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Front Cover: Colombian red-eyed tree frog (*Agalychnis terranova*), see article on p. 23.
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RESEARCH ARTICLE
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Persistence of a population of palmate newts *Lissotriton helveticus* in a saline environment on the west coast of Scotland

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ABSTRACT - A six year survey of a palmate newt (*Lissotriton helveticus*) population in rock pools on the west coast of Scotland indicates that this species has some tolerance of saline conditions. The newts were living with a mean salinity (conductivity) of 382 ppm (range 30.7 ppm to >4995 ppm). Other interesting observations include a variation in mating behaviour, in which normal open water behaviour is confined to crevices, and the occurrence of 'pelvic bumps' in some individuals that may indicate reduced body condition.

INTRODUCTION

Amphibians are at a significant disadvantage in saline environments as their highly permeable skin and eggs make them sensitive to water loss by osmosis. Nevertheless, 144 species of amphibian have been recorded in saline habitats worldwide (Hopkins & Brodie, 2015). Of those 144 species only 24 passed all their life history stages in saline waters; *Lissotriton helveticus* does not appear in this group. It is best known as an opportunistic inhabitant of neutral to weakly acidic waterbodies in northern Europe. There are a limited number of observations of other newt species in coastal environments, notably the smooth newt (*Lissotriton vulgaris*) in sand dunes in northern England (Hardy, 1943) and in a range of habitats on the coast of Croatia (Popovic & Veletanlic, 2018). However, the only record of *L. helveticus* in a saline environment is from Smith (1951) who associated the species with coastal brackish pools.

The present study was initiated following casual observations in the West Highlands of Scotland by Deichsel and Bennon (pers com.). On 25th August 2010, Deichsel observed both adult and larval *L. helveticus* in a supra-tidal pool by Loch Linnhe (56° 48' 59.33" N: 5° 06' 43.49" W), while on 15th September 2010, Bennon recorded adult newts in a pool one metre above the High-Water Spring tide mark at Kirton Bay near Lochalsh (57° 16' 17.1775" N: 5° 35' 39.2322" W). Such single observations raise many questions concerning inter-annual and intra-annual variability of a population in a habitat generally considered marginal for *L. helveticus*. These populations were still present when the sites were visited in July 2011 prompting a roughly monthly sampling survey for six years at the Lochalsh site.

MATERIALS AND METHODS

Survey site

The survey site was a complex of pools on a rocky platform of Torridonian sandstone. It sits one metre above the mean High Water Spring tide mark on Kirkton Bay, Lochalsh, West Highlands. The platform is backed by cliffs rising to 50 m above sea level and faces the Kylerhea narrows, Loch Alsh (Fig. 1). The surveyed pools ranged in surface area from 0.5 m^2 to 3 m^2 with depths of 0.25 m to 0.5 m; both surface area and depth varied greatly throughout the year. During the hot dry summer of June 2012 the pools were reduced to a few centimetres in depth. After high tides with storms and heavy rainfall the pools were overflowing and ran into each other.

Figure 1. The survey site showing the complex of rock pools on a Torridonian sandstone platform at Kirkton Bay

Newt population survey

The site was surveyed from 26th February 2012 to the 21st October 2017 with one visit each month, weather permitting. On the first survey every pool on the rocky platform was surveyed for *L. helveticus* activity. Those pools with adults or larvae received an identification number painted on a marker beside the pool and each pool was geolocated. On each survey the pools were watched for any signs of life: swimming adults or movements beneath rocks or within crevices. Notes were made on water clarity, vegetation growth (mostly filamentous green algae), presence or absence of any decaying seaweed (post extreme high tide and storm events), breeding and egg laying behaviour.

After observation, the pools were sampled with a pond net with fives sweeps and the contents of each netting placed in white trays with pool water. After the final netting, the trays were inspected, and the number of larvae and adults recorded. Larval data included the length and stage of growth in terms of number of legs and the presence or absence of external gills. Adult sex and body length were recorded, plus breeding condition and any obvious injury. Adult body length was measured in the surveys of 2015 and 2016 using a wide bore glass tube with a millimetre scale printed onto it. The glass tube method for measurement provides greater accuracy than a ruler and is faster so resulting in less stress to the newts. The tail filament of the adult male was not included in recording body length. Adults and larvae were returned to their pool immediately after assessment. Over time, the number of pools on the platform containing newts increased. In the first years, six pools were surveyed which had been observed to contain newts, over the years of surveying more pools were observed to have gained newts and by the time the surveys concluded an additional seven pools had adults and larvae being recorded on a regular basis.

Salinity and temperature measurements

To gauge variations of salinity and temperature over time and between pools, the conductivity and temperature of pools was measured using a HM Digital Combo Meter Com-100 multimeter. For conductivity measurements the multimeter was set for microsiemens with readings autocorrected to 25 °C and to salinity on the ppm500 (NaCl) scale (where conductivity reading is multiplied by 500 to give a guide as to the NaCl concentration). This is an approximation as true salinity can only be determined by chemical analysis. The multimeter could not record above 4995 ppm and any results above this were recorded as out of range of the meter. On the ppm500 scale, sea water is typically 27,000 ppm and drinking water less than 50 ppm. It should be noted that different salts will give different conductivities. Pools may contain dissolved salts from the surrounding rock and soil other than NaCl, but these are not necessarily taken into account when the multimeter is set on the ppm500 scale. From 2015 to 2017, 267 recordings of both temperatures and conductivity were made of 11 rock pools.

Water temperatures peaked in June, July and August, with a maximum of 25 °C recorded in July 2017. The lowest water temperature recorded was 5 °C below the surface in February 2016, when all the pools had full or partial surface ice.

RESULTS

Newt population survey

The results are based on those months when pools were accessible; accessibility was limited especially in some winter months. Adults are found in greatest number between April and June (Fig. 2) although a small number of individuals were found occasionally throughout the year. Larvae were encountered in small numbers primarily from May increasing in abundance to July and then slowly declining in abundance towards the end of the year (Fig. 2). In 2015, conditions permitted a survey in December and a small population of larvae was recorded (Fig. 2). In 2016, when surveying was possible in January, again a small population of larvae was found (Fig. 2). In the first year of surveying, six pools were observed to have *L. helveticus*, over six years four further pools were observed to have *L. helveticus* and included in the survey.

Body length measurements were made in 2015 and 2016 and the mean (± standard deviation) of males (n=67) was 70.3 ± 0.6 mm and females (n = 72) was 75.69 ± 0.67 mm. The majority of larvae recorded in October and later surveys possessed four legs and external gills, with a minority in the earlier metamorphic stage of two legs and external gills.

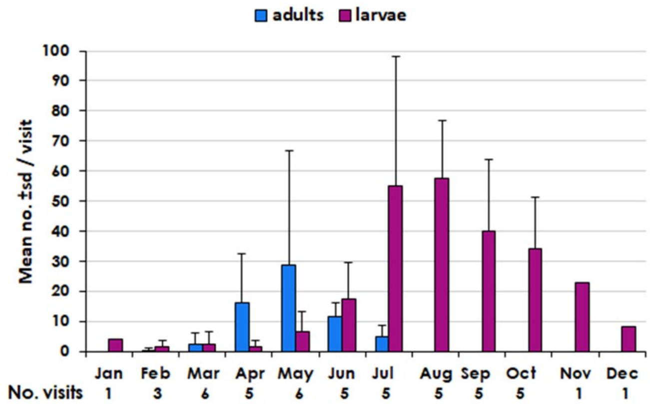

Figure 2. Mean (±sd) monthly catch of adults and larvae of *Lissotriton helveticus*, 2012 to 2017

Adults were observed with evidence of having survived an attack by a predator with partial limbs or missing parts of their tails. Adults were also found dead (9 on the 15th April 2012 and 1 on the 21st April 2014). Some adults were observed with a pair of raised white bumps, one on either side of the pelvis (Fig. 3). These were not obviously wounds or the blistered lesions typical of Amphibiocystidium infection that are distributed randomly over the body. In every other respect the affected individuals appeared healthy, with no discoloration anywhere else on their body or languor. The first observation of pelvic bumps was made 22nd May 2016 on a warm and dry day with an air temperature of 19.2˚ C. Out of ten pools sweep netted, six pools each had one adult newt with pelvic bumps, so that six newts (one male and five females) were affected from a total capture of 71 adults. The salinity of the six pools ranged from 81 ppm to 273 ppm. There were no further records of adults with pelvic bumps in any of the subsequent monthly surveys. However, in a separate rock pool survey beside the Coral Beach, Plockton (57° 20' 11" N: 5° 41' 04" W) on 25th June 2016, a single male was captured that exhibited exactly the same pattern, shape and form of pelvic bumps. There had been heavy rain the day before, but the pool was much reduced in size and had obviously dried out in the period of hot dry weather of the month before. The pool had a salinity of 1,100 ppm and the air temperature was 26.3˚ C. The pool had been surveyed on the 19th May 2016 where 12 larvae and 6 adults had been netted, at a pool salinity of 1070 ppm and an air temperature

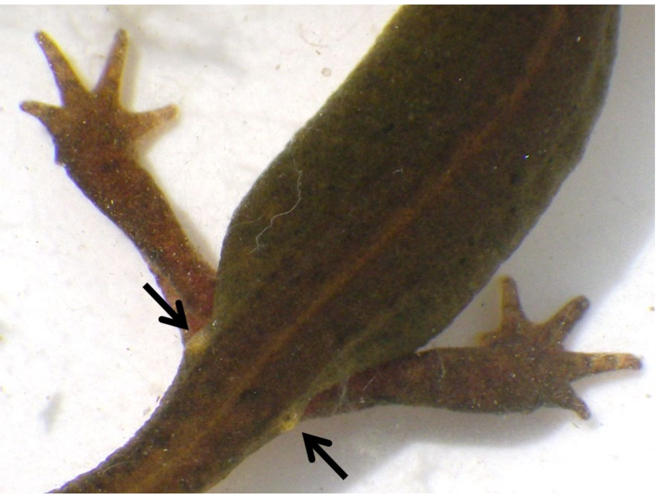

Figure 3. Adult female *Lissotriton helveticus* with two white bumps (arrows) in the pelvic region

of 19.0˚ C with no individual showing pelvic bumps. The symmetrical position of the pelvic bumps suggests that they are most likely to be part of the bone structures of the hip (the ileum). This would be indicative of reduced body condition resulting from lowered food supply and/or stress from the effects of excessive salinity.

Newt behaviour

Males arrived at the site before females and could be observed even when the pools still had surface ice. Observations of *L. helveticus* mating behaviour were made on the 21st April 2014 mid-afternoon in full sunshine; the males occupied a rock crevice on the side of the pool. They stayed by the crevice opening and when a female approached, they actively tail fanned towards her, sometimes leaving the opening to U-turn with a tail fan and go back to the entrance of their crevice. Females entered the crevice and the pair disappeared from view so that the final act of the courtship sequence and spermatophore transfer were not visible. This behaviour shows some differences from what is perceived as normal for adult palmate newts where the male initiates a courtship sequence by pursuing a female and uses a combination of tail fanning, waves and whips, followed by a series of movements by the male and female on the pool bottom and where mating is more prevalent at night (Halliday, 1977).

Females were commonly seen wrapping eggs in decaying vegetation that had fallen into the pools. They left these folded packages on the floor of rock pools. Other females were seen to lay their eggs in sediments at the bottom of pools. Newt eggs were found in the sediments recovered during netting.

Salinity measurements

From 2015 to 2017, at Kirkton Bay the salinity ranged between 30.7 ppm to greater than 4995 ppm (i.e. exceeding the scale reading of the multimeter) with an average of 382 ppm (not including any out of range meter readings). Five percent of pools had salinities that were out of the multimeter range, one such pool had a larval record, and 47.2 % of the pools within the range of 555 ppm to 3050 ppm had adults and/or larvae present. Salinity varied over the year with the potential for it to vary considerably according to the weather and tides. Salinities increased during periods of drought or extreme high tides. The survey shows that the larval and adult populations survived fluctuating salinity, but extremes of salinity over 500 ppm occurred only rarely (Fig. 4).

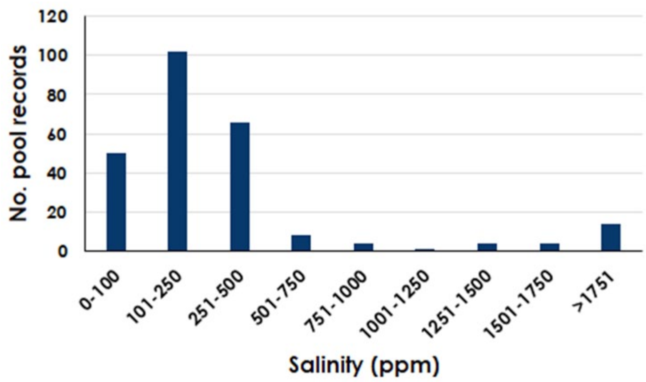

Figure 4. Frequency distribution of salinity ranges (derived from conductivity measurements) of Kirkton pools, 2015 to 2017

DISCUSSION

Over six years of surveying the rock pools at Kirkton Bay, it was observed that adult *L. helveticus* regularly visit the pools during the spring and that a larval population was observed to be present throughout each year of the study, peaking in the summer months. This suggests that *L. helveticus* have a persistent breeding population in this location.

The mating behaviour observed shows certain differences from the recognised courtship sequences for newts of the genus *Triturus* (Halliday, 1977; Arntzen & Sparreboom, 1989). Specifically, males occupied crevices in the rock pool sides and positioned themselves to look out into open water and tail fanned towards females. Females would then enter the rock crevice of the male of her choice, where the pair would disappear from view and any further mating behaviour and the act of spermatophore transfer could not be observed. These differences in behaviour may be related to the extremes of the physical conditions on site, but predation may also be a factor. The predators observed included otters and dragonfly nymphs that were seen in increasing numbers over the six years of surveying. The adult newts were possibly using the crevices as refuges from them. Nevertheless, there were adult newts with the signs of predator attack such as missing limbs and sections of tail. The presence of dragonfly nymphs in increasing numbers in these pools also questions their tolerance of salinity and how this may impact *L. helveticus* in this habitat. A low number of predators may be of vital advantage to a species with vulnerable small larvae (Beebee, 1992) and many of the predators of amphibian larvae are not found in the Kirkton Bay pools. Irregular seawater inundation prevents the establishment of most freshwater or marine species, invertebrate species diversity is minimal and macrophytes are mostly absent (Nicol, 1935). The crustacean

Gammarus duebeni was common in 7 out of the 11 pools; it is a species well known for its exceptional wide-ranging tolerance of salinity (Sutcliffe, 2000).

In terms of possible physical environmental stress, the multimeter showed a wide and fluctuating salinity in the pools, especially in the shallower rock pools. Pool salinity changes appeared to follow changes in weather conditions, storms and hot weather producing a rise in salinity and heavy rain lowering the salinity. In this fluctuating environment it was observed that a pool of high salinity that had no larval record one month could have larval records the next. Indicating larvae may escape exposure to higher salinities, possibly by physically moving into pockets of less saline water in the cracks and crevices of the rock pools. This migration would be worthy of further study.

Amphibians are particularly vulnerable to osmotic stress of saline conditions at all their life stages. Nevertheless, it is on record that the Lancashire coastal dune slacks have long been inhabited by the smooth newt (*Lissotriton vulgaris*), common frog (*Rana temporaria*), common toad (*Bufo bufo*), and the natterjack toad (*Epidalea calamita*) (Hardy, 1943). Furthermore, the closely related *L. vulgaris* is known to be tolerant of brackish waters as an adult (Speybroeck et al., 2016; Popovic & Veletanlic, 2018). *Lissotriton vulgaris* and *L. helveticus* overlap in range and although *L. helveticus* is more tolerant of acidic conditions it does share many habitat preferences with *L. vulgaris* (Cooke & Fraser, 1975). Neil (1958) inferred that salt tolerance may be widespread in amphibian species and Balinsky (1981) identified 61 species in 13 families of anurans and 12 species in four families of urodeles that inhabit or tolerate a moderately saline environment. *Epidalea calamita* on the North Solway coast salt marshes successfully bred within pools of 0.22 % to 14 % seawater that were not subject to seawater inundation (Beebee et al., 1992). It seems probable that palmate newts exhibit a similar range of tolerance although the observation of occasional individuals with pelvic bumps may indicate that this environment is not always conducive to good body condition.

When the weather and safe access did allow for winter surveys, larvae were found in the pools that had some vegetation growth or were more sheltered from high storm surge tides. This strongly suggests that larvae can survive in these pools in winter. It is known that larvae complete their metamorphosis by the end of summer, but it is also known that in years when environmental conditions are harsh, larvae experience a reduced growth rate (Speybroeck et al., 2016). Therefore, larvae can survive a slow growth rate by overwintering and resuming growth to adulthood when conditions allow in spring. The data shows larval numbers fall from July onwards, this is presumed to be due to predation and juveniles leaving the pools after metamorphosis. *Lissotriton helveticus* in this location appear capable of surviving in an environment that has varying salinity and is highly exposed to extremes of weather conditions. Their survival may be due to both physiological and/or behavioural adaptations although it is unknown whether these populations are specifically adapted to the harsh conditions on the coast. Interestingly, the adult body length data shows that males and females of this site achieve average body lengths greater than those found in the Weald of Kent in both heathland and non-heathland pools (Beebee, 1983). Adults in the Kirkton Bay pools appear to tolerate a relatively extreme coastal breeding site with no sacrifice in body length. However, 50 to 80 m above the survey site there is moorland with small freshwater bog pools and it is unknown what influence these may have on the wellbeing of the rock pool population. It would be useful to investigate whether there are movements of adult newts between the rock pools and bog pools and if so what benefits this may confer.

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RESEARCH ARTICLE
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Smooth newt *Lissotriton vulgaris* and common toad *Bufo bufo* populations persisting in the unusually small area of Sommers Island in the Baltic Sea

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ABSTRACT - Sommers Island is an abandoned 20 ha patch of land in the Baltic Sea. It is inhabited by isolated populations of smooth newt (*Lissotriton vulgaris*) and common toad (*Bufo bufo*). The island demonstrates the small area in which it is possible to have a stable population of newts and toads for a period of at least several decades, probably much longer. Most reproduction of both species occurred in one pool with an area of about 100 m^2. The total area of optimal terrestrial habitat is about 2.5 ha, with an additional 3.5 ha of sub-optimal habitat available. The island is inhabited by about 123 adult newts and several dozen adult toads.

INTRODUCTION

Habitat loss and fragmentation threaten the stability of animal populations (Haddad et al., 2017). Consequently, it is important to understand the minimum possible population size and minimal habitat area required for the survival of particular species (Reed et al., 2003). This information is urgently needed for amphibians due to the global declines of the past few decades. Even species that were considered to be common and abundant have experienced drastic reductions in their numbers because of pollution, climate change, the spread of the infectious fungus *Batrachochytrium*, and other factors (D'Amen & Bombi, 2009; Denoel, 2012; Houlahan et al., 2000; Spitzen-van der Sluijs et al., 2016). The minimal size of an amphibian population can be estimated by both monitoring and the modelling of reproductive rates (Petranka et al., 2004). Further information can be gained by examining extremely small populations that have been stable for a long time especially those on small, isolated islands. We discovered an apparently stable population of amphibians on the small Sommers Island located in the Baltic Sea. Herein we present our observations of the size of amphibian habitats and the number of breeding adults on Sommers Island.

MATERIALS AND METHODS

Study site

Sommers Island is located in the Gulf of Finland of the Baltic Sea. It occupies an area of about 20 ha and has a complex shape consisting of several headlands connected by small necks of land (Fig. 1). The distance to the mainland, i.e. to the northern coast of the Gulf of Finland, is 34 km. The other nearest land is Moschny Island, which is larger and 22 km away. The water around Sommers Island is brackish, salinity usually fluctuates from 2 – 3 ‰, but can reach 5‰ (Batalkina et al., 2007). Typical marine fishes (mainly herring) occur there (Popov, 2014).

The island represents a congregation of rocks, partly covered by a thin layer of soil and with sparse tree cover. General information on its environment is scarce, with few scientific publications on this topic. The island was annexed by Russia after the war with Sweden in 1722, at which time it was unpopulated (Bergholtz, 2018), and not used by people until 1808, when a lighthouse and a caretaker's house were built there. After the Second World War, a new lighthouse was built on the island, which still exists, and later on a military base was constructed. In 2005, an automatic radio tower was built and the island abandoned. Since then, the island is very rarely visited by people. Military specialists occasionally visit to maintain the radio tower, but visits by other people are usually not allowed. Access by researchers is difficult. The 'discovery' of the island in biological terms occurred only in 2017 during an expedition of the Russian Geographical Society. It turned out that animals had colonised or re-colonised the island, and a relatively rich fauna was forming: 23 species of birds, two species of mammals and two amphibian species were found. The two amphibians were the smooth newt, *Lissotriton vulgaris*, and the common toad, *Bufo bufo*. Although these two species are considered common and are widely distributed on the mainland, populations of smooth newt, and to a lesser extent common toad, have been reportedly declining (Kinne, 2006; Cooke, 1972; Carriera & Beebee, 2003). For newts, the main threats are destruction of small water bodies suitable for their reproduction, the introduction of fishes into their breeding sites, and pollution (Skei et al., 2006). Cases of the extinction of newt populations and other amphibians due to the invasive Chinese sleeper, *Perccottus glenii*, have been reported in adjacent regions (Reshetnikov, 2003).

Figure 1. Sommers Island: aerial photograph and scheme of its location (available from https://www.bing.com/ [accessed 8 December 2017])

Observations of breeding adults

We observed and photographed the island from the lighthouse. The lighthouse is 53 m high, and is located on a hill 16 m high. This height allows observation of the entire surface of the island. Visually and with the help of photographs, we determined the type and location of distinct habitats, including water bodies suitable for amphibian reproduction. We identified nine small pools that could be used for breeding by one or both amphibian species (Table 1). After identifying the pools, we visited them and delineated their boundaries using GPS Garmin Etrex 20. In this way, we composed a map of the habitats of the island (Fig. 2).

The time of year for which we were granted access to the island coincided with the breeding season of *L. vulgaris*; consequently, we were able to count breeding adults, which were concentrated in the shallow pools located in depressions in granite rocks. We surveyed for newts in the pools for eight days (7-14th June 2017). Two observers participated in the counts, walking around the perimeter of pools during daytime. Most newts were concentrated just in one pool, which is the largest one; we counted newts there each day. We inspected the other eight pools 2-5 times and performed the final observations on 14th June. We used the highest number counted as an estimate of the total number of breeding adults. The common toads had bred prior to our surveys, and tadpoles were already abundant in June; therefore, we were not able to count breeding adult toads. We estimated their distribution based on the locations we found adults on land and the tadpoles in the pools.

RESULTS

We identified several habitats on the island, including bogs, grasslands, different 'forest' types, plots of shrubs and barren land. We found evidence of amphibians breeding in nine pools, with newts breeding in all of them; however, we found toad tadpoles in only four pools (Fig. 2, Table 1). Most newts occurred in the largest pool, with a maximum area of 100 m² and a depth of 10-40 cm (Fig. 3). The size of the pool fluctuated depending on precipitation, but it cannot be larger than the maximum dimensions given here since, being on the top of a rock, any water added would just overflow. During periods of low air temperature, the newts were not active, but when the air temperature increased, we found dozens of them. During the eight days of the survey we counted the following numbers of newts in the largest pool: 5, 4, 16, 35, 55, 11, 10, and 93. Moreover, we found 18 recently dead adult newts. Probably, they became frozen (the frosts often happen in spring in the studied area). In the other pools, we usually found two or three newts, but did find as many as 15 (Table 1). The maximum number of breeding adults newts recorded was 123.

The pool with the highest number of newts was located in optimal habitat for summer activity on land. It was close to the alder 'forest' and grasslands originating from the abandoned settlement (military post) (Table 2). Here there is relatively fertile soil and dense vegetation, which could support small invertebrates suitable for newt consumption. Moreover, an accumulation of dead reeds and wood was found on the shore close to the alder 'forest'. It is also

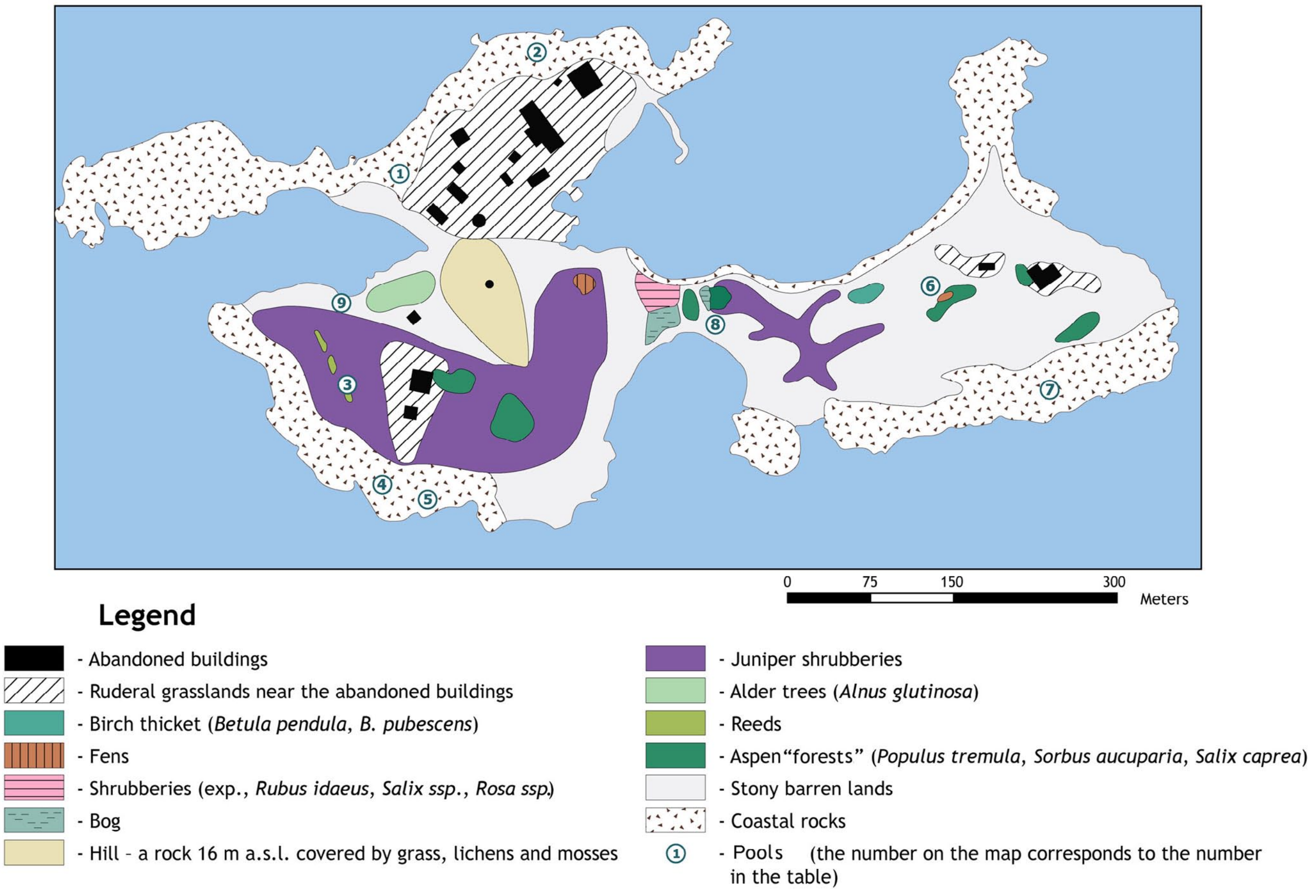

Figure 2. The location of habitats on Sommers Island

suitable for the summer activity of newts, one newt juvenile (3 cm long) was found there. The other pools are surrounded by less productive habitats. The trees and grasslands are sparser there. The main part of the island is hardly suitable for newts. There is no continuous soil and plant coverage, and rocks covered by a thin layer of lichens occupy the main part of the island. The total area of optimal habitat for newts is about 2.5 ha, which is supplemented by 3.5 ha of sub-optimal habitat (shrubs, aspen 'micro-forest' and birch thickets).

As for the toads, just nine adult individuals were observed. These were in the grasslands around the abandoned buildings in the western part of the island. In the pools nearby, several thousand toad tadpoles were observed. Moreover, a small number of tadpoles was noted in the eastern part as well; there are also small plots of grassland associated with the remains of fortifications. Like newts, the toads tended to occupy the habitats with relatively high productivity, so the terrestrial habitats may be the same for both species. Given the limited habitat, the total number of toads may be no more several dozen individuals.

Potential natural enemies of amphibians on the island are not numerous. Snakes were not observed, and mammals are represented by grey seal (*Halichoerus grypus*) and northern bat (*Eptesicus nilssonii*) which are not known to affect amphibians. Furthermore, the island is primarily an open area in which the mammals and snakes, or the indications of their activity, would be clearly visible. It is unlikely that any species would remain undetected. Among the birds, perhaps, some of them could occasionally eat amphibians. Currently, the number of birds on the island is growing and, possibly, in the future this could affect the amphibian populations. Now there are mostly gulls and terns that feed at sea, but also occasional ravens (*Corvus corax*), kestrels (*Falco tinnunculus*), and the red-backed shrikes (*Lanius collurio*).

Figure 3. The main breeding site of amphibians in the Sommers Island

Table 1. Details of pools and the presence of amphibians on Sommers Island

Pool	Co-ordinate	Area (m²)	Maximum number of newts recorded	Presence of toad tadpoles
1	N 60.20795 E 27.64005	100	93	+
2	N 60.20896 E 27.64225	5	3	+
3	N 60.20626 E 27.63923	49	2	-
4	N 60.20546 E 27.63991	26	3	+
5	N 60.20535 E 27.64051	23	15	-
6	N 60.20705 E 27.64841	17	1	-
7	N 60.20625 E 27.65041	7	3	+
8	N 60.20675 E 27.64511	6	1	-
9	N 60.20691 E 27.63911	16	2	-

Table 2. Areas of land habitats of Sommers Island

Habitat	Area (ha)
Alder thicket (*Alnus glutinosa*)	0.14
Hill – a rock 16 m a.s.l., covered by grass, lichens and mosses	0.59
Fens	0.03
Aspen 'forest' (*Populus tremula*, *Sorbus aucuparia*, *Salix caprea*)	0.37
Bog	0.08
Birch thicket (*Betula pendula*, *B. pubescens*)	0.04
Ruderal grasslands near the abandoned buildings	2.41
Shrubberies (*Rubus idaeus*, *Salix* ssp., *Rosa* ssp.)	0.09
Juniper shrubberies	2,95
Reeds	0.02
Abandoned buildings	0.37
Coastal rocks	6.82
Stony barren lands	6.26

DISCUSSION

Sommers Island demonstrates that amphibian populations can exist, at least temporarily, in a very small area: a few hundred newts and toads can live on a plot of land of effectively only about 2.5–6 ha. The size of the populations in the past is unknown, but was probably not significantly larger than during the last few decades. Since few people have lived on Sommers Island, there is no reason to believe that amphibians had more or less habitat than they do now. Rather, the presence of people inevitably leads to trampling down of grass, the destruction of trees and the direct extermination of animals. These impacts would be partly compensated for by kitchen gardens constructed around the houses, in which the amphibians could find suitable habitats, but the area is extremely small. Most of the surface of the island was, and still is, stony barren land. Unfortunately, there is almost no information about the historical condition of the island before its economic and military use. There is only a small note from 1723 stating that the island consists of sand and stones (Bergholtz, 2018).

Regular, active replenishment of these populations from elsewhere is unlikely since although the water in the Baltic Sea is brackish, the migration of amphibians for long distances through it is only likely to happen rarely if at all. It is possible that amphibians arrived in earlier periods during the formation of the Baltic Sea and/or arrived in the last one or two hundred years during the period of the island's economic development. They could have been transported accidentally or intentionally, although the latter is unlikely (the island was used predominantly by the military). In any case, the amphibians must have been established on the island before 2005 since that is when it was finally abandoned.

We believe that both the size of the stable amphibian populations on Sommers Island and the size of the habitat they occupy are the smallest currently known for amphibians. We searched the literature for occurrences of amphibian populations on extremely small islands (<20 ha); however, such populations either occupy larger areas, or are not as isolated from the mainland as the populations on Sommers Island. Populations of the common frog (*Rana temporaria*) and common toad have been reported living in small islands (1-16 ha) of the Baltic Sea (Seppa & Laurila, 1999), but these islands are located in 'skerries' – a zone of heavily rugged coastline, to which a large number of small islands adjoin. The distance to the mainland is relatively short, only about 0.6-2.5 km. Dispersal and gene flow between islands and from mainland to island were indicated for both amphibian species. Similar habitat is known on the Kumari Island of the Moonsund Archipelago of the Baltic Sea with an area of 16 ha and distance to the mainland of 5.35 km. There are populations of common toad, natterjack toad (*Epidalea calamita*), moor frog (*Rana arvalis*) and common frog (Lepik, 1995). Several small islands of the Lake Erie, USA, that are 3-312 ha and 1.1-19.1 km from the mainland are inhabited by the American bullfrog (*Lithobates catesbeianus*), mudpuppy (*Necturus maculosus*), American toad (*Anaxyrus americanus*), spring peeper (*Pseudacris crucifer*), green frog (*Lithobates clamitans*), northern leopard frog (*Lithobates pipiens*), red-backed salamander (*Plethodon cinereus*) (King et al., 1997). Since Lake Erie is a freshwater lake, migrations of amphibians to the islands take place rather often. As for the similar islands surrounded by salt water, at least four cases are known. Two of them have been reported for the San Francisco Bay: a population of arboreal salamander (*Aneides lugubris*) inhabits the S. Farallon Island, which is 28 ha and located 32.9 km from the mainland; a population of California slender salamander (*Batrachoseps attenuates*) inhabits the island Erba Buena which is 78 ha and 2.5 km from the mainland (Anderson, 1960). Two cases are known for the islands of the Atlantic coast of Spain: a population of fire salamander (*Salamandra salamandra*) occurs on the island San Martino which has an area of 143 ha and is 6.1 km from the mainland; populations of Bosca's newt (*Lissotriton *boscai*) and Iberian painted frog (*Discoglossus galganoi*) inhabit the island Salvora that has an area of 196 ha and is 3.7 km from the mainland (Cordero Rivera et al., 2007). In all these cases, there is not enough information about size of the populations; however, they might have been significantly larger than the number of newts and toads of the Sommers Island as the other islands are more favourable for amphibians; Sommers Island is an extreme habitat due to its cold climate and low biological productivity. Not only for newts or toads, but also for other vertebrates, the recorded numbers on Sommers Island seems to be close to the possible minimum.

The data we have obtained are interesting in the light they throw on the importance of metapopulations (Smith & Green, 2005) – populations existing as distinct units with exchange between them that facilitate long-term survival. Our data illustrate that amphibians can do without metapopulations when not disturbed. Isolated and genetically depleted populations are less resistant to environmental changes and less likely to survive (Allentoft & O'Brien, 2010; Lesbarreres et al., 2005). Nevertheless, with low levels of anthropogenic impact perhaps even very small populations can be stable. This suggests even very small areas may have value for the conservation of amphibians, provided they are subject to little anthropogenic impact.

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RESEARCH ARTICLE

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Cloacal anatomy and sex determination in *Tiliqua* sp.

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ABSTRACT - A rigid endoscope was used to examine the cloacae of an adult pair of *Tiliqua gigas gigas*, and single specimens of *Tiliqua gigas evanescens* and *Tiliqua* sp. (Irian Jaya form). Throughout the procedure the animals showed no signs of stress. Clear anatomical differences were observed between the sexes. Females presented the typical two pairs of papillae (ureteral and genital) and males a single pair of urogenital papillae. The observed differences were confirmed when both pairs bred successfully in the following year.

INTRODUCTION

Endoscopy can be used for direct observation of anatomy of the cloaca (Martínez-Silvestre et al., 2015; Oliveri et al., 2016; Spadola et al., 2009). The first report of endoscopy applied to reptiles was published in 1983 (Wood et al., 1983) but it is only in the last few years that endoscopy has become common in routine clinical practice. Normal procedures adopted are laparoscopy and coeloscopy (Schildger et al., 1999; Murray, 2000; Taylor, 2006), but other minimally invasive procedures such as gastroscopy, the assessment of the upper respiratory system, and cloacoscopy are now also routine (Schumacher, 2011; Knotek & Jekl, 2015, Spadola et al., 2009).

Endoscopy of the cloaca using a rigid endoscope with warm saline irrigation enables direct observation of the proctodeum, urodeum, and coprodeum, furthermore the papillae in the urodeum can be used to distinguish between the sexes; in lizards, females typically have two pairs of papillae while male have only one pair (Fox, 1977; Trauth et al., 1987; Vitt & Caldwell, 2009). Nevertheless, evaluation of the cloacal structures is not always easy due to a lack of detailed information on most of the 11,000+ reptile species. Consequently, this study describes the anatomy of the cloaca in both genders of specimens of *Tiliqua*, with attention to the urodeum in order to obtain a detailed description of the male and female cloaca. Such information may then be used for subsequent clinical comparisons or for sex determination.

MATERIALS AND METHODS

An adult pair of *Tiliqua gigas gigas* (3 years old) were investigated first followed by a specimen of *Tiliqua gigas evanescens* (5 years old) and an Irian Jaya form of *Tiliqua* sp. (unknown age but held for 3 years in captivity). The lizards were checked at Messina University Veterinary Teaching Hospital and Pombia Safari Park to ensure that they were in good health before their cloacae were inspected. To do this the animals were constrained manually in dorsal recumbency on an operating table equipped with a heat mat (Fig. 1). The cloaca was investigated with a rigid endoscope of 4 mm diameter and 0° optic inclination, connected to a Telecam DX camera II, all of which were connected to an all-in-one Karl Storz “TELE PACKTM” system, thus ensuring a light source and video control. In order to expand the cloacal chambers and display all its structures, cloacal washing was provided using a 60 ml syringe filled with warm saline solution (NaCl 0.9 %) connected to the endoscope. The entire procedure took about five minutes for each specimen and throughout the procedure the animals showed no signs of stress. Following the example for cloacoscopy in other reptiles (Perpiñán, 2018; Morici et al., 2017; Divers, 2014), we did not sedate the lizards as cloacoscopy is considered a ‘non-risky’ procedure for reptiles and anaesthesia poses greater risks for reptiles compared to mammals. After the procedure, all the animals were hospitalised and monitored for 24 h. These ethically approved procedures were undertaken as routine clinical practice with the owner’s permission.

Figure 1. Containment in dorsal recumbency of *Tiliqua* sp. (Irian Jaya form) during cloacoscopy

RESULTS

During cloacoscopy of the reproductive pair of *T. gigas gigas*, it was possible to highlight the anatomical differences between the sexes. Similar differences between females and males were also identified in the other two *Tiliqua* specimens.
Females

One of the *T. gigas gigas* and the *T. gigas evanescens* proved to be female. The insertion of the endoscope through the cloacal opening allowed us to see first the anatomy of the proctodeum. Proceeding cranially and dilating the walls by irrigation with saline solution, it was possible to observe a general display of the anatomy of urodeum and coprodeum and their relationship. Overcoming the horizontal envelope between coprodeum and urodeum, it was possible to see the urodeum; this was a blind-bottomed cavity whose wall presented four papillae, arranged in parallel and divided by a median crest (Fig. 2). These structures are the urethral papillae (more caudally) and the genital papillae (more cranially). It wasn't possible to see any structure attributable to the urethral ostium. Considering that a urinary bladder has been reported to adjoin the urodeum in the genus *Tiliqua* (Beuchat, 1986), this could suggest the presence of a rudimentary bladder with vestigial urethra, or differences between species of the genus *Tiliqua* not yet anatomically described. Observation of the coprodeum was possible by inserting the endoscope more cranially whilst at the same time it was possible to overcome the anal sphincter by washing with saline solution. From there the rectal ampulla could be observed and, cranially, the rectal colic valve and the caudal portion of the rectal canal. Furthermore, in the rectum the presence of uric acid as a white pulp mass was identified in both investigated animals. The passage of the aforementioned pulp was also documented from the ureteral papilla to the rectum, after cloacal contraction: this mechanism is typical of ophidians and saurians lacking a bladder.

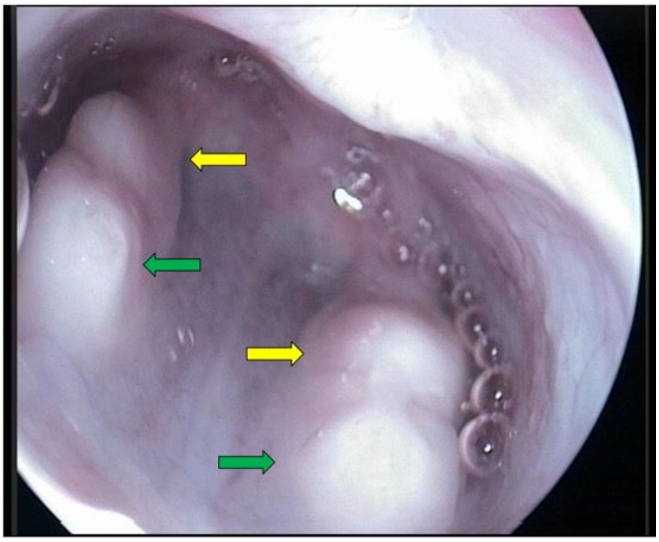

Figure 2. Urodeum of female *Tiliqua gigas gigas*, genital papillae (more cranial- yellow arrows) and urethral papillae (more caudal- green arrows)

Males

One of the *T. gigas gigas* specimens and the *T.* sp. Irian Jaya form were identified as male. During the introduction of the endoscope, it was possible to view the proctodeum, urodeum and coprodeum which appeared in the same general arrangement as for the females and likewise it was possible to dilate the urodeum by proceeding dorsally. The urodeum presented a single pair of urogenital papillae (urethral and genital combined) divided by a median crest, which can be used to diagnose the gender as male (Fig. 3A). During the visualisation of the structures in the urodeum it was possible to observe, as mentioned in females, the sequence of events that effect uric acid excretion and its conveyance through the anal sphincter in the coprodeum, preceded by cloacal contractions (Fig. 3B).

Neither pair of lizards had bred previously and the sex differences observed in this study were confirmed when both pairs bred successfully during the following year.

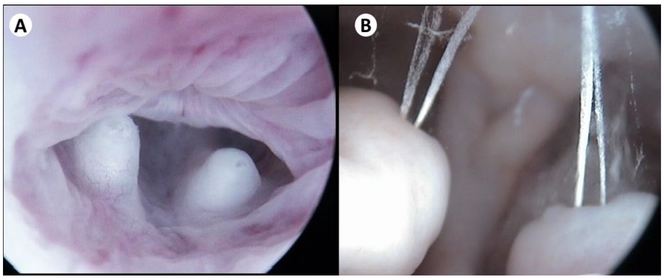

Figure 3. Urodeum of a male *Tiliqua gigas gigas* - **A**. Single pair of urogenital papillae that indicate that the gender is male, **B.** Uric acid discharged from urogenital papillae

DISCUSSION

Tiliqua gigas gigas, *T. gigas evanescens* and *T.* sp Irian Jaya have no obvious external features that distinguish males from females. For the first time, this study has demonstrated that when the urogenital system is viewed using cloacoscopy the presence of two pairs of papillae in the urodeum of females and one pair in males can be used as a reliable guide to sex determination. However, since only four individuals were investigated, intra- and extra-specific anatomical variability cannot be excluded.

Cloacoscopy is a minimally invasive technique that does not require sedation. It can be used to examine the cloaca for sex determination as well as for the diagnosis of common pathologies such as cryptosporidiosis or oviductitis (Rivera, 2008; Spadola & Insacco, 2009; Scullion & Scullion, 2009; Sykes, 2010). Furthermore, it can be used for minimally invasive surgical procedures (Mehler, 2011) of the gastrointestinal and urinary systems, such as removing cloacal calculus or intestinal fecalomas (Mans & Sladky, 2012a & b), obviating long post-operative periods and copious blood loss (Frye, 1972). It may also have a role in conservation medicine by way of assisted insemination as reported in snakes (Quinn et al., 1989; Mattson et al., 2007).

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RESEARCH ARTICLE
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Development and individual identification of captive Ziegler's crocodile newt *Tylototriton ziegleri*

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ABSTRACT - *Tylototriton ziegleri* is a newt native to Vietnam with a very limited range and assessed as Vulnerable by the IUCN. It is rarely found in captivity. Larval husbandry, based on field conditions, had mixed success with a high proportion of egg hatch and relatively rapid larval growth rates but also substantial larval mortality, probably due to high stocking density. Larvae started to hatch after 23 days and after 77-79 days had metamorphosed at a mean mass of 1.6 g; juveniles grew at an average of about 0.3 g/month. The primary animal carer was able to use wart patterns to distinguish between four individuals but scope for use on a larger scale was not supported when tested using computer-assisted individual identification software (WildID) or expert observers.

INTRODUCTION

The newt *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen 2013 (Caudata: Salamandridae) is native to northern Vietnam. The species is medium-sized with a snout vent length [SVL] of 54.4 - 77.7 mm in males and 70.8 - 88.85 mm in females (Nishikawa et al., 2013; Ziegler et al., 2018) and characterised by rough, black skin with fine granules over the body and distinct bony ridges on the head. The ventral side of the tail and the fingertips, toe tips, part of the palms, and soles are coloured orange (Nishikawa et al., 2013). The species is currently assessed as Vulnerable by the IUCN due to its small range and the ongoing decline of its natural habitat (IUCN SSC Amphibian Specialist Group, 2017). There are few specimens in captivity and these are almost exclusively held by private keepers; only six *T. ziegleri* are registered in zoos globally (Species360 Zoological Information Management System [ZIMS], 2020). Information on *T. ziegleri* is limited to morphology and genetics (Jiang et al., 2017; Nishikawa et al., 2013); basic ecology and larval development (Bernardes et al., 2017); and longevity (Ziegler et al., 2018). Consequently, data collection from captive newts is potentially a useful contribution to knowledge of *T. ziegleri*. We documented larval and juvenile husbandry, growth and development rates in captive bred newts and also trialled dermal wart patterns as a means of individual identification. Photographic identification of individuals in a population is widely used for tailed amphibians (Carafa & Biondi, 2004; Lunghi et al., 2019) but is likely less effective for species like *T. ziegleri* that lack colour patterns. Nevertheless, wart patterns have been useful in toads (Bindhani & Das, 2018).

MATERIALS AND METHODS

Development

Twenty four F2 captive bred eggs were acquired from a private breeder in the UK, descending from animals originally legally imported into Europe in 2010 by Max Sparreboom from Phia Oac, Cao Bang, Vietnam. The eggs were incubated for the first 20 days on damp kitchen towel at 20-22 °C and high humidity (cling film across the container was used to saturate the air) before being moved to water for hatching. Larvae were raised at 20-22° C (Digital LCD Thermometer, Lesai) in 7.5 pH, 8° gH and 15° KH tap water (6-in-1 Strips Aquarium to Test, Tetra) which was close to the field conditions reported by Bernardes et al. (2017; 7.18 pH, 7° gH and 6° KH), resulting in four surviving metamorphs (hereafter A, B, C and D). Following metamorphosis, the 4 juveniles were housed as a group in a 24.5 cm x 17 cm x 17 cm plastic fauna box (Komodo, UK). The fauna box was provided with a damp kitchen paper substrate, barks pieces forming a hide and a small plastic lidded container (IKEA, Sweden) with side entrance and lined with wet paper towels to give a high humidity hide. For the first few weeks after metamorphosis food offered was crickets (*Gryllus assimilis*) and fruit flies (*Drosophila melanogaster*). Subsequently, chopped earthworms (roughly 5mm long pieces, unidentified wild species, UK) were offered as a staple with the occasional addition of vitamin and mineral supplements (Nutrobal; Vetark Professional, UK). The newts were housed at temperatures between 12° C (winter minimum) and 26° C (summer maximum). Newts were weighed 48 h following the last feeding session at roughly weekly intervals between the 24th July 2020 and 13th December 2020 with a 0.01g precision scale (Wonolo Pocket Scale, Wonolo, China).

Photographic ID

Photographic IDs were developed for the four metamorphs using the skin granules on the dorsal surface of the head. This area does not contain soft tissue between skin and bone, so is not affected by weight gain/loss due to feeding, and is flat or roughly parallel to the surface where the newt rests, which facilitates photography without the need to manipulate and restrain the animal. Photographs were taken with a DSLR camera (Canon 700D, Canon, Japan) equipped with a macro lens (Tamron SP AF 60mm F/2.0 Macro 1:1, Tamron, Japan). A LED panel video light (Newest Pixel G1s) was used to provide strong, consistent light between photos. A tripod (AmazonBasic Lightweight Tripod, Amazon, USA) was also used to hold the camera steady. The camera was positioned horizontally over the top of the animals allowing for a clear shot of the whole head. The light was positioned next to the lens to minimise shadows on the head. The newts were first photographed from day 92 after oviposition (= day 15 after leaving the water).

After using the photographic IDs to monitor individual animals, the individual identification system was validated using both the computer programme WildID (Bolger et al., 2012) and with human observers. WildID was used to compare the initial individuals identification photographs of each of the four newts taken after metamorphosis in July with sets of photographs of each individual taken at monthly intervals thereafter until December, representing five monthly intervals after metamorphosis. We also presented seven professional herpetoculturists (Herpetology Team, ZSL London Zoo) with the same photographic set used to test WildID. Each person was tasked to assign an ID to each photo based on the original individual identification photographs. Individuals completed this task independently of one another. Inter-rater agreement was calculated with Fleiss' fixed-marginal Kappa (Fleiss, 1971; a statistical measure for assessing the reliability of agreement between a fixed number of raters when classifying items), and mean success determined as the proportion of correct ID assignments across all seven observers, overall and for each month after metamorphosis.

RESULTS

Development

Larval hatching occurred between 23 and 30 days after oviposition, giving 19 larvae from 24 eggs (79 % hatch rate). Only four larvae survived to metamorphosis (corresponding to Stage 45 as defined by Bernardes et al., 2017) which occurred 77 to 79 days after hatching. The metamorphs had a mean mass of 1.60 g. All individuals followed similar growth trajectories (Fig. 1), increasing to a mean mass of 3.38 g after 6 months, giving a mean growth rate of about 0.3 g/month.

Photographic ID

The lead author (JC) was able to consistently individually identify the 4 surviving metamorphs by using easily identifiable differences in their skin granules and found little variation in the location and shape of the granules in the first 5 months following their metamorphosis, although all wart patterns varied to some degree over time. The defining patterns of granules over the midsection of the skull remained more-or-less constant but as the newts aged the granules located on the bony ridges on the side of the head were subject to drift and movement with the growth and widening of the area. Granule location and the space

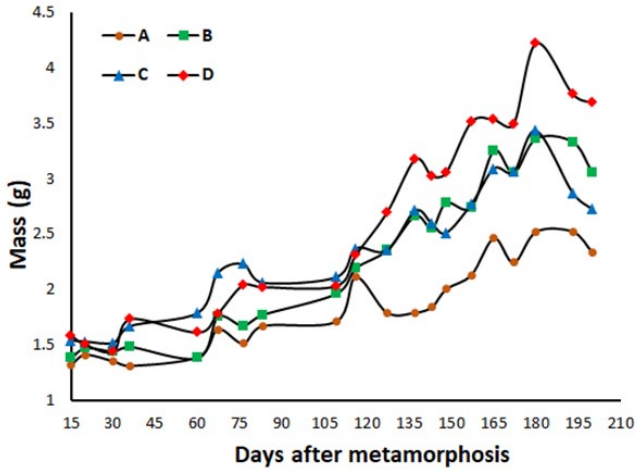

Figure 1. Change in mass of four *Tylototriton ziegleri* (labelled as A, B, C, D) and their mean mass trend. Tracking of the weight for each animal was only possible thanks to photographic individual identification at the time of weighing.

between granules tend to change slightly with time, as the head widened, but identifiable patterns of granules remain distinguishable, at least over 6 months post metamorphosis. The WildID test proved unsuccessful with a mean False Rejection Rate (FRR) of 85 % over the 6 months period when using 0.1 as the positive identification score threshold (Bolger et al., 2012), which increased to 100 % when comparing November and December photos to the original July photos. When lowering the positive benchmark score threshold to 0.05, average FRR decreased to 70 %, with a FRR of 75 % for October onwards. There were no positive identifications so no False Acceptance Rate could be calculated.

Using human raters, mean ±SD agreement and kappa were 63.4 ± 15.2 % and 0.54 ± 0.2, respectively. Mean agreement and kappa (in parenthesis) for months one to five after metamorphosis were, respectively, 72 %(0.63), 85.71 %(0.81), 53.57 %(0.38), 47.62 %(0.3), 67.86 %(0.57). The mean proportion of success for the same time periods was, respectively, 0.86, 0.93, 0.68, 0.71, 0.39, and overall mean ±SD success rate was 0.71 ± 0.2. One individual (D) with the most striking wart patterns (see Figs. 2 & 3) received a higher success rate than the other three individuals.

DISCUSSION

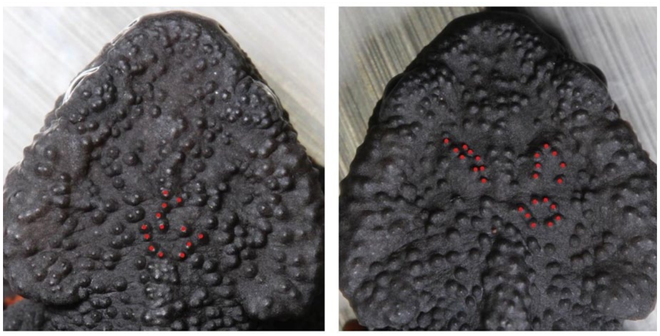

Figure 2. Example of different characteristic wart patterns of two different newts, their most recognisable pattern are highlighted with red dots

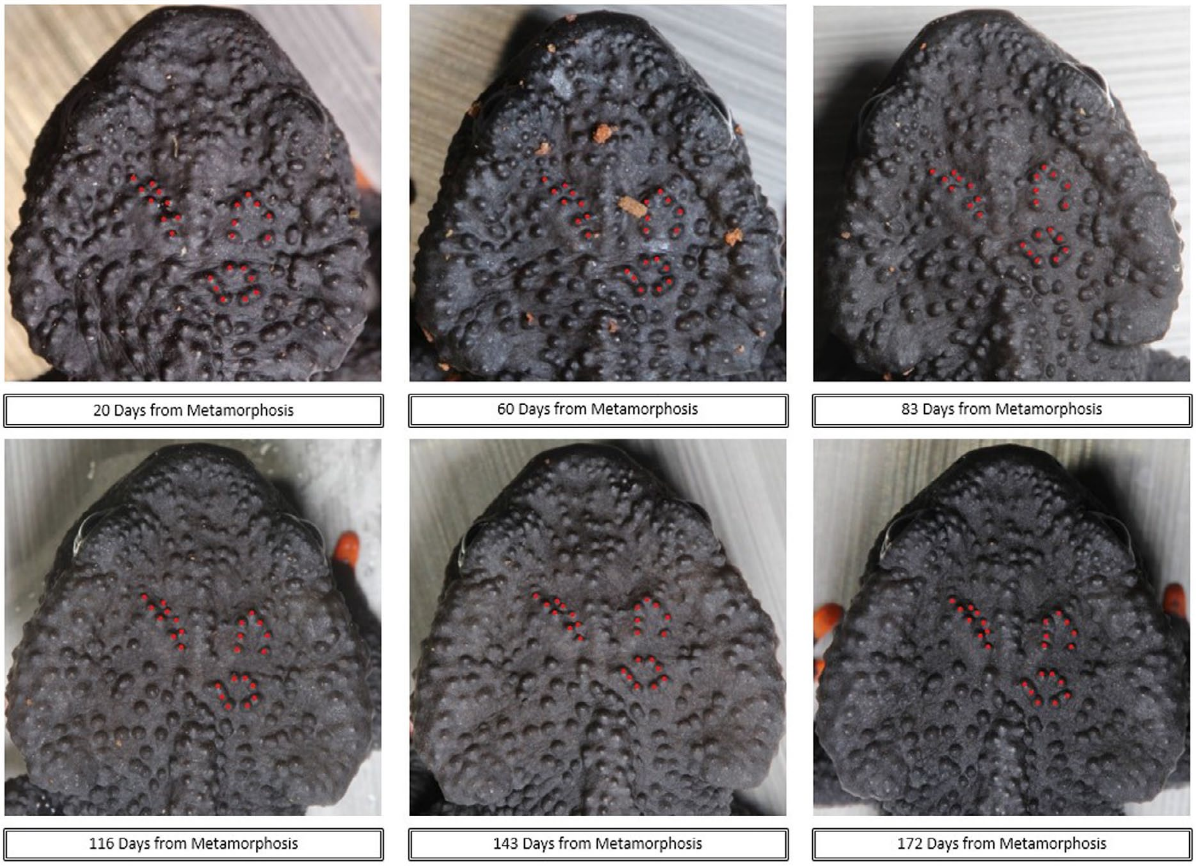

Figure 3. Example of consistency of a wart pattern through the growth of one newt, the same pattern is highlighted in red at each post-metamorphic time interval

Development

Observations on the development of *T. ziegleri*, in an ex-situ facility in Vietnam, have been reported by Bernardes et al. (2017). Their egg incubation period was similar to ours with larvae hatching from 20 days. However, some larvae took substantially longer to hatch (>30 days) but hatched at a more developed stage. They did not determine the duration of the larval period, so our observation of 77-79 days provides novel information. Their mean mass at metamorphosis was only 0.6 g, in our study this was exceeded by 166 % (1.6 g) and for two specimens raised the previous year by the lead author, but under less favourable conditions, was exceeded by 136 % (1.42 g). This suggests that conditions provided in captivity may radically affect larval growth rate and therefore the fitness of metamorphs. Their larval hatch rate (58 % for a collected clutch and 77 % for field clutch) is broadly similar with the 79 % hatching rate that we observed. Our observed larval survival rate was only 21 % but no information on larval survival rate was provided by Bernardes et al. (2017). It is unclear why body mass at metamorphosis of captive newts was so much greater than those in the wild and why so few of captive larvae survived. Our captive conditions were designed to match those reported from the field as closely as feasible. We suggest that larval density was too high in the 13 L container, leading to either competitive inhibition of some larvae, stress due to larval aggression or compromised water quality due to nitrogenous waste (data on the latter were not collected). If this was the case, then as stocking density fell due to mortality, conditions would have improved and mortality rate fallen. Larval survivorship rates are not known for the species in nature or in other ex situ contexts, so relative success here cannot be quantified.

Even under favourable captive conditions, post-metamorphic growth rates were slow, reflecting the relatively slow developing and long lived nature of this species and sub-genus of newts (Ziegler et al., 2018). If the growth rates observed in this study were maintained, the newts would take approximately five years to attain mean adult size (Nishikawa et al., 2013; Bernardes et al., 2017; Ziegler et al, 2018), which matches maturation estimates provided by Ziegler et al. (2018). Consequently, there would be slow replacement of adult populations should adults be lost from wild populations, for example due to collection from the wild. This threatens many species of *Tylototriton* (Nishikawa et al., 2013). No aggression between the animals was recorded following metamorphosis.

Photographic ID

Although individual identification was feasible for a single expert familiar with the individuals and species in question, validation of the photographic IDs via WildID and by other herpetologists was more problematic. WildID likely failed due to the monochrome skin colour of the newts, small differences in lighting between photographs casting shadows of skin granules in different directions, as well as other challenges of photographing tiny animals in a standardised way. These issues would likely be difficult to correct under field conditions. Expert human observers who routinely use amphibian photo IDs also struggled in some cases to use wart patterns to identify individuals. The seven observers showed good, but not excellent, kappa values for some months, and poor for others (following definitions for these terms presented by Fleiss, 1981), and the proportion of photographs correctly identified only exceeded 0.9 once, i.e. was never perfect. As with WildID, the last month showed the worst success rate and agreement Kappa.

Observers reported that identifications proved difficult due to the complex patterns of warts (especially for those individuals with less obvious 'marker' patterns) and changes in patterns between months, which was consistent with head morphology changes described in this species by Ziegler et al. (2018). These data result from a test involving a small number of newts; should this system be applied to greater umbers of animals, its success would have been even lower (Gamble et al., 2008). Overall, our data suggest that wart patterns may only be viable for the individual identification of numbers of newts if limitations of computer software to process images from monochrome animals can be overcome. Our data specifically compared initial post metamorphic photographs with subsequent pictures with a view to the individual identification of translocated juveniles. It may be that photographic individual identification using wart patterns would be more effective in adult animals and this would be a useful future study to facilitate monitoring adult populations in the field. Currently, therefore, if individuals need to be identified, especially in a field setting, more invasive methods such as VIE (Visible Implant Elastomer) or microchipping may be required (Tapley et al., 2019), although no other marking method has been trialled in this species.

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SHORT NOTE
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Extended range and observations on the natural history of the casquehead lizard *Laemanctus julioi* from Honduras

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INTRODUCTION

The family Corytophanidae, or casquehead lizards, comprises three genera and a total of 11 known species, nine of which occur in Honduras (Uetz et al., 2020). Two of the four *Laemanctus* species are endemic to Honduras (McCranie, 2018), *Laemanctus julioi* McCranie, 2018 and *Laemanctus waltersi*. *Laemanctus julioi* is found on the Pacific versant of south-central Honduras, in the Departamento de Francisco Morazán (holotype) and with an alleged distribution in Departamento de El Paraíso based on a casual observation in 1998 (McCranie, 2018). It has a reported altitude range of 650 to 1000 m, and it is restricted to Premontane Dry Forest (McCranie, 2018). There appears to be no information on the reproduction or feeding ecology of the species, although McCranie (2018) mentioned that these aspects are known for the related species *Laemanctus serratus*. Here, we increase the known geographical range of *L. julioi* into additional forest zones and to greater altitude, present new observations on behaviour, nutrition, reproductive ecology and report on total body length.

MATERIALS AND METHODS

During May and June 2017, May 2018, and October 2019, we obtained five new records for *L. julioi* in different locations in south-central and south-west Honduras. Four locations were documented with voucher photographs deposited in the Amphibian and Reptile Diversity Research Center of the University Texas Arlington, Texas, USA (UTADC) and one location documented with voucher photograph in the Colección de Registros Fotograficos de Vertebrados of the Universidad de San Carlos, Ciudad de Guatemala, Guatemala (USACF). We took morphometric data on UTADC 9461–9477 and USACF000003_1–5, photographed them, and identified each one using McCranie´s taxonomic keys (2018). Using a Garmin eTrex 10 Global Positioning System, we obtained their geographical coordinates (WGS 84) and altitude and mapped them using QGIS version 2.18. The forest types mentioned in this text are described by Holdridge (1967) and modified by McCranie (2011). We kept USACF000003_1–5 in captivity for eight months in the Centro Nacional de Conservación y Recuperación de Especies Rosy Walther to obtain nutritional and behavioural data. The colour codes used to describe the specimens are based on Köhler (2012).

RESULTS

We found *L. julioi* in five different locations (Fig. 1), as follows:

1) Lempira, Gracias, Parque Nacional Montaña de Celaque (PNMC), south-west Honduras; (14° 33'38" N, 88° 39'18" W) 1750 m a.s.l.. Three adult females UTADC 9478–9479 (Fig. 2A) were found laying eggs on the ground within an approximate 1 m^2, at 04:27 h on May 16th, 2017.

2) Francisco Morazán, San Antonio de Oriente, Escuela Agrícola Panamericana Zamorano, south-central Honduras; (13° 59'15" N, 86° 58'54" W) 770 m a.s.l.. One subadult of unknown sex UTADC 9480 (Fig. 2B), was found basking in a patch of Premontane Moist Forest near the Escuela Agrícola Panamericana Zamorano, at 17:43 h on June 30th 2017.

3) Intibucá, Jesús de Otoro, CA-11a highway, in west-central Honduras; (14° 32'04" N, 87° 58'32" W) 750 m a.s.l.. A gravid female UTADC 9481–9484 (Fig. 2C) was found as a road-kill, at 10:40 h on May 2nd 2018.

4) Lempira, Gracias, PNMC, south-west Honduras; (14° 33'46" N, 88° 38'34" W) 1,410 m a.s.l.. A gravid female UTADC 9461–9477 (Fig. 2D) was found basking on the decking of a visitors' center with a portion covered by ferns. At the time of capture this individual was lime green in colour (105) but after two days in captivity its tonality changed to cinnamon-rufous (31), found at 07:54 h on May 8th 2019.

5) Lempira, Gracias, Thermal Waters, south-west Honduras; (14° 33'31.54" N, 88° 34'16.32" W) 890 m a.s.l.. An adult female USACF000003_1–5, (Fig. 3A–C) was found in a lethargic state after falling from a *Ficus benjamina* tree on the edge of a pool, it was cinnamon-rufous (31) in colour, the

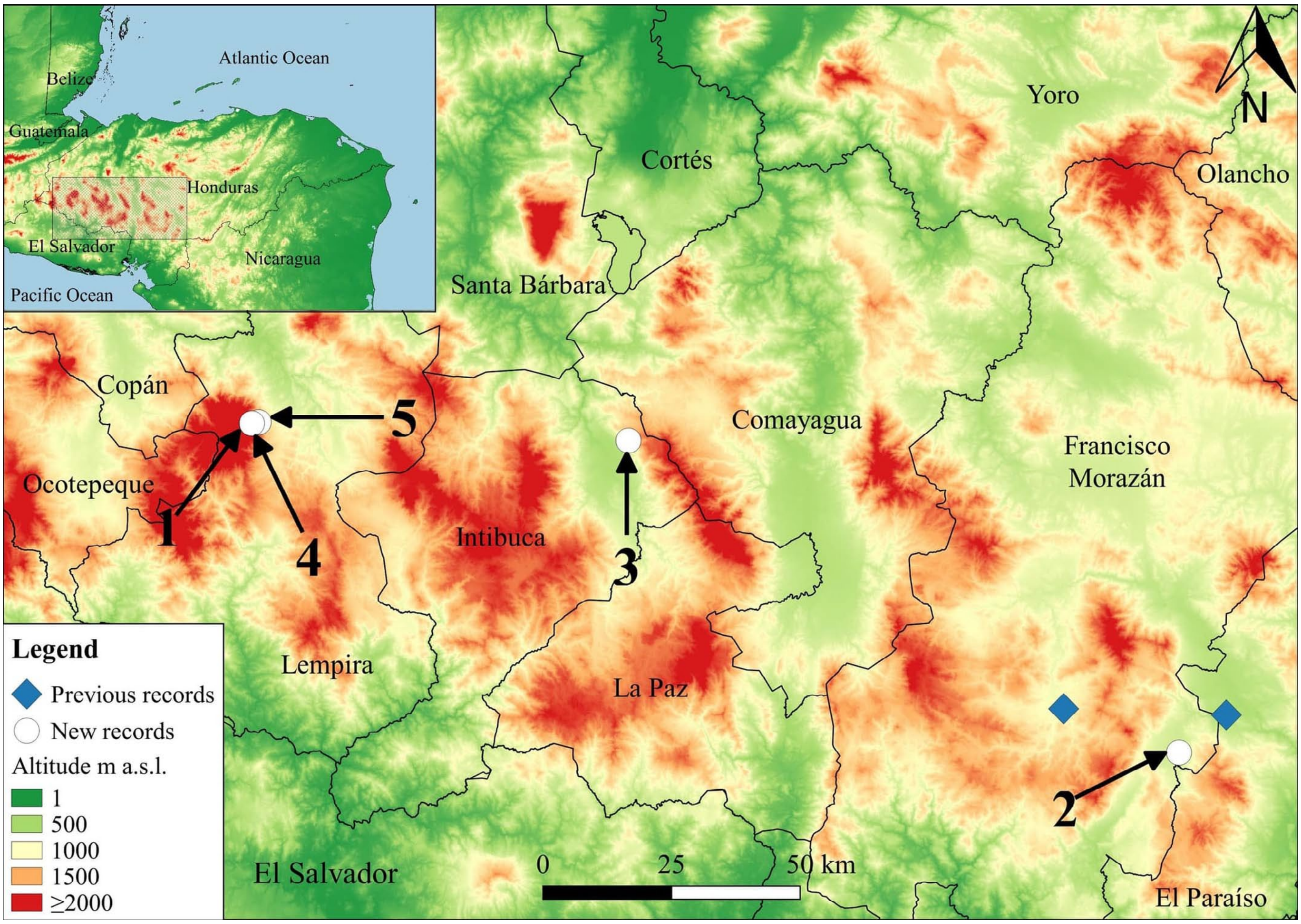

Figure 1. New locality records for *Laemanctus julioi* in Honduras, localities 1, 3, 4 and 5 are on the Atlantic versant. Numbers on the map match the locality records in the results section.

Figure 2. Aspects of *Laemanctus juloi* natural history- **A.** Three females UTADC 9478 in a group depositing their eggs close together, **B.** Subadult of sex unknown, UTADC 9480 in San Antonio de Oriente, Departamento de Francisco Morazán, Honduras **C.** An adult female of UTADC 9481 found deceased on the road, Jesus de Otoro, Departamento de Inti bucá, Honduras, **D.** Adult female UTADC 9477, from the PNMC, Sierra de Celaque, Departamento de Lempira, Honduras

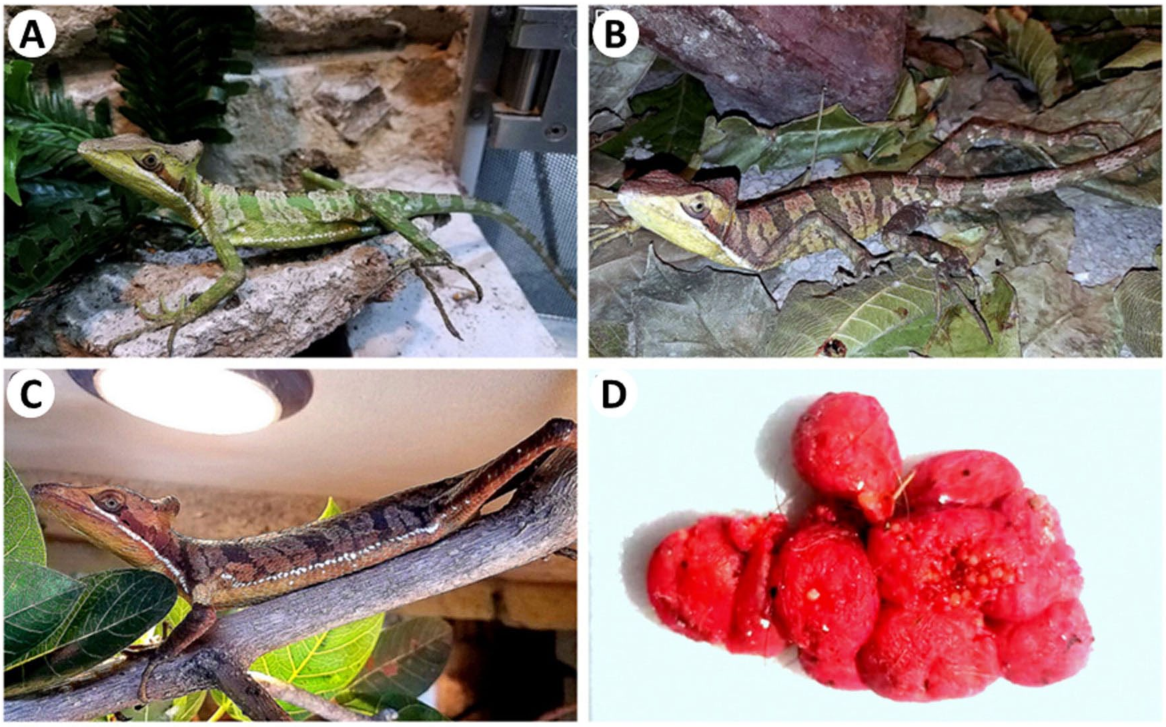

Figure 3. Aspects of *Laemanctus julioi* natural history – **A.** Adult female USACF000003_1 lime green in colour, **B.** USACF000003_2 with an intermediate shade of colouration between lime green and cinnamon-rufous, **C.** USACF000003_5 cinnamon-rufous in colour, **D.** Fruits of *Ficus benjamina* regurgitated by USACF000003_1–5 after its capture

next morning it regurgitated food remains inside its collection bag (Fig. 3D) and changed colour to lime green (105), found at 22:00 h on October 3th 2019.

DISCUSSION

We provide two new departmental records for *Laemanctus julioi*, Intibucá, and Lempira. The latter represents the westernmost distribution point in the Parque Nacional Montaña de Celaque. Also, for the first time, we record the presence of this species on the Atlantic versant, previously it was considered exclusive to the Pacific versant (McCranie, 2018).

We added two new types of forest habitat for the species, Lower Montane Moist Forest (UTADC 9461–9479 and USACF000003_1–5) and Premontane Moist Forest (UTADC 9480). Previously, individuals of this species were only recorded in Premontane Dry Forest (McCranie, 2018, in whole with our data from UTADC 9481). Our record UTADC 9478 made at 1750 m a.s.l. represents the highest altitude known for the species; 750 m above the highest altitude reported by McCranie (2018). Finally, USACF000003_1–5 is the longest measured individual with a snout-vent length of 145 mm making it distinctly longer than the holotype, another female measuring 115 mm. Compared with the holotype reported in McCranie (2018), this specimen also has a higher ratio tail to snout vent length, 4.10 compared to 3.82, and a higher ratio of helmet length to snout vent length, 0.30 compared to 0.29. These new data suggest that a taxonomic review of *Laemanctus* from western Honduras may be warranted, since the observations on external morphology and ecological observations seem to show some differences from those reported by McCranie (2018).

We consider this species may also be found in the Valle de Comayagua, which could be a link between the Valle de Choluteca and the Valle de Otoro (Wilson & McCranie, 1998). It may also be present in protected areas and their surroundings that connect to recorded populations, such as the Parque Nacional Montaña de Comayagua, Reserva Biologica Montecillos, Reserva Biologica Uyuca, Reserva Biologica Yerba Buena, and the Refugio de Vida Silvestre Mixcure, within the region of the Cordillera Sur de la Serranía. In which case, the species would have a distribution pattern similar to that of other lizards in the middle and upper zones such as *Sceloporus squamosus*, *Diploglossus bivittatus* and *Abronia salvadorensis* (McCranie, 2018). Having an overlapping habitat, previously or currently, with *L. longipes* in the north-west of their distribution and/or with *L. waltersi* in the north (see McCranie distribution, 2018), could result in competitive exclusion between species as mentioned by Lee (1980), although to test this assumption, it would be necessary have greater clarity of the distribution limits of each species.

We observed that female *L. julioi* lay their eggs between May and June; this is within the range reported for *L. serratus* (Lee, 1996; Köhler, 1999). There is no information on the timing of copulation. UTADC 9478 demonstrated communal oviposition, as far as we know the first report of such behaviour in the family Corytophanidae, although it is considered common in oviparous reptiles (Burghardt & Rand, 1982; Gillingham, 1987; Gregory, 1984; Gregory et al., 1987; Graves & Duvall, 1995). We suggest that this phenomenon may occur due to - 1) mutual attraction to features of the environment with limited availability, which may occur incidentally, and/or 2) a mutual attraction among individuals (Graves & Duvall, 1995).

The specimen USACF00003_1–5 was maintained in captivity after its capture. Before it's capturing we observed the individual in a *Ficus benjamina* tree. Once inside the cloth bag it regurgitated fruits from the same tree. Consumption of small amounts of plant material has been reported in two other genera of the family Corytophanidae, *Corytophanes* and *Basiliscus* (Andrews, 1979; Cooper Jr. & Vitt, 2002). Typically, *Laemanctus* feeds on a wide variety of invertebrates; *L. longipes* in captivity ate crickets (McCranie, 2018). The stomach contents of *L. serratus* included snails, arthropods, insects (coleopterans and orthopterans), Anolis lizards (Martin, 1958), caterpillars, and the remains of leaves and stems of a monocot plant (Peters, 1948), the latter being the first report of ingestion of plant material. Therefore, we present the first data on *L. julioi* feeding in captivity and in its natural environment and what we consider to be the first record of fruit feeding in the genus *Laemanctus*. The intake of this fruit might be due to 1) direct intake, as it is a small easy to swallow fruit that is rich in sugar that may provide a high energy food source during hot days or in dry and poor trophic locations (Pérez-Mellado & Corti, 1993; Valido & Nogales, 1994; Passos et al., 2013; Brock et al., 2014; Mačát et al., 2015), or 2) accidental ingestion at the time that the lizard was actually feeding on insects attracted to fruit, as mentioned in the case of *Sceloporus occidentalis* (Clark, 1973). In captivity, USACF000003_1–5 fed on crickets and new born white mice (pinkies); however, the second offer of a pinkie after a few months was ignored by the individual, which thereafter fed only on crickets. Occasionally, the lizard was offered fruits of *Ficus benjamina* attached to a branch, but it never accepted any.

USACF000003_1–5 displayed several colour changes before it fell from a tree and was collected. In a lethargic state it showed a cinnamon-rufous coloration (31), whereas the next day, being in the water or after several hours without being manipulated, it presented a lime green coloration (105). Vaillant (1896) suggested that in the case of *L. longipes* such colour changes were due to sun exposure or emotional conditioning (stress), although patterns such as the black spots on the back or the white line behind the eyes remain unchanged. We exposed USACF000003_1–5 to the sun after being inside a terrarium with low lighting and an average temperature of 24 °C. Initially, it had a cinnamon-rufous coloration (31) but after one hour it had become lime green (105), we also observed that this individual presented light tones when slightly submerged in the water. In other observations, before the caretaker opened the terrarium facilities, the individual was lime green (105), despite the low temperatures at night. Once the manager manipulated objects inside the terrarium, the specimen became cinnamon-rufous (31). According to these data presented, we consider that the tonality changes in *L. julioi* (Fig. 3A–C), may be responses to stress stimuli caused by the presence of a possible threat, temperature changes and light or the availability of food and water. Rapid colour changes of this kind involve the movement (dispersion or concentration) of pigments within chromatophores, for example, the dispersion of melanosomes (melanin pigment-containing organelles) through the long dendritic processes ("arms") of the starry melanophores, causing the darkening of the reptile's skin (Olsson et al., 2013).

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SHORT NOTE

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First record of Colombian red-eyed tree frog *Agalychnis terranova* from the Sierra Nevada de Santa Marta, Colombia with a description of its advertisement call

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ABSTRACT - Based on collected specimens and phylogenetic information, we provide the first record of the Colombian red-eyed tree frog, *Agalychnis terranova*, from the Sierra Nevada de Santa Marta in northern Colombia. This species is known from several localities on the middle Magdalena river valley and the Pacific lowlands. With this new record, the known geographic distribution of this frog is extended about 370 km north-west from its previous northernmost record. Additionally, we describe for the first time the tonal advertisement call of this species, which consisted of one or two notes with a total duration of 0.52 s and dominant frequency of about 1.74 kHz.

INTRODUCTION

The genus *Agalychnis* Cope, 1864 (Anura: Phyllomedusidae) includes 14 recognised species, ranging from the lowlands of the south Pacific coast of Mexico, extending through the lowlands of Central America, along the Pacific coast to north-west Ecuador, and the lowlands along the Eastern Andes from Colombia and Venezuela to Peru and the Upper Amazon basin. Half of the known species of *Agalychnis* occur in Colombia: *A. callidryas* (Cope, 1862), *A. buckleyi* and *A. lemur* (Boulenger, 1882), *A. spurrelli* Boulenger, 1913, *A. psilopygion* (Cannatella, 1980), including two species restricted to the country: *A. danieli* (Ruiz-Carranza, Hernández-Camacho & Rueda-Almonacid, 1988) and *A. terranova* Rivera-Correa, Duarte-Cubides, Rueda-Almonacid & Daza, 2013.

The Colombian red-eyed tree frog *Agalychnis terranova*, was described using material collected from three localities on the middle Magdalena river valley (Magdalena province), Colombia *(*Rivera-Correa et al., 2013). Belonging to the *A. callidryas* group, *A. terranova* is characterised by a red iris with golden reticulations in the eyelid membrane, usually showing a slim dorsally green body that sometimes has white warts, and with a head that is wider than the body. The ventral body area is cream-coloured and the body extremities and flanks are orange with some reduced white warts. The head is slightly sloping in lateral view, with a rounded snout in dorsal view. This medium sized frog does not have calcars or tubercles on the legs, and the parotid glands are absents (Rivera et al., 2013).

Currently, this species has been reported in different localities in the middle of the Magdalena river valley region, in the departments of Antioquia, Cundinamarca and Santander. (Rivera-Correa et al., 2013; Guarnizo et al., 2015). Later, its distribution was extended to the Chocó province (Palacios-Rodríguez et al., 2016).

Figure 1. Adult male of *Agalychnis terranova* (CBUMAG:ANF:01173, SVL 43.7 mm) from south-western foothills of the Sierra Nevada de Santa Marta

During an amphibian field survey between 3rd to 5th September 2019, we discovered a new population of *Agalychnis terranova* (Fig. 1.) in the south-western foothills of the Sierra Nevada de Santa Marta, near the rural school of vereda Tierras Nuevas, Corregimiento Villa Germania, Municipio de Valledupar, Departamento del Cesar, Colombia (10° 14’25’’ N, 73° 45’27’’ W, 874 m altitude, Fig.

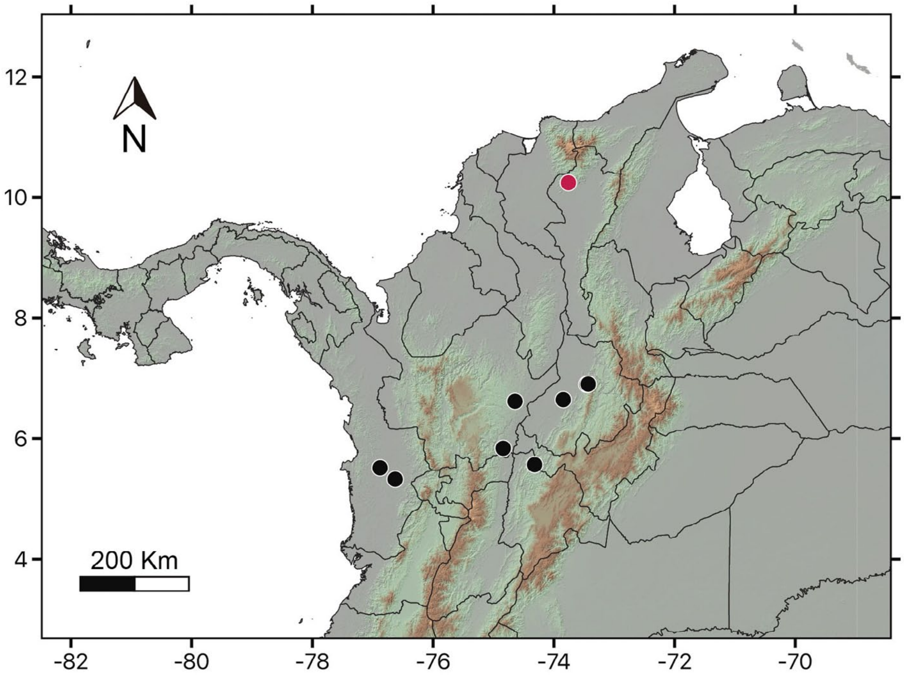

Figure 2. Known distribution of *Agalychnis terranova* in Colombia- black circles = previous records; red circle = new record in the Sierra Nevada de Santa Marta

Figure 3. Habitat of *Agalychnis terranova* in the Sierra Nevada de Santa Marta, Colombia 874 m altitude

2; Supplementary Material Table S1). This population was found in a small and degraded dry forest fragment where heavy rains had occurred in previous days, flooding much of the soil (Fig. 3). At this location, about 5 males were heard calling from the branches of trees at more than three meters high. Two individuals were captured, euthanised with a 2 % lidocaine solution, fixed in a 10 % formaldehyde solution, stored in 70 % ethanol and deposited in the herpetology section of the Centro de Colecciones Biológicas de la Universidad del Magdalena (vouchers CBUMAG:ANF:01173 and 01174). Prior to formalin fixation, muscle tissue was collected from these voucher specimens and stored in 98 % ethanol.

To confirm the species identity using genetic information, we conducted a phylogenetic analysis using a fragment of the ribosomal 16S gene. Nine species of *Agalychnis* with available genetic information were included (Supplementary Table S2). PCR and sequencing followed the protocols by Rivera & Daza (2020). The assembled matrix comprised 33 terminals and 839 sites, including 11 outgroups within Phyllomedusidae. The tree was rooted using *Cruziohyla calcarifer*. The best model of evolution was obtained using ModelFinder (Kalyaanamoorthy et al., 2017) and a maximum likelihood tree was obtained using IQTREE 1.6.12 (Nguyen et al., 2015). Nodal support was obtained using the ultrafast bootstrap method after 5000 pseudoreplicates (Hoang et al., 2018). Intraspecific variation within *A. terranova* was estimated from the ml distance matrix from IQTREE.

The inferred phylogenetic tree (lnL=-4532.8) agrees with previous studies within *Agalychnis* and confirms the identity of *A. terranova* on the Sierra Nevada de Santa Marta (Fig. 4). Intraspecific variation including the sample from the new locality is very low ranging from 0.0 to 0.58 percent.

Calls from three individuals were recorded with a Sennheiser ME66/K6 directional microphone connected to a Zoom H4N digital recorder. Recording settings were 96 kHz sampling rate and 16-bit resolution. The air temperature and relative humidity were also recorded using a thermo-hygrometer RH 101 Extech IR. All recordings were analyzed using Raven Pro 1.5 software for Windows (Bioacoustics Research Program 2014 in Hann's sampling window, FFT window size of 512 points. Call features definitions follow Köhler et al. (2017). According to Köhler et al. (2017), call dominant frequency was considered as the frequency with the greatest amount of acoustic energy, note as the sound unit produced by a single expiratory event of the frog, and pulse as a single unbroken wave train isolated in time by amplitude

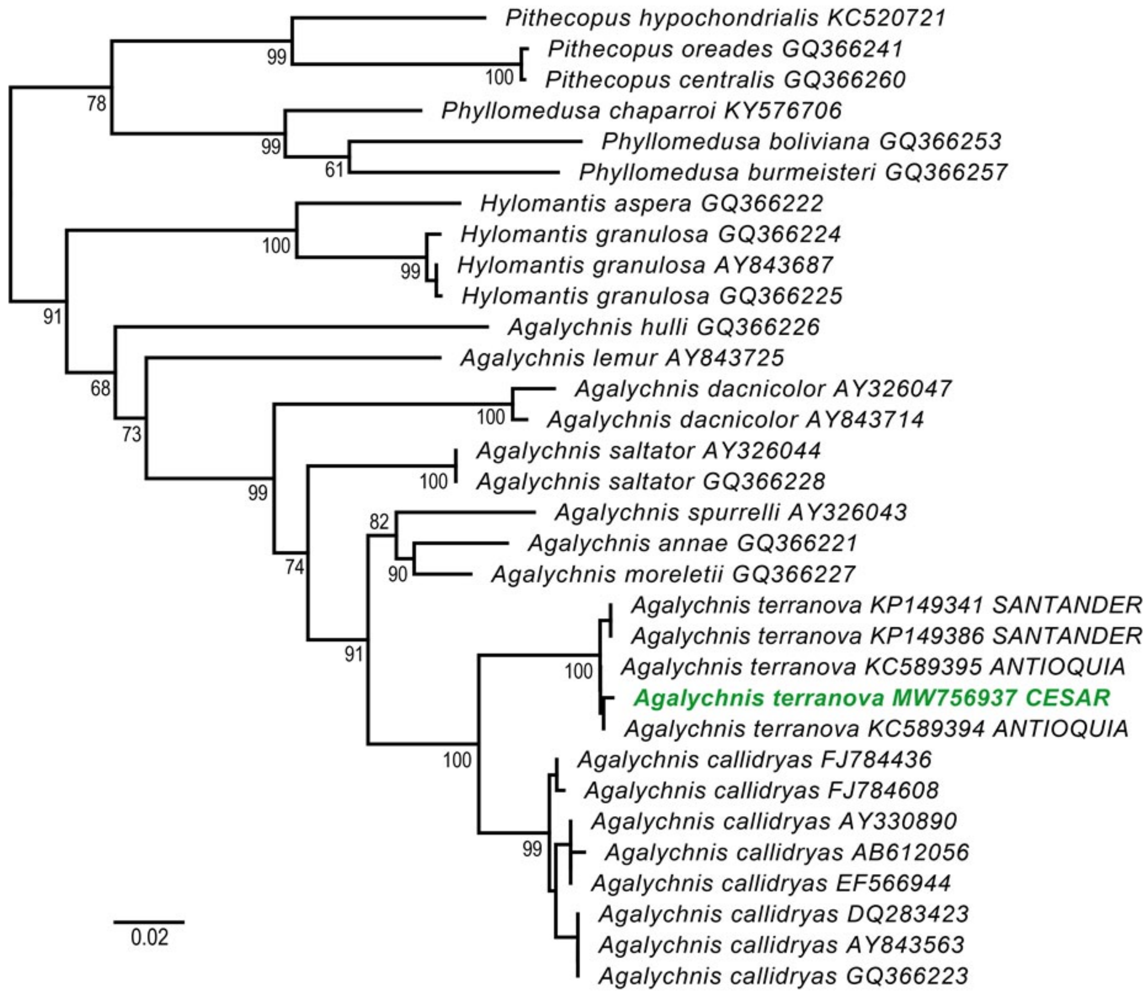

Figure 4. Maxium likelihood tree of Phyllomedusidae showing the phylogenetic position of *Agalychnis terranova* from the south-western foothills of the Sierra Nevada de Santa Marta. Numbers next to nodes represent ultrafast bootstrap support. See the Supplementary Material, Table S2.

reduction. We report numerical call features as mean ± SD and the respective range in parenthesis. The temporal call features (i.e., call duration, number and duration of notes and pulses) were measured in oscillograms; we used power spectra diagrams to calculate the call dominant frequency, and frequency bandwidth hereafter referred to as low frequency and high frequency, which were measured at 10 dB (re 10 mPA) below the peak intensity of the dominant call frequency. Graphs of oscillograms, spectrograms, and power spectra were elaborated with R 2.15.1 software using Seewave package (Sueur et al., 2008) settings (i.e., window name (Fourier transform window) = Hann; window length = 512 samples; and overlap = 90 %). The values of each call feature are presented with mean ±standard deviation (minimum - maximum). Five recording are housed at Fonoteca Zoológica (MNCN-CSIC): FZ SOUND CODE 12964 to 12968.

The tonal advertisement call of *Agalychnis terranova* (Fig. 5) consisted of one or two notes with similar dominant frequency of approx. 1.74 kHz and total call duration of 0.52 s, N = 7 calls. These calls were recorded from three solitary males perched between five and seven meters from the riparian vegetation. A summary of acoustic features is presented in the Table 1. According to the guild classification system proposed for anuran advertisement calls (Emmrich et al., 2020), *A. terranova* has an advertisement call of the guild E type, which corresponding to multi, uniform and no modulated notes.

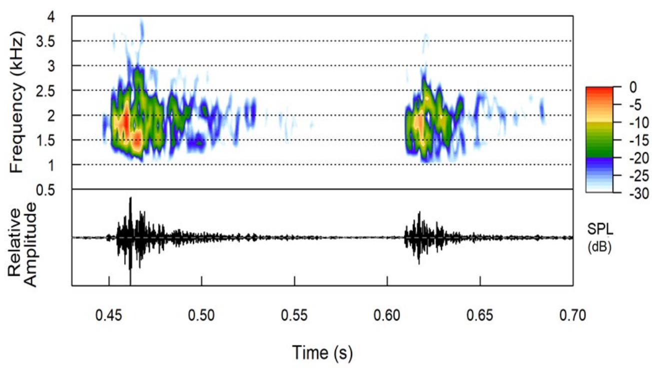

Figure 5. The tonal advertisement call of *Agalychnis terranova* (voucher CBUMAG:ANF:01173, recording time 18:10 h, air temperature 26.8 ˚C, air humidity 88 %) from the Sierra Nevada de Santa Marta, Colombia. Spectrogram (above), oscillogram (below).

Table 1. Summary of tonal call features for *Agalychnis terranova* from the Sierra Nevada de Santa Marta, Colombia. Data of seven calls from three individuals (including voucher CBUMAG:ANF:01173); air temperature 26.8 ˚C, relative humidity 88 %.

	Call duration (s)	Inter-Call intervals (s)	1˚ Note duration (s)	2˚ Note duration (s)	Inter-Note interval (s)	1˚ Note Dominant frequency (kHz)	1˚ Note Low frequency (kHz)	1˚ Note High frequency (kHz)	2˚ Note Dominant frequency (kHz)	2˚ Note Low frequency (kHz)	2˚ Note High frequency (kHz)
Mean	0.52	29.925	0.227	0.173	0.087	1.739	1.160	2.300	1.741	1.128	2.193
SD	0.15	2.07	0.05	0.06	0.03	0.20	0.06	0.16	0.19	0.08	±0.07
Min	0.03	17.97	0.03	0.047	0.03	1.37	1.060	2.660	1.360	1.040	2.080
Max	0.46	22.11	0.15	0.20	0.10	1.89	1.280	2.120	1.890	1.250	2.280

Table 2. Tonal call features in the *Agalychnis callydrias* species group. Species are sorted according to the phylogeny in Figure 4.

Species	Notes/Call	Call duration (s)	Dominant frequency (kHz)	Source
Agalychnis saltator Taylor, 1955	1 –2	0.08–0.12	1.844–1.89	Duellman (1970)
Agalychnis spurelli Boulenger, 1913	1	0.13–0.36	0.75–1.051	Cossio & Medina-Barcenas (2020)
Agalychnis annae (Duellman, 1963)	1	0.16–0.44	1.044–1.295	Duellman (1970)
Agalychnis moreletii (Duméril, 1853)	1	0.022–0.088	1.046–1.396	Duellman (1970); Briggs (2010)
Agalychnis terranova				
Rivera-Correa, Duarte-Cubides, Rueda-Almonacid & Daza, 2013	1–2	0.03–0.46	1.36–1.89	This Study
Agalychnis callidryas (Cope, 1862)	1–2	0.08-0.24	1.488–2.4	Duellman (1970); Lee (1996)

The advertisement call of *A. terranova* is similar to that of its sister species *A. callidryas* (Duellman, 1970; Lee, 1996); both show one or two notes and their call durations and dominant frequencies overlap. Likewise, when the call characteristics of *A. terranova* are compared with the other species of the *A. callidryas* group, several similarities are observed (Table 2).

This is the first record of *A. terranova* in the Sierra Nevada de Santa Marta and extends its geographical distribution over 370 km in a straight-line from San Vicente de Chucurí, Santander, Colombia, the north-easternmost locality previously reported in literature (Guarnizo et al., 2015). Furthermore, this record extends the presence of this species to a new biogeographic region, the Maracaibo province (Morrone, 2001), adding to the other two provinces (Chocó and Magdalena) where the species has been found. With the description of advertisement call of *A. terranova*, we contribute to acoustic studies of Colombian anuran species, mainly those that inhabit the dry forest (Vargas-Salinas et al., 2019).

The Colombian red-eyed tree frog, *A. terranova*, is included in the Near Threatened (NT) IUCN red list category in the most recent Colombian amphibian assessment. This is due to the continuous decline in the extent and quality of habitat in the few localities that are known for this species due to several human activities including mining and the construction of dams (IUCN SSC Amphibian Specialist Group 2017). Similar to the other known localities for the species, the south-western foothills of the Sierra Nevada de Santa Marta are being severely deforested to make way for livestock, crops, and mining, which may be affecting this particular population of *A. terranova*.

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Population status and distribution of mugger crocodile *Crocodylus palustris* in the Similipal Tiger Reserve, Odisha, India

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The mugger crocodile *Crocodylus palustris* (Lesson, 1831) is one of the 27 extant crocodilians (Grigg, 2015). It is distributed across six countries, Iran, Pakistan, India, Nepal, Sri Lanka and possibly also Bangladesh (Choudhury & de Silva, 2013) although formally it was also found in Bhutan and Myanmar. In India, the mugger has been reported at altitudes up to 420 m a.s.l. in Corbett Tiger Reserve (Whitaker & Whitaker, 1984; Whitaker, 1987) which is listed as its highest altitude by IUCN (Choudhury & de Silva, 2013) but is also present in the lower hill streams up to 700 m a.s.l. (Daniel, 1983; Rao, 1993). In Nepal, most records are from below 200 m a.s.l. (Schleich & Kästle, 2002), while in Sri Lanka it has been recorded at 450 m a.s.l. (Whitaker & Whitaker, 1979).

The International Union for Conservation of Nature (IUCN) recognises the mugger as vulnerable, and in India it is included in Schedule I of the Wildlife (Protection) Act, 1972. Throughout its range, the mugger is highly adaptable and has been found to co-exist with humans in urban landscapes (Vyas, 2012). The mugger is widespread in India except its north-eastern states and it is known to occupy various habitat types such as rivers up to 3-5 m depth, lakes, marshes, human-constructed ponds, reservoirs, irrigation canals, as well as estuaries and coastal saltwater lagoons (Whitaker & Whitaker, 1984; Whitaker, 1987; Whitaker & Andrews, 2003). Similipal Tiger Reserve (STR) in India is a part of the Deccan Peninsula Biogeographic zone and Chhotanagpur Biotic province (Rodgers & Panwar, 1988) that covers 2750 km^2 in the Mayurbhanj district of Odisha state (Fig. 1). The major forest types in the STR are Tropical Moist Deciduous Forest and Tropical Semi-evergreen (Champion & Seth, 1968). The temperature here ranges from 4˚ C in winter to 34˚ C in summer. It is a very moist landscape with an average annual rainfall of 2000 mm, and the landscape is a matrix of numerous perennial streams (Nayak, 2014).

In Odisha, the mugger is found in river systems of the STR (Sagar & Singh, 1993), in the Mahanadi river system of the Satkosia Wildlife Sanctuary (Dutta et al., 2009), the Ghodahada reservoir in Ganjam district, and a few individuals have been recorded from the Saberi river in Koraput district (Debata et al., 2018). In around 1980, the mugger was extirpated in the STR. Possible reasons for this include fishing using explosives and nylon nets and fires at nesting sites

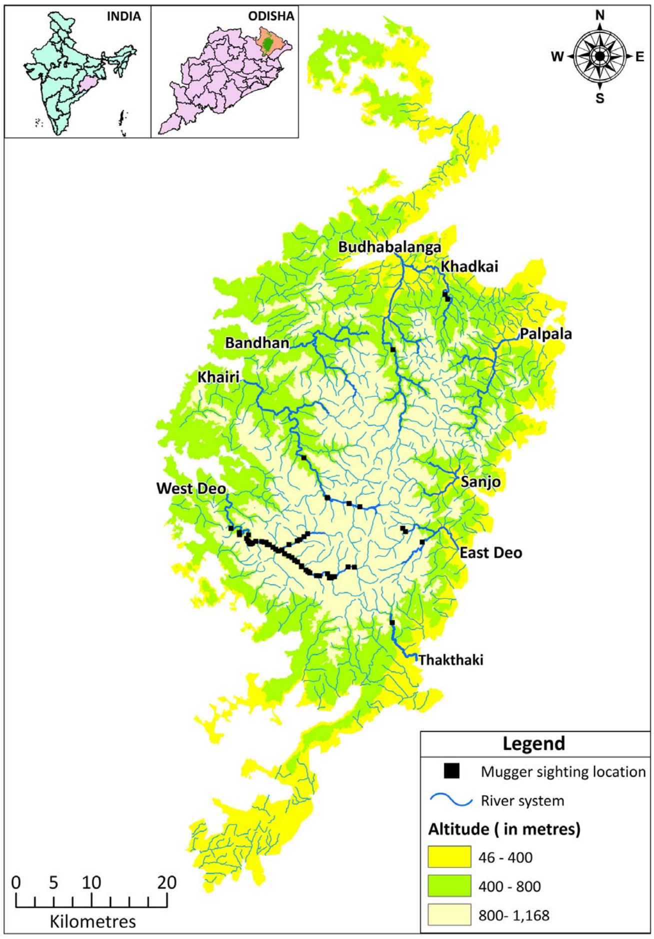

Figure 1. Map of Similipal Tiger Reserve along with the distribution of mugger crocodiles across different river systems and altitudes

(Sagar & Singh, 1993). In the past, the core of the STR had villages with fishing folk but now all but one village has been relocated outside the STR boundary, thereby significantly minimising fishing pressure. A mugger reintroduction programme took off in the STR's river systems after the establishment of the Ramatirtha Captive Breeding Center in 1980. A few individuals from the Madras Crocodile Bank, Tamil Nadu and Nandankanan Zoological Park, Odisha were also released. From 1981-2010, 812 muggers were released

Figure 2. An adult mugger and a hatchling (in the inset) basking near a pond at an altitude of 822 m a.s.l.

in the water bodies of the STR, which consist primarily of river systems and a few pools (Mishra et al., 2013). As per the unpublished records of the Odisha Forest Department, 55 %, 21 % and 16 % of these muggers were released in West Deo, Budhabalanga, Khairi river systems respectively. The remaining individuals were released in Khadkei and East Deo river systems.

We assessed the current population status and distribution of the mugger in the STR as a contribution to better conservation management of the species. We counted the basking individuals in winter on 9th - 10th January 2019, as at this time of the year the crocodiles spend many hours of the day basking on the banks or partially exposed rocks in the rivers resulting in reliable counts. The survey was conducted by the trained frontline force of the STR, which includes range officers, foresters, beat guards and protection assistants. The smallest administrative unit here is called the beat (approximately 12 km^2 under the charge of a beat guard). The survey was conducted at beat level and all the teams headed by a forester/beat guard covered the entire stretch of the river systems in their respective beats. Thus the cumulative effort of the frontline staff of the STR led to intensive sampling of a large geographical area in just two days. Boats were not used because the rivers here are shallow, narrow, and rocky, which makes them un-navigable, instead the teams walked along the banks of the rivers to record the number of muggers, the GPS location of each sighting, and the muggers' body size classes based on the approximate ocular length following the method of Santiapillai & de Silva (2001) in Sri Lanka.

In total, we recorded 82 individuals from all the rivers of the STR during the survey. Of these, 70 individuals (85 %) were recorded from the West Deo river system. In 2005, 85 muggers were counted of which 50 (59 %) were from the West Deo river and approximately 40 % were from the other four river systems (Sahu et al., 2007). A survey in 2011 detected 85 individuals in the rivers systems of the STR with 64 (75 %) coming from the West Deo River (Mishra et al., 2013). The total number of muggers recorded in our survey and those reported in previous studies remained fairly constant with the bulk of the observations coming from the West Deo river system. It is suggested that there are greater number of muggers in the West Deo river due to it being longer that the other rivers, with greater availability of fish than the others, the majority of the river lying in the core of the STR thereby greatly reducing the anthropogenic interference (Sahu et al., 2007), and because more than 50 % of the reintroduced stock was released in the West Deo river.

During our survey, 90 % of individuals observed were recorded above an altitude of 750 m a.s.l. with the lowest record being from 380 m a.s.l. (Fig. 1). In 2004, a dead sub-adult mugger was reported at an elevation of 800 m in the Khairi river system of the STR; this was the highest recorded altitude for a mugger in India (Sahu & Swain, 2004). In the current survey we observed an adult and three hatchlings basking near a pond (21° 38'59.44" N, 86° 18'1.45" E) in the vicinity of West Deo River at an altitude of 822 m a.s.l. (Fig. 2).

From 1981, the reintroduction programme for muggers in the STR, coupled with the relocation of much of the human population away from the reserve, has successfully resulted in the establishment of a sustainable population of muggers. Furthermore, this population occurs up to an altitude of 822 m making it the highest altitude habitat for this species in India. The STR population provides a unique opportunity for research on various facets of mugger ecology.

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Pit viper *Bothrops pauloensis*: Reproduction and comments on the colour and pattern of neonates

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INTRODUCTION

Bothrops pauloensis Amaral 1925, a species in the *Bothrops neuwiedi* group, is a medium sized terrestrial pit viper that ranges from the central, south-east and central-west regions of Brazil (Silva, 2004; Silva & Rodrigues, 2008) to Paraguay and eastern Bolivia (Nogueira et al., 2019). It is primarily nocturnal and inhabits mainly dry savannah (Cerrado) (Valdujo et al., 2002; Fiorillo et al., 2020) and open areas of the Atlantic Forest (Silva, 2004; Silva & Rodrigues, 2008) where it frequently uses burrows and cavities for shelter, thermoregulation and foraging (Valdujo et al., 2002; Fiorillo et al., 2020). It feeds mainly on small mammals and lizards but has been recorded consuming birds, snakes, amphibians and centipedes, with ontogenetic shifts according to diet (Martins et al., 2002; Valdujo et al., 2002).

Information about the reproductive cycle, litter size and relationship between female size and fecundity has been published (Valdujo et al., 2002). Here we present data on the length, mass and sexual dimorphism in neonate *B. pauloensis* and determine relative clutch mass (RCM) expressed as the total litter mass, including any atretic eggs, divided by the post parturition mass of the mother (after Shine, 1980). The RCM is used as an estimate of the reproductive investment by females, and may be related to the body condition of the female and/or environmental factors during pregnancy.

Two female *B. pauloensis* collected when pregnant were brought and kept in captivity in Museu Biológico, Instituto Butantan (IB). The snakes were housed individually in plastic boxes (650 mm wide; 400 mm in deep; 450 mm high), with a temperature gradient of 24-27 °C, fed regularly with mice (*Mus musculus*), and offered water ad libitum. The females and the neonates were measured with a millimeter ruler and weighed using a semi-analytical balance with a precision of 0.01 g (maximum capacity 3200 g). Statistical comparisons were made using t-tests.

The first female was collected in Uberlândia, MG (18˚ 55’08” S, 48º 16’37” W) on 20th May 2019. A few days before parturition, the female measured 815 mm snout-vent length (SVL), 115 mm tail length (TL). On 20th December 2019, it gave birth to 4 live young (Litter 1: 3 males, 1 female) and 4 atretic eggs. The mass of the female after parturition was 212 g, and the total litter mass was 48.11 g, so giving an RCM of 0.22. The second female (SVL - 716 mm, TL 85 mm) was collected in Araraquara, SP (21° 47’ 38” S, 48° 10’ 33” W) on 20th December 2019. On 27th December 2019, this female gave birth to 10 live young (Litter 2: 8 males, 2 females). The mass of the female after parturition was 140 g, and the total litter mass was 113.8, giving an RCM of 0.81.

Biometric information of the neonates was obtained on the day of birth and summaries of each litter, and the litters combined, are given in Table 1. In *B. pauloensis* the adults are

Table 1. Biometric details of *Bothrops pauloensis* neonates (litters 1 and 2)

Mother (SVL+TL)	Litter 1	Males (N=3)	Females (N=1)
	SVL (mm)	230 ± 8.6 (220 - 235)	225
815+115	TL (mm)	41 ± 1.7 (40 - 43)	32
	Mass (g)	8.2 ± 0.2 (8 - 8.4)	8.13
Mother (SVL+TL)	**Litter 2**	**Males (N=8)**	**Females (N=2)**
	SVL (mm)	264.3 ± 12.9 (245 - 285)	250 ± 21.2 (235 - 265)
719 + 85	TL (mm)	46.2 ± 4.8 (38 - 50)	37.5 ± 3.5 (35 - 40)
	Mass (g)	11.7 – 1.7 (8.9 ± 13.1)	9.9 ± 2.8 (7.9 – 11.9)
	Litter 1 & 2	**Males (N=11)**	**Females (N=3)**
Litters combined	SVL (mm)	255 ± 19.7 (220 - 285)	241.6 ± 20.8 (225 - 265)
	TL (mm)	44.8 ± 4.8 (38 - 50)	35.6 ± 4 (32 - 40)
	Mass (g)	10.7 ± 2.2 (8 - 13.1)	9.3 ± 2.2 (7.9 - 11.9)

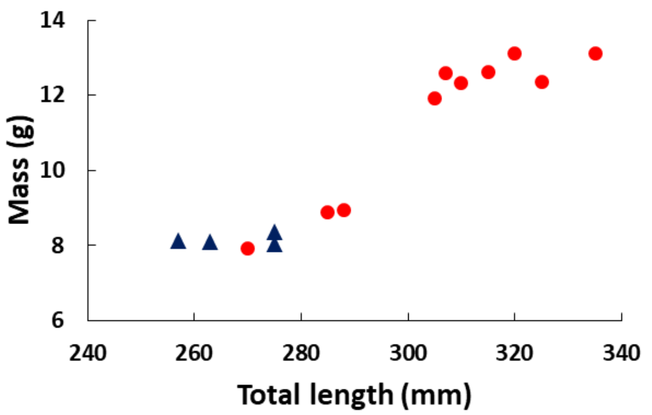

Figure 1. Relationship between total length (mm) and mass (g) in two litters (litters 1 and 2) of *Bothrops pauloensis*. Blue triangles – Litter 1; Red circles – Litter 2

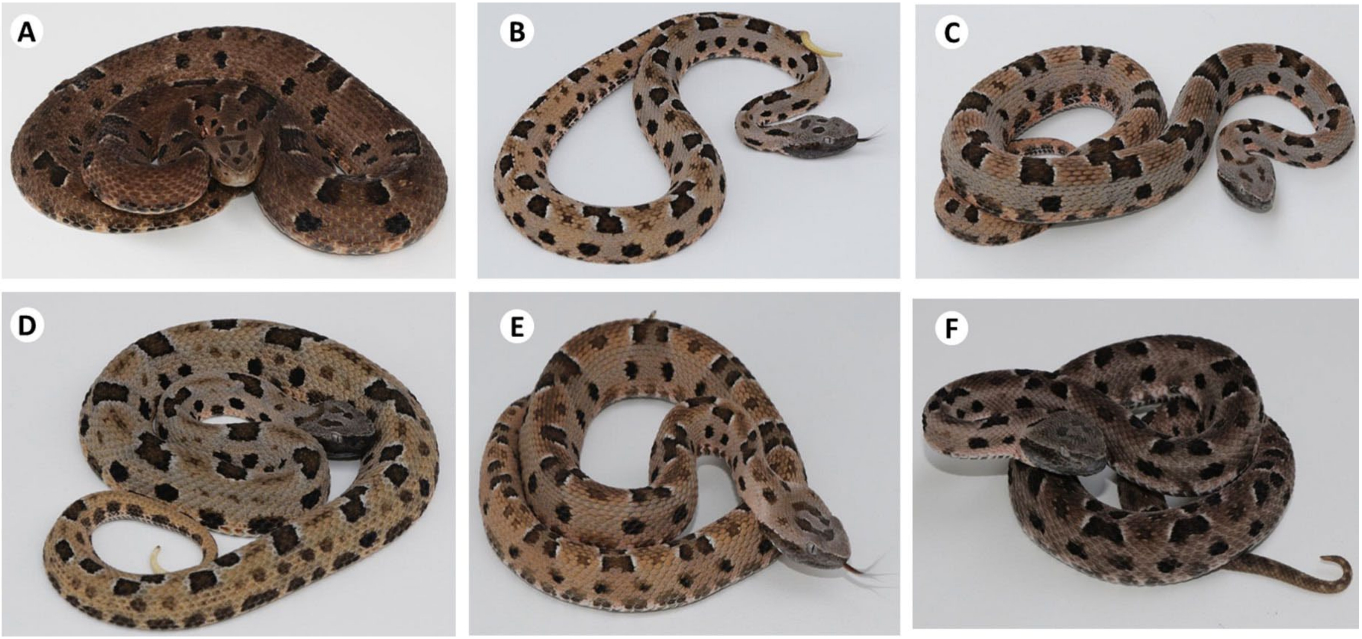

Figure 2. Different skin patterns and skin tones of neonate *Bothrops pauloensis* from a single litter (litter 2): **A.** mother; **B.** orange; **C.** greyish-orange; **D.** yellowish; **E.** light brown; **F.** dark brown

already known to be sexually dimorphic, with females being larger, heavier and with shorter tails than males (Valdujo et al., 2002). Our observations on neonates in the two litters combined, suggests that male and female neonates did not differ significantly in snout-vent length ($t_{0,05\ (2),12} = 1.034$; $p = 0.3213$) or mass ($t_{0,05\ (2),12} = 0.9770$; $p = 0.3479$), but were dimorphic in that males had longer tails than females ($t_{0,05\ (2),12} = 3.017$; $p = 0.0107$) (Table 1).

The RCM differed greatly between the two litters (0.22 litter 1; 0.81 litter 2), and the value obtained for the second litter (0.81) can be considered high for *B. pauloensis*, and other vipers. Despite coming from a larger litter, the young of litter 2 had significantly greater snout-vent lengths ($t_{0,05\ (2),12} = 4.178$; $p = 0.0013$) and were significantly heavier ($t_{0,05\ (2),12} = 3.171$; $p = 0.0080$) than those in litter 1 (Fig.1).

The number of neonates per litter is known to vary widely in *B. pauloensis*; 4 to 20 neonates per litter (N = 15 litters) (Valdujo et al., 2002). Our observations fit within this range as well as two unpublished records - a litter with 4 neonates and 1 atretic egg from a female collected in Itirapina, SP, that gave birth one day after arriving at the IB (S. Cardoso, pers. observation), and a litter with 6 neonates from a female collected in Alcinópolis, MS on January 2020 (Information Scales of Biodiversity Project, FAPESP: 2016/50127-5).

As for the RCM, other aspects of fecundity (number in a litter, size of the young, and reproductive frequency) in the same species of snake can vary, especially in relation to the size and mass of the females and their geographical origin which likely reflects climatic variation and food availability (Vitt, 1983; Seigel et al., 1986; Seigel & Ford, 1987; Jordão, 1996; Travaglia-Cardoso, 2011). In the case of long-term captive females, the effects of captivity on litter size cannot be ruled out.

For *B. pauloensis*, it is known that there is considerable individual variation in colour and skin pattern, even within the same population (Campbell & Lamar, 2004). In the litters analyzed, the neonates of litter 1 were similar. However, in litter 2, we identified 5 different patterns and colour tones - orange, greyish-orange, yellowish, light brown and dark brown (Fig.2 A-F). Female snakes may mate with more than one male in a reproductive season, producing litters with multiple paternity and consequently greater genetic variability (Duvall et al., 1992; Madsen et al., 1992). As a possible explanation for the differences in skin patterns and tones between neonates in litter 2 (and the absence of these differences in litter 1), we can suggest that the mother of litter 2 may have copulated with more than one male, generating a litter with greater variability. This can only be confirmed by genetic testing.

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The advertisement call of the Perak spadefoot toad *Megophrys aceras* from Langkawi Island, Kedah, Peninsular Malaysia

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Megophrys aceras Boulenger 1903 (Anura: Megophryidae), is distributed in Peninsular Thailand to the northern and the western part of Peninsular Malaysia, at an altitude of up to 1500 m (Chan-ard, 2003; Grismer et al., 2006; Thong-aree et al., 2011; Sumarli et al., 2015). Frogs of the genus *Megophrys* produce a loud advertisement call to attract females during the mating season. We follow Mahony et al. (2017) in the treatment of Megophryinae as a single genus, *Megophrys*, with a subgenus-level classification due to the lack of reliable morphological characters that can be used to define all previously proposed genera. There are nine *Megophrys* species in Malaysia (Frost, 2021), and call descriptions have been published for *Megophrys nasuta* (Malkmus et al., 2002), *M. kobayashii* (Malkmus et al., 2002), and *Megophrys baluensis* from Sabah (Malkmus & Riede, 1996; Malkmus et al., 2002).

The advertisement calls of frogs are species–specific (Pavan, 2008; Köhler et al., 2017) and it is well known that there is a strong relationship between anuran phylogenetic relatedness and the acoustic similarity of their advertisement calls (Blair, 1958; Gingras et al., 2013). Consequently, information on advertisement calls can help to identify cryptic species in the field (Inger et al., 2017) and is a valuable tool for taxonomy, systematics and biodiversity research. Furthermore, frogs from the genus of *Megophrys* contain an undiagnosed diversity of morphologically cryptic species and this information is essential especially in species delimitation. However, call descriptions for many species within this genus are still lacking. Hence this study describes the characteristics of the advertisement call of *M. aceras* from Langkawi Island, Kedah, Peninsular Malaysia.

Acoustic Encounter Survey (AES) was used to detect the presence of *M. aceras*. The advertisement calls of *M. aceras* were recorded as uncompressed/raw .wav files using a rechargeable portable digital voice recorder (8GB, OEM, Rechargeable Dictaphone MP3 player, AS331C_V1000) with attached mini microphone. The advertisement calls were recorded within 20 cm of the frog and analysed using Adobe Audition CC 11.0.0.199 software (Dehling, 2010). The figures of spectrograms and oscillograms were exported using Raven Lite 2.0 audio editing software.

The calling individual was photographed in-situ using a digital camera (Nikon COOLPIX L840) and then collected for further identification and measurement. This species can be identified by its truncated snout, distinct tympanum (three quarter diameter of the eye), a short dermal projection on the eyelids, a large triangular mark between the eyes, slightly swollen fingertips, and short limbs with dark crossbars (Smith, 1930). The frog was preserved as a voucher specimen (20USM/LA/MA03) in 70 % ethanol and deposited at School of Pharmaceutical Sciences, Universiti Sains Malaysia, for future reference. This frog was euthanised by injecting absolute ethanol straight into the frog's heart using a sterilised needle and syringe for immediate death. This procedure was approved by the USM institutional Animal Care and Use Committee, USM/IACUC/2019/(121)(1039).

Figure 1. Male *Megophrys aceras* (20USM/LA/MA03) from which acoustic recordings were made, in-situ at the calling site

The advertisement call of the male *M. aceras*, was recorded at 20:40 h on the 2nd November 2020 in Gunung Raya Forest Reserve (6.39098, 99.79596; 323 m a.s.l.), Langkawi Island, Kedah, Peninsular Malaysia. The air temperature and relative humidity of the sampling site were 28° C and 83 %, respectively (obtained from the AccuWeather application, android version). The specimen was measured using a digital Vernier calliper (Fisher scientific), and was weighed using an analytical balance (Ohaus). The morphometric measurements (after Watters et al., 2006) were tibia length (the length of the flexed knee's outer surface to the heel/tibiotarsal inflexion) - 26 mm; snout–vent length (the distance from the snout tip to posterior margin of the vent) - 55 mm; head width (the

Table 1. List of the call parameters measured in this study and observed values for *Megophrys aceras*

Parameter	Description	Parameter range (mean ± sd)
Call group	A single group of calls produced during a single expiration	2.24 – 20.94 s (11.13 ± 3.83, N = 51)
Call groups per minute	Number of the call groups per minute	2 – 4
Call group interval	Time interval between each call group	5.43 – 56.46 s (13.81 ± 7.83, N = 50)
Call number	Numbers of calls per call group	31 – 111 calls (72.74 ± 25.08, N = 51)
Call duration	The time between the beginning and end of a single call	74 – 94 ms (64.04 ± 4.63, N = 228)
Inter-call interval	The time interval between each call	73 – 112 ms (86.50 ± 7.12, N = 226)
Pulse number	Number of pulses contained in a single call	13 – 24 pulses (21.92 ± 24, N = 228)
Pulse duration	Interval time between each peak of each pulse	2 – 3 ms (2.27 ± 3, N = 228)
Dominant frequency	Dominant occurrence of call frequency	3.75 kHz

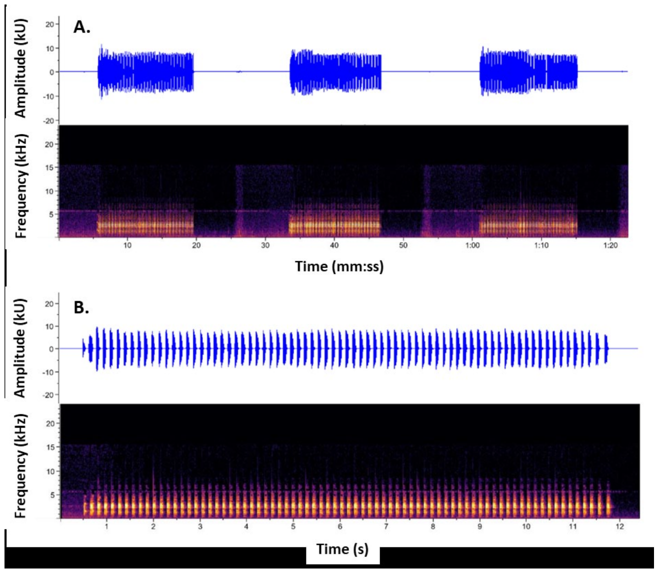

Figure 2. Oscillogram and spectrogram representation of a *Megophrys aceras* advertisement call - **A.** Three call groups, **B.** One call group

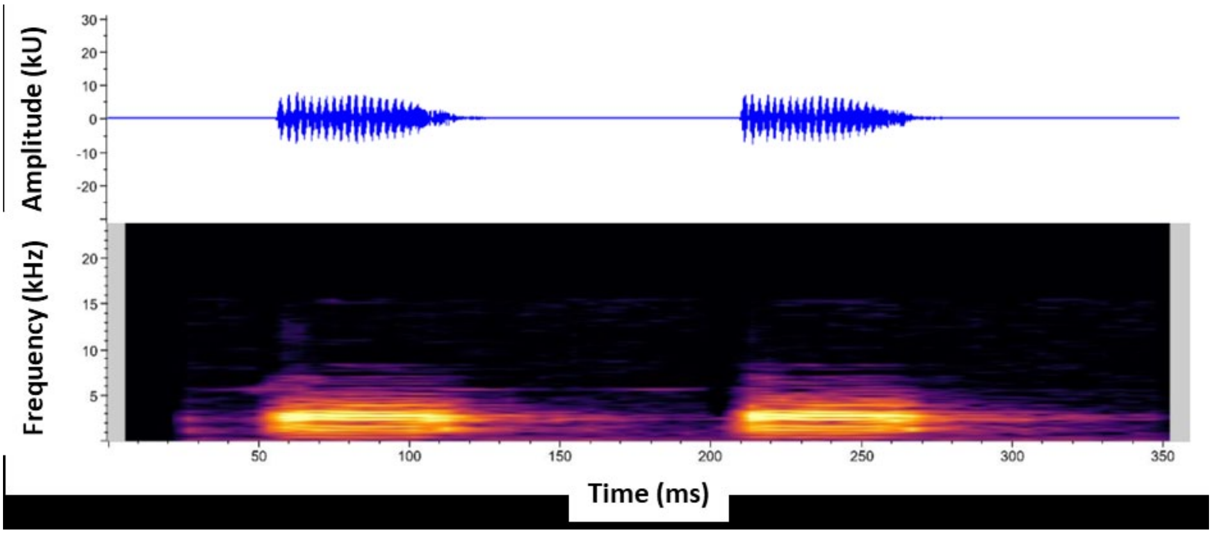

Figure 3. Oscillogram and spectrogram representation of a *Megophrys aceras* advertisement call (2 calls)

widest portion of the head or angle of the jaws) - 25 mm, and weight - 27 g.

The calling frog was recorded while sitting in the water of a sandy spring-water creek, beside the road at a forest edge (Fig. 1). The call parameters analysed are listed in Table 1 following Duellman (1970), Cocroft & Ryan (1995), Brown & Richards (2008), and Tapley et al. (2017). A total of 51 call groups were recorded and analysed, with 2 – 4 calls per minute. The call group of *M. aceras* has a repetitive "EARK" sound. The oscillogram and spectrogram of the advertisement call are illustrated in Figures 2 and 3. The values for the call parameters are shown in Table 1. This call has been deposited in FonoZoo (recording number F_SOUND_CODE 12963)

In South-east Asia and north-east India, there are 61 *Megophrys* species (Mahony et al., 2020; Frost, 2021). However, the advertisement calls of only sixteen species of five subgenera have been analysed and described from this region (Table 2). Whereas, only a call description has been reported for a frog in the subgenus *Xenophrys* (*M. oropedian*) (Mahony et al., 2013) (Table 2). A constant range limit of dominant frequency is notable in each subgenus. For example, frogs from the subgenus *Xenophrys*, have a dominant frequency limit of above 3 kHz. Frogs from the subgenus *Brachytarsophrys*, have the lowest dominant frequency, followed by *Pelobatrachus*, *Xenophrys*, *Panophrys*, and *Ophryophryne*. This order may reflect body size since the calls of larger frogs (e.g. *Brachytarsophrys* and *Pelobatrachus*) are expected to be at a lower dominant frequency. The sequence of these subgenera is aligned with the maximum likelihood tree by Mahony et al. (2017). Subgenus *Brachytarsophrys* diverges the earliest compared

Table 2. The advertisement calls of congeneric species of *Megophrys* from South-east Asia and north-east India

Subgenus	Species	Ambient temp. (˚C)	Dominant frequency (kHz)	Call duration (ms)	Inter-call interval (ms)	References
Xenophrys	*M. aceras*	28.0	3.75	64.04±4.63 (74–94)	84.50 ±7.12(73–112)	This study
Xenophrys	*M. oropedion*	18.0	3.10–3.25	44.6±7.33 (30–55)	-	Mahony et al. (2013)
Pelobatrachus	*M. baluensis*	17–19	2.8	90–100	550–700	Malkmus & Riede (1996); Malkmus et al. (2002)
Pelobatrachus	*M. nasuta*	Not reported	2.0–2.5	160–170	150–160	Malkmus et al. (2002)
Pelobatrachus	*M. kalimantan-ensis* Type 1	20.7	2.1±0.15 (1.8–2.3)	137.9±16.18 (116.2–211.5)	185.8±23.53 (141.8–233.8)	Munir et al. (2019)
	Type 2	20.7	2.04±0.1 (1.9–2.2)	536.9±195.08 (268.7–885.6)	434.7±75.93 (323.7–535.1)	
	M. kobayashii	21.0	-	163 (135–188)	150 (134–182)	Malkmus et al. 2002
Panophrys	*M. fansipanensis*	15.3–18.3	3.6–4.7	42.0 (34.0–49.0)	204.4 (180–290)	Tapley et al. (2018)
Panophrys	*M. hoanglienensis*	18.5	3.0 (2.8–3.0).	102.9 (96.0–108.0)	274.2 (178–565)	Tapley et al. (2018)
Panophrys	*M. jingdongensis*	18.5	2.4 (2.4–2.6)	132.7 (117.0–147.0)	113.0 (102.0–127.0)	Cutajar et al. (2020)
Panophrys	*M. minor*	14	3.5 (3.4–3.5)	90.8 (75.0–110.0)	253.0 (213.0–363.0)	Jiang et al. (2002)
Panophrys	*M. rubrimera*	21.0–22.9	3.2 (3.2–3.4)	74.2 (56–83)	207.0 (190–235)	Tapley et al. (2017)
Brachytarsophrys	*M. feae*	13.0	1.38	340.0–474.0	293–482	Wogan et al. (2004)
Brachytarsophrys	*M. intermedia*	21.4	0.56–0.82	99.0-240.0	267-611	Tran (2013)
Ophryophryne	*M. elfina*	11.3–17.5	4.03–4.92	73 ± 0.23 (25–112)	207 ± 2.06 (96–942)	Poyarkov et al. (2017)
Ophryophryne	*M. gerti*	11.3–17.5	4.45–5.10	104 ± 0.56 (75–152)	421.54 ± 4.17 (275–813)	Poyarkov et al. (2017)
Ophryophryne	*M. synoria*	11.3–17.5	3.60–3.89	62 ± 0.46 (37–85)	143 ± 3.32 (56–528)	Poyarkov et al. (2017)

to other subgenera (Mahony et al., 2017). Based on this comparison we suggest that the dominant frequency of calls between each subgenus to be a phenotypic character expressing phylogenetic time divergence. Further research on the calls of *Megophrys* species is required to determine whether call parameters can be used to delimited *Megophrys* subgenera.

As frogs are ectotherms, the temporal character of call duration and inter-call interval are dependent on temperature (Platz 1989; Gerhardt & Bee, 2006). Call duration increases with temperature (Gerhardt, 1978; Gerhardt & Huber, 2002) while inter-call interval decreases (Gerhardt, 1978; Gerhardt & Huber, 2002). Call duration and inter-call are distinct in some species within the same subgenus (Table 2) but this may be due to differences in ; ambient temperature at the time of recording (Licht, 1969; Pough et al., 1983; Cree, 1989; Navas, 1996). This may account for the higher call duration of *M. aceras* compared to *M. oropedion* (Table 2).

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NATURAL HISTORY NOTE

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First cases of predation of *Bufo spinosus* by two leech species in Algeria

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Leech predation of amphibians is a common phenomenon, but in North Africa is documented in just a few short reports (Beukema & de Pous, 2010; Ben Ahmed et al., 2014; Merabet et al., 2017). In the Maghreb, intensive leech predation in synergy with the continuing decline of amphibian habitats may ultimately lead to the decline of amphibian populations (Beukema & Philip de Pous, 2010; Samraoui et al., 2012). In fact, the proliferation of freshwater leeches may affect amphibian survival directly by predation or indirectly by contributing to the spread of pathogens and secondary parasitic organisms. This may especially be the case for vulnerable amphibians with highly fragmented populations such as the newt *Pleurodeles nebulosus* (Ahmed et al., 2014).

We report here predation of *Bufo spinosus* Daudin, 1803 in Algeria by two species of leech, *Hirudo troctina* Johnson, 1816 and *Batracobdella algira* (Moquin-Tandon, 1846). On the evening of 8th March 2019, we encountered several adult *B. spinosus* in amplexus near a large permanent pond known as Lac Noir situated at 1260 m asl (36° 41'48.5" N, 4° 36'08.1" E) in Akfadou forest in Kabylia (Fig.1). In one of the couples, the male had a single specimen of *B. algira* attached to his right flank and the female had one sanguinary leech *Hirudo troctina* at the insertion of the anterior right leg (Fig. 2). Leeches were photographed and then identified on the basis of external features.

In Tunisia, it is relatively common to find *B. algira* on amphibian hosts during the anuran-breeding season (Ben Ahmed et al., 2014) and in Algeria *B. algira* has been observed on *Pelophylax saharicus* (Billet, 1904). We observed an abundance of unattached *H. troctina* in the permanent pond which suggests a significant threat from this species. Together with other threats, the presence of *H. troctina* with vulnerable amphibians such as the *P. nebulosus* may result in significant population decline, as has already been reported for this predator/host combination in a permanent pond close to this region (Merabet et al., 2017).

Figure 1. Lac Noir in Akfadou forest (Kabylia), where observations on *Bufo spinosus* and leeches were made

Figure 2. Predation by *Batracobdella algira* and *Hirudo troctina* upon a pair of *Bufo spinosus* in amplexus (arrows indicate positions of leeches)

Although the current observations do not show whether or not leech predation is associated with mortality in the case of *B. spinosus*, further study of the impacts of leeches on amphibian populations in this pond would be of value especially in view of the lack of knowledge on the ecology and distribution of *B. spinosus* in Algeria (Mateo et al., 2013).

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Predation and ingestion of a barred grass snake *Natrix helvetica* by a rainbow trout *Oncorhynchus mykiss* in Lot, France

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Barred grass snakes (*Natrix helvetica*) are found throughout north-west Europe (Kindler & Fritz, 2017) and potentially grow to 1.5 m but rarely attain this size (Speybroeck et al., 2016). They are thought to feed primarily on amphibians and are adept swimmers although rarely seen hunting in open water (Gregory & Isaac, 2004).

The rainbow trout (*Oncorhynchus mykiss*) has been introduced to many European countries and is valued as a game fish and for its economic importance as food (Crawford & Muir, 2008). Despite the introduction, the species has rarely formed self-sustaining populations in the wild in France (Stanković et al., 2015). However, rainbow trout predation has been shown to reduce amphibian populations, e.g. *Rana muscosa* in the USA (Vredenburg, 2004) and *Rana temporaria* in Sweden (Nyström et al., 2001).

At around 16:00 h on the 18th July 2020, a rainbow trout, recently released for the purpose of recreational fishing, was caught by HO while angling along the Saint-Matré (a small stream) in Grézels, Lot, France (GPS: 44° 28’ 03.7” N, 1° 09’ 01.8” E). The fish was a mature specimen, roughly 30 cm long. The trout was gutted minutes after capture and surprisingly, a live hatchling barred grass snake was found inside the gastrointestinal tract (Fig 1). The hatchling snake measured approximately 20 cm long and appeared sluggish, but otherwise relatively unharmed. After a quick visual inspection, the snake was released onto a patch of grass where it remained still for approximately 20 minutes. Subsequently, it moved directly to a nearby pond and swam away.

It’s reasonable to assume that the hatchling snake must have been consumed a short time prior to the trout’s capture for it to have remained alive until discovery. It is also likely that the snake was swallowed whole whilst it was traversing the small river. The occurrence of fish within a grass snake diet is well documented (Gregory & Isaac, 2004); however, there are no scientific reports of fish predating *N. helvetica* or other *Natrix* spp. There are, however, online references to grass snakes forming part of the diet of Pike (*Esox lucius*).

Barred grass snakes are well known for their anti-predatory responses such as feigning death and musking (Hagman et al., 2015), however, these behaviours would be less effective in the aquatic environment. What evidence there is suggests that the consumption of *N. helvetica* by fish is rare and restricted to small specimens. The current observation suggests that it would be worthwhile investigating whether grass snakes close to commercial fisheries are at a significantly increased risk of predation.

Figure 1. Hatchling barred grass snake head and neck clearly seen coming out from the gastro-intestinal tract of the captured trout

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An unusually high number of Italian wall lizards *Podarcis siculus campestris* entering Great Britain as stowaways

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The Italian wall lizard *Podarcis siculus campestris* occurs in north and central Italy, on the eastern Adriatic Coast and in Corsica (Speybroeck et al., 2016). It is a medium sized lizard, with a total length of about 26 cm of which about 17 cm is accounted for by tail (Speybroeck et al., 2016). Two dorsal green stripes distinguish *P. s. campestris* (Fig. 1) from *Podarcis sicula sicula*. *Podarcis siculus campestris* is active throughout most of the year in the south of its range, which may be challenging in terms of food availability in the autumn and winter months.

Figure 1. A male and female *Podarcis siculus* campestris shortly after being rehomed

Over a period of almost five weeks starting in early October 2020, eight shipments of grapes originating from the Abruzzo region of central/southern Italy were found to contain a total of 29 stowaway *P. s. campestris* (Table 1). The shipments of grapes all came in by sea and the lizards were all discovered in distribution centres by workers. A large supermarket chain reported the interceptions to the National Centre for Reptile Welfare (NCRW), which arranged for all but one of the lizards to be rehomed. This isn't the first time that a shipment containing Italian wall lizards has been reported (Hodgkins et al., 2012), but this is the first report of a closely spaced series of interceptions.

Feeding experiments on the rehomed lizards have shown that they readily feed on grapes; they completely ignored offerings of mango, persimmon and banana. The relatively large numbers found over an extended period in the grape consignments suggests that they were gaining some nutritional benefit from hiding in the bunches of grapes. Mačát et al. (2015) reported *P. siculus* feeding on a range of fruit in Croatia at a time when invertebrate prey was still available. Given that the grapes were imported in October and November when invertebrate food is less abundant it seems plausible that the stowaway *P. s. campestris* were feeding on grapes before being accidentally packed with them.

Table 1. A record of when and where consignments of grapes containing *Podarcis siculus campestris* were intercepted in England, as well as the number of lizards in each shipment

Date	Location	Number of lizards
07/10/2020	Manchester	1
14/10/2020	Leeds	5
22/10/2020	Leeds	5
27/10/2020	Leeds	10
03/11/2020	Essex	1
03/11/2020	Leeds	4
04/11/2020	Gloucester	1
09/11/2020	Leeds	4

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NATURAL HISTORY NOTE

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Arboreal behaviour and reproductive biology of the northern banded coffee snake *Ninia pavimentata*

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The northern banded coffee snake *Ninia pavimentata* (Bocourt, 1883) is a small, semi-fossorial dipsadine snake (max. 421 mm) (Köhler, 2008). It can be identified by slate-grey and black bands, and a checkerboard ventral pattern (Fig. 1 A&B). It is differentiated from its close relative *Ninia maculata* by having a significantly higher segmental count, more subcaudals, and a different head shape (Smith & Campbell, 1996; Köhler, 2008; McCranie, 2011). *Ninia pavimentata* occurs in pine-oak and cloud forests of the Central Highlands of Guatemala and western Honduras between 1120 to 1825 m a.s.l. (Campbell & Smith, 1996; Townsend et al., 2005; Townsend et al., 2008). This nocturnal and primarily terrestrial species is thought, like other *Ninia* spp., to feed on earthworms and other soft-bodied invertebrates (Green, 1975; Savage, 2002; Köhler, 2008). However, very little has been reported about the ecology or reproductive behaviour of this species. Herein, we report on natural history observations of *N. pavimentata* from Alta Verapaz, Guatemala.

On 19th April 2017 at 19:00 h, we encountered an adult female *Ninia pavimentata* during a herpetofaunal survey of a cloud forest canyon near the town of Santa Cruz Verapaz, Alta Verapaz, Guatemala (15˚ 21'36'' N, 090˚ 20'47.9'' W, WGS 84). The snake measured 239 mm snout-vent length and 96 mm tail length (total 335 mm) and was moving through the leaf litter on the forest floor. The air temperature was 18.5˚ C and relative humidity 95.4 %. During measuring the snake, we noticed that the body close to the vent was highly swollen (Fig. 1B). Upon gentle palpation we recognised four well-formed eggs close to parturition. In the interests of conservation and good field practice we returned the snake to its habitat without further molestation. Several other species of Ninia have been documented to lay between 1 and 5 eggs (Greene, 1975; Savage, 2002; Angarita-Sierra & López-Hurtado, 2020). Our observation confirms that *N. pavimentata* may align with such clutch sizes of other *Ninia* spp.

On 2nd July 2018 at 21:33 h, during a survey of the same cloud forest canyon we observed climbing behaviour in *Ninia pavimentata*. Upon sighting, the individual was 100 cm above ground ascending a stem 0.5 cm in diameter, the individual continued to climb to a height of ca. 200 cm before stopping (Fig. 2). A further display of arboreal climbing behaviour was recorded on 9th August 2018 at 21:07 h, in the same canyon 180 cm above the ground on a stem. A final observation was recorded on 18th August 2018 at 21:34 h during a survey of a nearby walnut plantation perched on a branch, diameter 1 cm, at a height of 100 cm.

Figure 1. Female *Ninia pavimentata*, Alta Verapaz 2017 – **A.** In-situ photo on left, **B.** Ventral view showing the proximity of egg bulge to cloaca

Figure 2. Climbing behaviour in *Ninia pavimentata*. This individual was observed to climb from 1 to 2 m above the ground.

The multiple displays of *Ninia pavimentata* climbing above a height of 100 cm, within different environments, suggests this behaviour occurs more commonly than realised, at least at this location. *Ninia* have been highlighted as an important part of leaf-litter herpetofauna (Köhler, 2008) with *N. pavimentata* repeatedly described as semi-fossorial (Smith & Campbell, 1996; Townsend et al., 2005). So, our observations of arboreal climbing between 100-200 cm appears significant for a snake of this genus. Other species of terrestrial/fossorial snake have also been known to exhibit climbing behaviour (Keller & Heske, 2000; Brown et al., 2018; Viteri & Arrivillaga, 2019). Viteri & Arrivillaga (2019) postulate that foraging is a possible explanation for such arboreal venturing by another Guatemalan dipsadine snake - *Adelphicos veraepacis*. Both *A. veraepacis* and *N. pavimentata* are potential invertebrate predators so this may be a plausible explanation, although would need to be confirmed by further behavioural observations in the field. To the best of our knowledge these are the first observations of both reproduction and arboreal climbing behaviour for *N. pavimentata*.

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NATURAL HISTORY NOTE

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Very long northern vipers *Vipera berus* from Norway

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Vipera berus (Linnaeus, 1758) is a widely distributed species covering nearly the entire Palaearctic realm. It is both the most widely distributed terrestrial snake and the most northerly with populations living north of the Arctic Circle in Sweden (Andersson, 2003). The species is sexually dimorphic in both coloration and size; males generally attain total lengths of 50-55 cm whereas females average 60-65 cm (Otte et al., 2020). However, the longest males may measure about 70 cm and the longest females about 90 cm. Very large individuals are more often observed in northern Europe than in other parts of the distribution (Nilson et al., 2005). Herein, we report two Norwegian vipers, one female and one male, that exceed these lengths.

On 20th April 2007, PS found a road-killed female viper on the roadside on the west side of lake Årungen (59˚ 41'38" N, 10˚ 44'9" E, altitude 37 m) near the town Ås, county (fylke) of Viken, 25 km south of Oslo. The habitat consisted of mixed forest and arable land. He kept it in a freezer for 13 years. In 2020 it was given to KHO who measured, weighed and photographed it before it was stored in alcohol. It had a total length of 93.5 cm, of which 10.2 cm was tail, and weighed 158 g. It was melanistic without any sign of a zigzag pattern (Fig. 1). Melanistic individuals (with or without visible trace of zigzag pattern) are regularly observed in this area. The specimen was deposited at the Norwegian University of Science and Technology, Trondheim, voucher number NTNU-VM-HE-2657 (Hårsaker & Dolmen, 2020).

Figure 1. The dead melanistic female *Vipera berus*, found 25 km south of Oslo, total length 93.5 cm

On a field trip on 13th March 2020 to Jeløy island (59˚ 25'11" N, 10˚ 35'21" E, altitude 10 m), county (fylke) of Viken, 50 km south of Oslo, a very long male adder was captured by KHO just 40 m from the sea (Fig. 2). It had a total length of 78.5 cm, of which 10.6 cm was tail, and weighed 206 g. The ground colour was greyish-brown with a normal dorsal black zigzag. The habitat was volcanic rock with scattered shrubs and other sparse vegetation. This male had been observed regularly for several years (G. Hermansen pers. comm., 2020) and was found at its usual location.

Figure 2. The live male *Vipera berus*, found 50 km south of Oslo, total length 78.5 cm

Long females have been mentioned by both Nilson et al. (2005) and Otte et al. (2020), one from Thuringia in Germany with a total length of 87.5 cm and one of unknown origin measuring 89 cm. Citing R. Kreüger with reference to the journal Ornis Fennica from 1930, Vainio (1931) mentioned a 94 cm long female that was captured in the summer 1929 in the Finnish part of Sápmi at latitude 68° N. However, nothing further was apparently ever published in Ornis Fennica or elsewhere. Smith (1919) reported that in August 1914 an individual of 104 cm was found in Härjedalen, Central Sweden. But no documentation exists for these very long northern vipers. Moreover, we have been informed that *V. berus* in populations east of Kiruna 150 km north of the Arctic Circle in northern Sweden may grow to a very large size, a result of their great longevities (Andersson & Madsen pers. comm. 2020-2021). The longest female measured by

Andersson (pers. comm., 2021) in that northern area had a total length of 86 cm, of which 8 cm was tail, and the longest male was a total of 79 cm, of which 10 cm was tail. The national record for Norway has until now been 86 cm total length (Engdal, 1978). Record sizes of males have seldom been published although Vainio (1931) records a male from Finland of total length 72.5 cm.

A number of females longer than our new record of 93.5 cm have been reported, however our Norwegian female probably constitutes the longest vouchered or otherwise properly documented specimen to date. Nevertheless, females longer than 93.5 cm may potentially be encountered in northern environments in the future. We believe that our Norwegian male of 78.5 cm total length is the second longest male *V. berus* recorded, only exceeded by the above-mentioned male from the Kiruna area.

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NATURAL HISTORY NOTE

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A series of unlikely events: from washing machine to new species

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On 13th December 2015, a guttural toad (*Sclerophrys gutturalis*, Fig. 1) entered our care after a series of unlikely events. The toad reached the Cambridge area after hitching a ride back to Great Britain from Mauritius as a stowaway in the bags of a couple of elderly holidaymakers. Native to most of sub-Saharan Africa (Channing, 2001), this toad species does not regularly enter Britain in this manner. In Mauritius, where there are no native amphibians, *S. gutturalis was* introduced in 1922 as a biocontrol agent for the cane beetle *Phyllophaga smithi* (Cheke & Hume, 2008).

Figure 1. The female guttural toad *Sclerophrys gutturalis* shortly before parasitic worms were discovered in its faecal pellets

The holidaymakers were unaware of the toad's presence until they washed the contents of their suitcase and removed them from the washing machine, at which point the female *S. gutturalis* was discovered. The toad was quickly packaged in a temporary quarantine facility (a plastic sweet container with damp paper towels as a substrate) following guidance from JWW, before MJG collected the toad from the confused couple. During the time that the toad was in our care, it shed a number of parasitic worms which were later confirmed to be a new species (Smales et al., 2020). The toad was identified as a female as it did not respond to call playbacks of its own species, trialled on multiple occasions. The toad also failed to call during its time in captivity which also supports this earlier assumption.

Sclerophrys gutturalis is an extremely adaptable species. This has facilitated its range expansion since it can inhabit niches that are intolerable to other amphibian species and can out-compete other species for resources. The species may also adapt its breeding behaviour to better suit the climate in which it finds itself, helping to maximise reproductive output (Vimercati et al., 2019). This may lead to alarming declines of native amphibians (Measey et al., 2017).

Whilst in our care during the quarantine period, the toad was housed in a 12 litre terrarium (Exo Terra Faunarium) filled to approximately 25 mm depth with hydrated coconut fibre substrate. A further 15 mm of Sphagnum moss was added to this to provide better cover for the toad and also to help maintain the humidity. A small Tupperware container, 15 x 22 cm in size, was used to create a water bath at one end of the terrarium. Brown crickets (*Gryllus assimilis*) made up the majority of the diet of the toad during this period, although the diet was supplemented with other invertebrates when available. At the time of rehoming, the *S. gutturalis* was the only species of amphibian in our care.

Over the course of the initial 21 days, the toad was closely monitored to ensure it was feeding and was not suffering any ill effects of its journey. During this time, the new habitat was kept moist (sprayed every 24 h), abundantly supplied with food, and with constant access to Asda Still Natural Mineral Water. On the 15th December 2015, several parasitic worms were found drowned in the water bath within the remains of a faecal pellet. These were carefully extracted and preserved in 70 % ethanol before the water was changed. Over the course of the following two weeks, the toad shed more parasitic worms (all of which were found in the water bath deceased). Again, these were collected to allow for formal identification. To the untrained eye, they resembled tiny beansprouts with no discernible head end. In total, 27 whole or partial parasitic worms were shed. These were later identified as a new species of the family Echinorhynchidae and named *Pseudoacanthocephalus goodmani*, after MJG (Smales et al., 2020). This species of this family are known to live in the intestines of fish, amphibians and reptiles.

The toad was later rehomed by JWW and maintained in isolation from February 2016. Throughout that time, it failed to shed any more parasitic worms which may indicate that the flushes in late 2015 removed all these parasites. The *S. gutturalis* was not tested for any pathogens and as a consequence no other captive amphibians were brought into contact with the terrarium or its contents. Despite our initial fears over potential stunted growth due to a high parasite load, the activity patterns and appetite of the toad appeared to be normal. The toad continued to grow to a near-mature size but unfortunately died in early 2020. The fact that the toad didn't grow to the full mature size was attributed to the recently documented island dwarfism displayed by populations introduced into Mauritius and Réunion (Baxter-Gilbert et al., 2020).

Should the toad have been released in northern Europe, it is unlikely that it could have easily become established in the wild. We suggest that the stress of being washed in the washing machine (along with the chemical detergents) resulted in the parasites being shed. If the *P. goodmani* were not deceased at the point of expulsion (or shortly after) and the toad had escaped into the wild then it is at least possible that these could have been transmitted to native species. Nothing is currently known of the lifecycle or potential intermediate hosts of *P. goodmani*.

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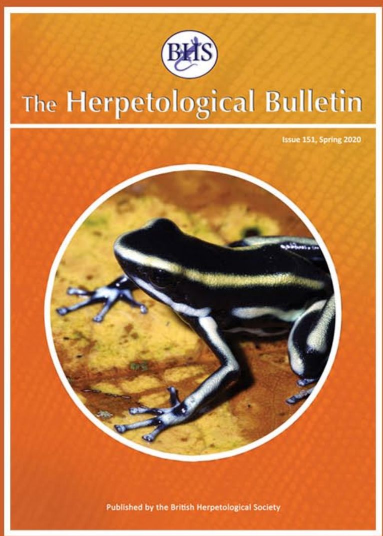
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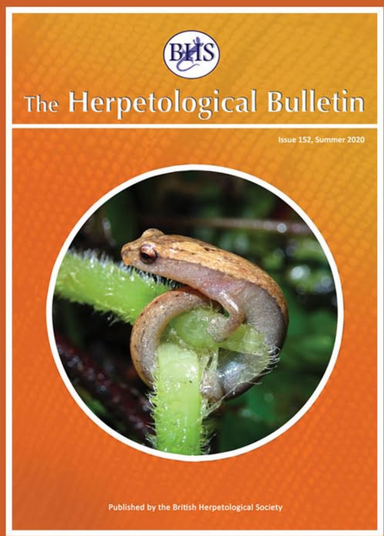
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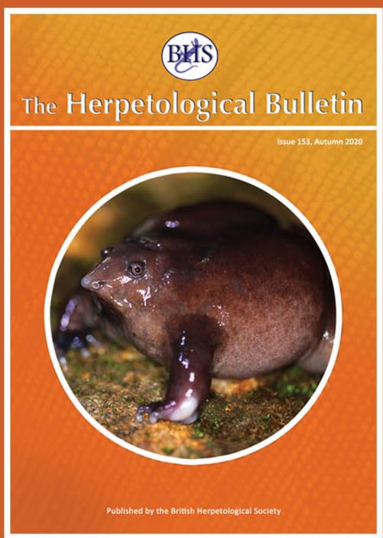
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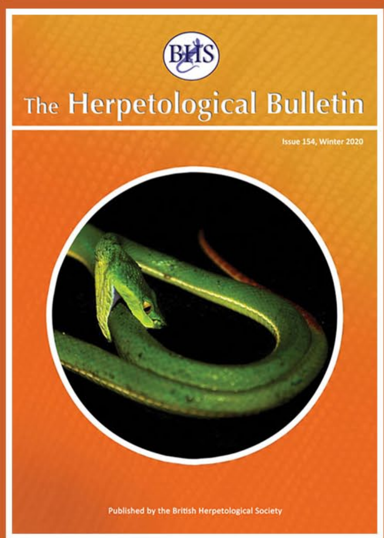
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