond in 2001 (n = 1), 2006 (n = 1, observed spawning) and 2020 (n = 2) within a relatively large population (for a garden pond) with spawn clump counts of approximately 30 to 100 per year. The recurrence of this aberrant coloration at the same location suggests a genetic basis rather than a result of trauma during development. Unusual coloration in common frogs may be

more apparent in garden populations than in the countryside due to genetic drift caused by population fragmentation within developed areas (Beebee, 1997). In this case the pond is in a village bordered to the north-east by a major road (the A14), which is presumably a significant barrier to amphibian migration.

Piebald appears to be a rare colour variation in the common frog. Nicholson (1997) collated responses to two press releases issued by Cornwall Wildlife Trust in 1994 and 1995, regarding orange or albino common frogs and requesting reports of other unusually coloured specimens (Mark Nicholson, pers. comm.). Responses covering 124 different locations in southern Britain were regarded as reports of either albino or partial albino frogs. In fact most of these were probably xanthochromic, as described by Allain and Goodman (2017). Presumably the orange/red eyes of some xanthochromic frogs has led to them being labelled as albino. Three of the sites (2.4 %) reported 'variegated' frogs and these may have been piebald specimens. Records of piebaldism in other amphibians are also scarce (García-Padrón & Bosch, 2019) although in Britain Sewell (2007/2008) describes a possible case of piebaldism in the great crested newt Triturus cristatus.

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A case of leucism in the slow worm (Anguis fragilis)

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The slow worm (*Anguis fragilis*) is a limbless lizard found across Europe and south-west Asia (Beebee & Griffiths, 2000). Colour variations and abnormalities in slow worms are rarely observed which may in part be due to their cryptic nature. Examples of such abnormalities include melanism (Gleed-Owen, 2012), albinism (Knight, 1966) and blue ventral coloration (Hails, 2017). However, only one case of leucism has been recorded previously; in a new born slow worm (Jablonski & Purkart, 2018). Leucistic reptiles have diminished numbers of iridophores in the skin associated with a low number of, or no, melanophores and xanthophores (Bechtel, 1991). Here we report a further case of leucism, this time from a sub-adult slow worm.

On the 25th April 2020 a routine reptile survey was undertaken of a derelict plant nursery (51° 37'57.7" N 0° 04'24.9" W) located in a predominantly urban area. The site had recently been cleared of brambles and scrub and was thought to support a small population of slow worms. During the survey, a leucistic sub-adult *A. fragilis* (Fig. 1) was found under a 1 m² square of bitumen roofing felt, laid to assist the survey effort. At the time, the weather was overcast and warm.

The leucistic *A. fragilis* was approximately 21 cm in length with an intact tail. The coloration can be described as an almost translucent pale pink which transitioned to a very pale pink towards the tail. This coloration made the dark eyes of the individual stand out more than normal. No vertebral stripe or lateral coloration were visible (Fig. 1). After photographs were taken, the individual was returned to the point of capture. A juvenile and an adult male slow worm with typical coloration were also seen under other artificial refuges within the same locality during the survey.

Jablonski & Purkart (2018) comment that leucism is rare in *Anguis* spp. However, among other reptiles it is not uncommon (Krecsák, 2008) and leucistic individuals are particularly favoured by snake breeders. In the wild, it is reasonable to assume that leucism would increase the chances of predation so that in the case of this sub-adult slow worm, the likelihood of it reaching sexual maturity would be reduced. Both recorded cases of leucism in *A. fragilis* have come from observations in England despite the species' large geographic range. Jablonski & Purkart (2018) suggested that the particularly low recording rate of leucistic individuals may be due to their misidentification as albinos.



Figure 1. Sub-adult leucistic slow worm (A. fragilis)

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Predation and ingestion of a viviparous lizard (*Zootoca vivipara*) by the common wall lizard (*Podarcis muralis*) in England

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The common wall lizard (*Podarcis muralis*) is a small-medium sized lacertid that attains a snout to vent length (SVL) of about 7.5 cm and varies considerably in colour or pattern. Its diet includes a wide range of invertebrates and small lizards including its own young (Speybroeck et al., 2016). The species ranges widely in continental Europe but has been introduced into the Netherlands, Germany and Britain (Speybroeck et al., 2016). Common wall lizards are thought to impact the native viviparous lizard (*Zootoca vivipara*) through competition and displacement (Mole, 2010) but this has yet to be quantified.

The viviparous lizard is a particularly cold tolerant species occupying a wide range of habitats across an expansive range that extends from Ireland to Japan but absent from much of southern Europe (Speybroeck et al., 2016). They too vary in colour but often tend to be brown, with females possessing a dorsal stripe and the males a brightly coloured belly (Beebee & Griffiths, 2000). Viviparous lizards are the smallest and most widespread lizard species in the British Isles but their populations are declining for a variety of reasons (Beebee & Griffiths, 2000). They prefer open habitats that are undisturbed and have exposure to the sun such as railway embankments, cliffs and heathland (Inns, 2011). This can lead to them coming into direct contact with *P. muralis*.

The common wall lizard has numerous established populations in England (Quayle & Noble, 2000) and Wales. One of the most well-studied is at Boscombe Cliffs in Dorset (SW England) (Gleed-Owen, 2004; Mole, 2010). Using genetic analysis this population of *P. muralis* was found to originate from either France or Italy (Michaelides et al., 2015), whereas the nearby Seacombe population, which happens to be closest to the sighting described here, was found to be of Venetian origin (Michaelides et al., 2015).

At 15:19 h on 13 May 2020 an adult *P. muralis* was observed by PS capturing and ingesting an adult *Z. vivipara* among boulders and foliage in Winspit Quarry, Dorset (GPS: 50° 35'05.5" N 2°01' 59.1" W). The *Z. vivipara* was grasped just behind the front legs (Fig. 1) before the whole lizard was swallowed head-first in its entirety. The process lasted approximately 5 minutes, with *P. muralis* ingesting *Z. vivipara* in stages with pauses of about 30 seconds at a time, returning to a rock in direct sunlight to complete ingestion after having been briefly disturbed by a passer-by.

To our knowledge this is the first observation of *P. muralis* feeding on *Z. vivipara* although this behaviour is consistent with the known habit of this species to eat small lizards. It would seem that *P. muralis* is not only a potential



Figure 1. An adult male common wall lizard (*P. muralis*) holding a viviparous lizard (*Z. vivipara*) in its mouth shortly before consuming the smaller lizard whole

competitor of the viviparous lizard but it is also a predator. Besides competition and predation common wall lizards may potentially threaten viviparous lizard populations through transfer of pathogens or parasites although this aspect has still to be investigated.

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