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Front Cover: Adult female *Emys orbicularis* found in the Palude de San Genuaio Nature Reserve during 2016.
See article on page 36. Photographed by Riccardo Cavalcante.

Natural history observations on the endangered turtle *Geoemyda spengleri* in Tay Yen Tu Nature Reserve (Vietnam), with notes on other sympatric species

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ABSTRACT - Vietnam is one the most biodiverse countries of the world in terms of turtle species richness, with 27 species being freshwater and terrestrial. The natural history of these species is very little known, but it is thought that the great majority of them are seriously threatened by habitat loss and overhunting. Here, we report on a survey employing both interviews with local people and field transects through the forest in the Tay Yen Tu Nature Reserve, on natural history information on *Geoemyda spengleri* and on other sympatric species. Using pictures of 14 different species of chelonian, at least 10 % of the interviewees recognised seven species. Of these *G. spengleri* and *Cuora mouhotii* were by far the most frequently recognised by interviewees, and were also the most frequent in a sample of 40 turtle individuals that were observed on local trade. Both species were also the only two species that were observed during field transects. Individuals of *G. spengleri* were observed from 10:47-13:45 h, in all cases in sunny weather after rain, at 25-30 °C temperature range. All individuals were seen in montane bamboo forests, often inside or nearby rocky caves, and in sites with forest cover being 70 %-90 %, at slope angles varying from 5-45 °.

INTRODUCTION

The Socialist Republic of Vietnam is ranked 9th among the most biodiverse countries of the world in terms of turtle species richness with a total of 32 species, 27 being freshwater and terrestrial and five being marine (Turtle Taxonomy Working Group et al., 2017). Unfortunately, the conservation status of the Vietnamese turtle fauna is uncertain as 85.1 % of the native freshwater and terrestrial taxa are currently listed as Threatened in the IUCN Red List (IUCN, 2018). For most species, habitat loss and overhunting (for consumption as human food, traditional medicine, religious merit) have been the main threats (Stanford et al., 2018). Indeed, the forest cover in Vietnam has decreased from 43 % to 36 % from 1945-1990 (De Jong & Hung, 2006). Although the country's forest cover has been recently increased due to the national-scale reforestation of Vietnam since 1992 (Meyfroidt & Lambin, 2009), the forest quality still remains poor. Indeed, the recovery of forest coverage was due in part to the ban of industrial logging in natural areas and the displacement of wood extraction to neighboring countries, but also to extensive programs of homogenous tree plantations (Meyfroidt & Lambin, 2009). The latter are clearly unsuitable for turtles, whereas the suitable remnant natural forest is still heavily fragmented and isolated (Fox et al., 2004). The second main threat to Vietnamese turtles is trade for domestic consumption, with massive numbers of individuals being exploited in the 1980s-2000s (Van Dijk et al., 2000; Nijman, 2010) also to supply the food and traditional medicine markets of China (Cheung & Dudgeon, 2006; Turtle Conservation Fund, 2002). According to IUCN (2018) assessments, the populations of most Vietnamese turtle species have declined by from 50-90 %. The Black-breasted leaf turtle (*Geoemyda spengleri*), that is the main

target of this paper, is not an exception and we also present some comments on the other sympatric turtle species.

Geoemyda spengleri (Fig. 1) is among the smallest *Geoemydidae* species worldwide. It is native to southern China, Northern Vietnam and Laos (Yasukawa & Ota, 2010; Turtle Taxonomy Working Group et al., 2017), and is classified as endangered by IUCN (2018) due to habitat loss and trade (Gong et al., 2005, 2009). However, the population size and distribution are poorly known; for instance, *G. spengleri* has recently also been recorded in Laos (Stuart et al., 2011) but there have been as yet no ecological studies (Yasukawa & Ota, 2010) with behavioural data being recorded only in ex-situ research (Henze et al., 2004). In Vietnam, the presence of *G. spengleri* has been documented in Tam Dao National Park, (Bourret, 1934; Nguyen & Ho 1996; Yasukawa et al., 2001), Tuyen Quang, Son La, Yen Bai, Vinh Phuc, Bac Giang, Quang Ninh, Thanh Hoa, Nghe An, and Ha Tinh provinces (Bourret, 1934; Le, 2001; Nguyen et al., 2010). The southernmost boundary of its distribution range lies in Da Nang and Quang Nam (Le, 2000; Le & Nguyen, 2003). However, the coastal city of Da Nang may serve as a collection center rather than a field locality (Yasukawa & Ota, 2010). Few conservation actions have been implemented to manage the remnant free-ranging populations of this species. Our aim in this paper is to present natural history data on *G. spengleri* in a protected area of Vietnam (Tay Yen Tu Nature Reserve) to give a better understanding of the field ecology and conservation of this threatened species and also to present additional notes on the turtle fauna of the study area.

Study area – We conducted the field survey in the Tay Yen Tu Nature Reserve (coordinates: 21°10'N and 106°43'E; Fig. 2). In this nature reserve (NR), a remnant portion of the natural habitat of the study species still



Figure 1. Black-breasted leaf turtle (*G. spengleri*) in the wild (male top image, female bottom image)

exists (Fig. 3) (Forest Protection Department of Bac Giang Province, 2010). Established in 2002 with a total area of 13,002 ha, the Tay Yen Tu NR consists of two parts: Tay Yen Tu and Khe Ro. The NR is the largest remnant natural forest patch in the Quang Ninh and Bac Giang provinces, and is mainly located in Luc Nam and Son Dong Districts, Bac Giang Province. In the Tay Yen Tu section, the mountain peak is 1068 m a.s.l. (Mount Yen Tu) while in the Khe Ro section the mountain peak is 886 m (Khe Ro; Forest Protection Department of Bac Giang Province, 2010). The main forest type of Tay Yen Tu is the lowland evergreen broad-leaved tropical forest (Averyanov et al., 2003). The NR is known to be home of 728 plant and 285 animal species (Dong, 2016; Forest Protection Department of Bac Giang Province, 2010), including 76 species of amphibians and reptiles (Hecht et al., 2013). There are five types of forest cover in the NR. Shrub and grassland are dominated below 100 m a.s.l., followed by small trees mixed with bamboo at the elevation of 100-200 m. From 200-900 m the forest is evergreen broad-leaf tropical forest mixed with bamboo forest. Above 900 m is the broad-leaved forest with large trees dominant (Forest Protection Department of Bac Giang Province, 2010).

METHODS

We decided to carry out field surveys for *G. spengleri* at the study area after having verified, in the years 2017-2018, that many individuals were offered for sale on the internet (Facebook, etc.) and were reported to come from Tay Yen Tu NR by the interviewed traders. Thus, the research consisted

of both interviews with selected people and field surveys in natural habitats that were potentially inhabited by the study species.

Interviews – We conducted semi-structured interviews, with identification photos of *G. spengleri* (from hatchling to adult) and 13 other potentially sympatric chelonian species (*Platysternon megacephalum*, *Mauremys sinensis*, *Mauremys mutica*, *Cuora galbinifrons*, *C. cyclornata*, *C. mouhotii*, *C. zhoui*, *Indotestudo elongata*, *Sacalia quadriocellata*, *Palea steindachneri*, *Pelodiscus sinensis*, *Rafetus swinhoei* and *Pelochelys cantorii*) with 72 people in 11 communes in June and July, 2018. The dominant interviewees were traditional medicine collectors and hunters, who were presumed to be familiar with the species (Online Appendix 1). There were 11 interview days from 17th-27th June 2018 and 28th July 2018. We also interviewed 38 randomly selected people (neither traders, traditional medicine practitioners nor hunters by profession). We used the following nine questions to each interviewee:

- (i) Do you recognise any turtle from your region?
- (ii) If yes, what is their local name?
- (iii) What do they look like (carapace colour, hardshell or softshell, size of the animal, aquatic or terrestrial)?
- (iv) What do they eat?
- (v) When did you see it?
- (vi) How many people buy/sell turtles or what do they do with the turtles obtained?
- (vii) Which morphological characteristics do you use to determine the different species (hinged (for *Cuora* spp.), serration, size of the head, length of the tail (for *Platysternon megacephalum*)?)
- (viii) What is the habitat in which the turtle is found?

After completing the interview, to confirm their description, we showed them the ID photos of turtle species. Each interview lasted from 5-30 minutes. We then recorded the location of the interview sites using a GPS device (see Fig. 2 for the map of the study sites).

During the interviews, we also recorded captive turtles held by villagers. For each individual, we asked how do they intend to use the animal, for how much they sell/buy it, and we measured carapace length, carapace width and shell height by a caliper (30 mm), and weight by an electronic kitchen scale, with the accuracy of 0.1 g.

Field research – We conducted the first section of the field survey in Dong Ri commune, Son Dong district from 19th-22nd July 2018 with 5 people and two hunting dogs and the second section in a forest in Tuan Mau commune, Son Dong district from 23rd-27th July 2018 with 6 people and 3 hunting dogs. Following Dawson's (2016) data, combining with local experience, we selected only forest patches situated at above 400 m elevation as field survey sites.

To locate wild turtles, we used local people (researchers/assistants), and two or three hunting dogs in eight days, all of them being employed to follow non-linear transects crossing through the potential turtle habitats (see Fig. 2 for the spatial arrangement of the non-linear transects). The beginning and ending time of each survey, and the GPS points were recorded at the beginning and at the end of the transect. Each person followed the transect by keeping a nearly constant five metres distance between each surveyor. Hunting dogs were used to improve the searching effort.

We also recorded for each individual (i) the GPS

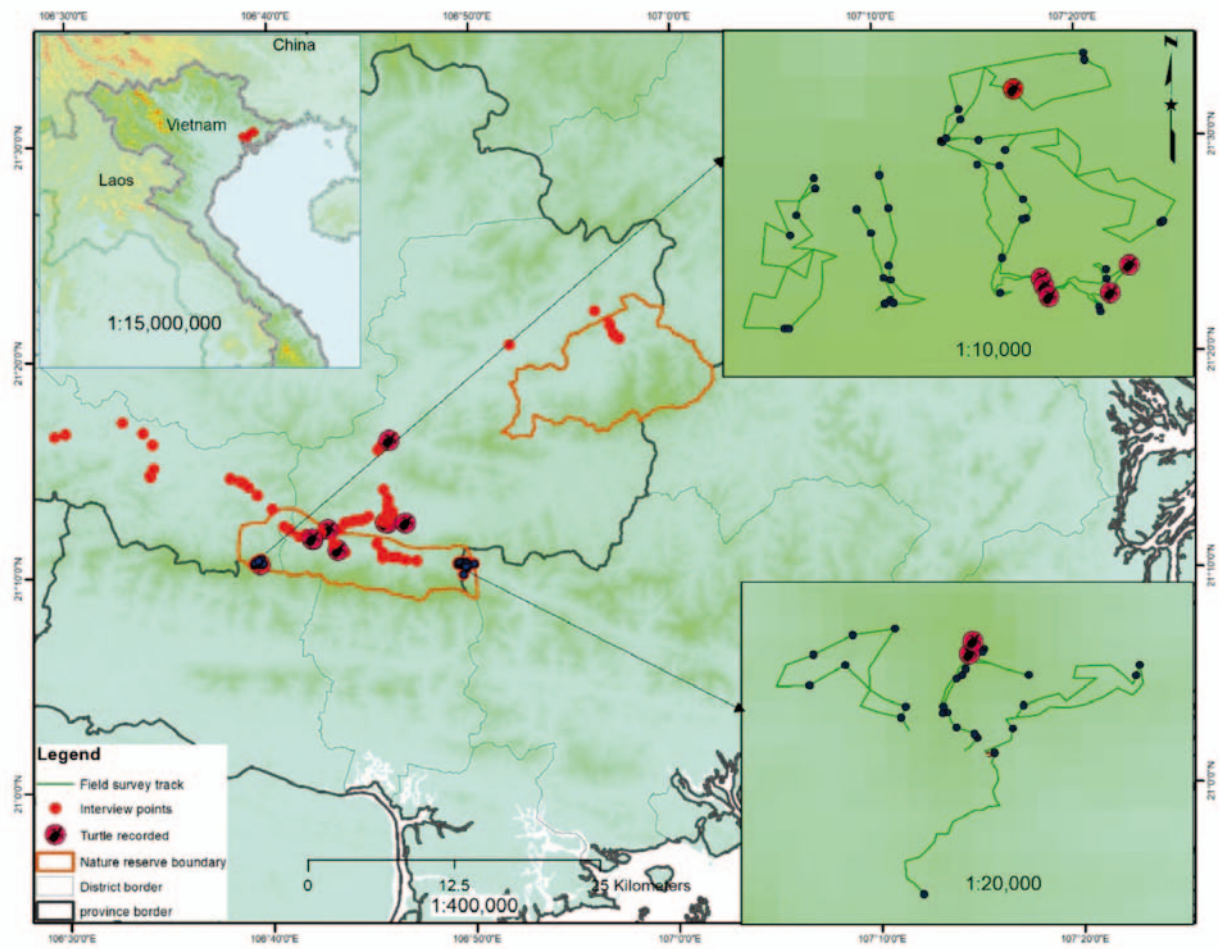


Figure 2. Map of Vietnam, showing the position of the interview points and of the presence sites of turtles at the Tay Yen Tu Nature Reserve. The field survey tracks are also indicated.

Table 1. Synopsis of the results of the interview surveys at Tay Yen Tu Nature Reserve, by species and by type of usage as declared by the hunters/traders, including the turtle price. No. Individuals = Number of turtle individuals observed during the interview surveys; Pet = number of individuals used as pet; For sale = number of individuals that will be sold in China; Food = number of individuals used as food; Medicine = number of individuals used for traditional medicine; No. People confirming presence = number of interviewees (and their relative percentage, %; total $n = 72$) claiming the presence of the various turtle species at the study area. The price of *C. cyclornata* and *C. zhoui* claimed to be 1990s, the rest are current price.

| Species | No. Individuals | Pet | Sell to China | Food | Medicine | No. People Confirming Presence | % | Price Range (\$) |
|----------------------------------|-----------------|-----|---------------|------|----------|--------------------------------|------|------------------|
| <i>Geoemyda spengleri</i> | 10 | 3 | 7 | 0 | 0 | 30 | 41.7 | 4.55-9.09 |
| <i>Cuora mouhotii</i> | 14 | 2 | 12 | 0 | 0 | 32 | 44.4 | 27.27-40.92 |
| <i>Cuora cyclornata</i> | 1 | 0 | 0 | 1 | 0 | 7 | 9.7 | 3076.90- 3846.12 |
| <i>Cuora galbinifrons</i> | 7 | 0 | 1 | 1 | 5 | 18 | 25.0 | 68.13-150.00 |
| <i>Indotestudo elongata</i> | 1 | 0 | 1 | 0 | 0 | 1 | 1.4 | unknown |
| <i>Palea steindachneri</i> | 5 | 0 | 2 | 3 | 0 | 20 | 27.8 | 29.50-31.82 |
| <i>Pelodiscus sinensis</i> | 2 | 0 | 0 | 2 | 0 | 15 | 20.8 | 22.72-27.27 |
| <i>Platysternon megacephalum</i> | 0 | 0 | 0 | 0 | 0 | 10 | 13.9 | 181.81-227.22 |
| <i>Sacalia quadriocellata</i> | 0 | 0 | 0 | 0 | 0 | 11 | 15.3 | 90.90-136.31 |
| <i>Mauremys mutica</i> | 0 | 0 | 0 | 0 | 0 | 5 | 6.9 | unknown |
| <i>Cuora zhoui</i> | 0 | 0 | 0 | 0 | 0 | 2 | 2.8 | 307.61-1538.42 |
| <i>Cyclemys oldhamii</i> | 0 | 0 | 0 | 0 | 0 | 2 | 2.8 | 90.91 |
| Total | 40 | 5 | 23 | 7 | 5 | | | |

coordinates, (ii) the time (Hanoi Standard Time), (iii) the weather, (iv) the ground temperature (°C) the habitat type, (v) the elevation (m a.s.l.), (vi) slope, and (vii) forest cover. We also determined its (a) sex and (b) age class. We determined sex by secondary sexual characteristics (tail morphology and head patterns; Yasukawa & Ota, 2010; see Fig. 1), and classified the age classes as follows: carapace length ≥ 80 mm = adult, 60-80 mm = subadult, 40-50 mm = juvenile, < 40 mm = hatchling. When a turtle individual was found, we recorded (i) habitat type, (ii) slope angle, (iii) forest cover, (iv) ground temperature (°C). Slope angle was determined by the Angle Meter version 1.0 software in an Android 8.0 system smartphone. Forest cover was estimated by Canopy Cover 1.03 version software, also in an Android 8.0 system smartphone. The ground temperature was measured by a thermometer, by placing the tip of the thermometer on the surface in the exact position of the turtle.

The dogs are commonly used by local villagers, especially minority ethnics to hunt for the wildlife in protected areas in Vietnam and trained to bark when they find a turtle (Espenshade & Le, 2002). However, we could not include them in our calculations of survey efforts as they run to search without control from the owner. A mean of 45.67 hours survey effort was made per person, with the searching team including 5-6 people and 2-3 dogs on each day. Thus, excluding dogs and considering the searches by each person as independent along the transects, the total field effort was 251.2 h. Field surveys were conducted in two areas of Tay Yen Tu NR, with 11,280 m of transects being walked by the researchers. The minimum and maximum elevation of our transects were 416 m and 795 m a.s.l. respectively, with the average elevation being 604.5 m a.s.l. Transects were walked in all weather conditions, from sunny and dry to cloudy and rainy, including also sunny weather after heavy rains. When a free ranging turtle was encountered, it was photographed and measured as mentioned above. We released all individuals unharmed after data processing. We did not survey any aquatic species

Statistical analyses – To calculate turtle relative frequency of observation for each transect, we used the following formula:

$$S = \frac{x}{a \cdot b}$$

with S = turtle relative frequency of observation, x = number of turtles found in a given transect; a = number of people involved in the time search along a given transect; b = total time spent for a trip along a given transect.

The χ^2 test was used to evaluate (i) the frequency differences of occurrence of the various species in the hands of hunters/traders, and (ii) the frequency differences among the various turtle species in terms of the number of interviewees reporting them to be present at the study area. Differences between wild-caught and traded samples of *G. spengleri* in terms of body size measurements (carapace length, carapace width, shell height and weight) were assessed by Student t-test. In the text, the means are given with ± 1 standard deviation. All statistical analyses were performed by Past 3.0 software, with alpha set at 5 %.

RESULTS

Interview surveys: At least 10 % of the interviewees identified the presence of seven species, whereas for the other seven species the number of people claiming their

presence was very low and thus considered unlikely (Table 1). The various species differed significantly in terms of frequency of the interviewees reporting them to be present ($\chi^2 = 133.6$, $df = 11$, $P < 0.0001$), with significantly more interviewees knowing well *G. spengleri* and *Cuora mouhotii*. Interestingly, no species was confirmed to be present by more than 50 % of the interviewees (Table 1), thus indirectly suggesting that all species should be rare in the study area. Concerning *G. spengleri*, the various interviewees concurred that the species weighs less than 100 g, has serration, lives in bamboo forest (they called it 'the bamboo turtle' (rùa trúc)) and, because of the reddish skin coloration, is also called the 'fire turtle' (rùa lửa). According to their information, the species inhabits the evergreen forest at high elevation, even on the top of the mountains. Hunters also claimed that the species is normally seen when weather change from rain to sunny or vice versa, and that they can be located more easily in the rainy season (April to July) when turtles are very active above-ground while searching for food. Based on the interviewees' reports, it appeared that *G. spengleri* was the cheapest of all the locally traded species (Table 1).

During the interviews, we observed 40 individuals, belonging to seven native species (*G. spengleri*, *Cuora mouhotii*, *C. cyclornata*, *C. galbinifrons*, *I. elongata*, *P. steindachneri*, and *P. sinensis*) in the hands of the hunters/traders (Table 1). The frequency of occurrence of the various species was significantly different from equality ($\chi^2 = 142.2$, $df = 6$, $P < 0.0001$), with *G. spengleri*, *C. mouhotii* being the two dominant species. Overall, 57.5 % of the observed individuals were going to be sold to China, followed by the local consumption as food (17.5 %), traditional medicine (15 %) and pet (12.5 %) (Table 1). In our observed sample, six individuals of three species (one *G. spengleri*, four *C. mouhotii* and one *I. elongata*) were confiscated by rangers of the NR (Online Appendix 2). Body size measurements taken from both wild-caught and traded turtle individuals are reported in Online Appendix 3. All the observed species were listed as Threatened species by IUCN (2018).

Field surveys: During the field transects, we recorded seven individuals of *G. spengleri* and one of *C. mouhotii* (Online Appendix 4). Individuals of *G. spengleri* were observed at an average elevation of 699.7 ± 31.6 m a.s.l. (range = 651-725 m, median = 710 m). Their relative frequency of observation was 0.037 individual/person/hour. Among our observed *G. spengleri* individuals, four were adults, one sub-adult, one juvenile and one hatchling. Two were males (one adult and one sub-adult), four were females (all adults) and one was a hatchling of unidentified sex.

Out of seven free-ranging *G. spengleri*, only one individual was found by a hunting dog. The turtles were found from 10:47-13:45 h, in all cases when the weather was sunny following heavy rains. Turtles were observed at 25-30 °C temperature range. Three turtles were found in rocky caves while four turtles were found in a forest patch dominated by bamboo (*Arundinaria* sp) (Fig. 3). The individuals found outside of rocky caves were not too far from a rocky area (range from 10-50 m) when first seen and all were found on but not hiding under leaf litter. The forest cover at the location of *G. spengleri* ranged from 70 %-90 % (mean = 76.4 ± 7.4 %, median = 75 %), thus showing that this species inhabits dense forest patches. However, there were no turtles found in wet forest areas with canopy cover higher than 90 %. The slope angles where turtles were found varied considerably, from 5-45 °, with an average of 24.6 ± 17.5 ° (median = 25 °).



Figure 3. Some typical habitat types where *G. spengleri* were encountered during the field surveys. Live turtles were found in forest dominated by bamboos, with three individuals found in small rocky caves, and four individuals found on leaf litter.

The single individual of *Cuora mouhotii* was an adult male. Two subspecies of *C. mouhotii* are currently recognised. The plastron markings and carapace shape of this specimen agree with published descriptions of the subspecies *C. m. mouhotii* (Das et al., 2016). This individual was observed at 607 m a.s.l., in secondary forest dominated by bamboo trees, with a 5 % slope and 50 % forest cover. It was found by a hunting dog.

DISCUSSION

Overall, seven turtle species were observed during our surveys, including field records and animals encountered while interviewing people. All of these species are listed as Threatened by IUCN (2018). Five other species (*Platysternon megacephalum*, *Sacalia quadriocellata*, *Mauremys mutica*, *C. zhoui* and *Cyclemys oldhamii*) were mentioned as occurring in the study area by at least one interviewee, but without any firm evidence. Three of these species (i.e. *M. mutica*, *C. zhoui* and *Cyclemys oldhamii*) were mentioned to occur by so few respondents (< 7 % of the interviewed sample) that we consider their presence in the study area very unlikely. Concerning *C. zhoui*, this species has never been observed in the wild until now, and the two interviewees claiming its presence were elderly, reporting that they had collected it in the 1980s. However, their descriptions could

have been of similar species that may now be extinct.

Among the interviewees, there were three former turtle hunters who quitted their activity due to the significant reduction of wildlife inside the NR. One of these collectors claimed that he would collect a bag of 10-20 kg of turtles per hunting day in 1980s-1990s, whereas he was able to find fewer animals since the 2000s. He also claimed that, during 1980s-1990s, there were several hundred tons of turtles being collected to sell to China. Nowadays, collectors can only find a single turtle occasionally. One also claimed that he was forced to give up the job because there were too few turtles in the Tay Yen Tu NR due to overharvesting whereas, in 2010-2015, he was able to fill a tank with 150-200 *G. spengleri* individuals to sell as pets or for religious release in Tay Yen Tu Buddhist festival from February to April annually.

Our study suggests that *G. spengleri* and *C. mouhotii* are the least rare among the various terrestrial turtle species of the Tay Yen Tu Nature Reserve as they (i) were the most frequently cited species to be present according to our interviewees (>40 % of the responders), (ii) were the most abundant species in the hands of the hunters/traders (accounting for 68.3 % of the total recorded sample), and (iii) were the only species directly observed by us in the field. The presence of adults of both sexes and of a hatchling in our small sample suggests that the *G. spengleri* population is still viable although more field research is needed to assess its conservation status.

All of our *G. spengleri* sightings were on the ground, thus confirming anecdotal literature claiming that this species is primarily terrestrial, but occasionally enters streams (Fang, 1930). The linear distance of our observed turtles from the nearest streams ranged from 50-80 m. Moreover, our habitat data showed that this species is clearly linked to hilly and montane forests with dense canopy. In this regard, our data mirror Pope's (1935), who considered that this turtle prefers intact, forested, montane environments (indeed we did not find any individual at 416-704 m a.s.l.). Regarding the elevation, Dawson (2016) considers that these turtles in Hainan normally inhabit sites ranging 700-1200 m elevation. Our results, although fitting well with Dawson's (2016) observations, also would indicate that *G. spengleri* can be found even at slightly lower elevations than supposed (two individuals found at 651 m and 659 m, respectively). In Vietnam, according to Hendrie (2000), *G. spengleri* is a highland, terrestrial and forest species, that is replaced at lower altitudes by *C. mouhotii*. Our study demonstrates that the two species can be at least contiguously sympatric in Vietnamese montane forests. Indeed, the *C. mouhotii* individual was still found at a slightly lower elevation than all *G. spengleri* individuals, so there is likely not a firm cutoff between the species but rather a transition where they overlap slightly (the same being observed in Hainan; J. E. Dawson, unpublished data). In addition, the forest habitat of our *C. mouhotii* individual was less covered than that of *G. spengleri*, although also bamboo forest. So, it is not certain that the populations of these species can be completely sympatric. Further studies should explore how frequently truly sympatric populations of these two species occur in Vietnam. In conclusion, our study documents that *G. spengleri* is still found with likely viable populations in Tay Yen Tu NR, but all the evidence presented here would suggest a decline in population sizes of turtles inside the protected area. The full extent of this supposed decline remains unknown.

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Herpetofaunal survey in rainforest remnants of the Western Ghats, India

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ABSTRACT - We undertook amphibian and reptile surveys in six rainforest remnants of the Anamalai Hills in the Western Ghats, India. Over a two-month period, 36 species of herpetofauna were recorded from these remnants, including one species of caecilian, 19 frog species, 8 lizard species and 8 species of snake. Six species were either critically endangered or endangered. We also recorded one species of frog (*Nyctibatrachus acanthodermis*) outside of its type locality for the first time since its original description. The study demonstrated the presence of several threatened species of herpetofauna in these small forest remnants, the protection and restoration of which are important for the conservation of biodiversity in the Western Ghats.

INTRODUCTION

With increasing fragmentation of tropical forests and conversion to other land uses, there has been an increased interest in the conservation of biodiversity in fragmented landscapes (Wade et al., 2003; Harris & Silva-Lopez 1992; Chazdon et al., 2009). This is especially relevant in areas of high biological diversity, such as tropical rainforests. The Western Ghats of India, a biodiversity hotspot, has lost most of its original habitat due to land-use conversion related to human activities (Bhupathy et al., 2016). Despite this, recent studies indicate that even in their fragmented and degraded state, remnants of forests in the Western Ghats can sustain high levels of biodiversity (Balaji et al., 2014; Anand et al., 2010; Sridhar et al., 2008; Karanth et al., 2016).

The Anamalai Hills in the southern Western Ghats is rich in herpetofaunal biodiversity and endemism, having at least 83 species of amphibians and 82 species of reptiles and is an important area for conservation of biodiversity in this region (Smith, 1935 & 1943; Kumar et al., 2002; Deepak et al., 2009; Deepak et al., 2016; Subramanian et al., 2013; Frost, 2018). Approximately 45 % of amphibian species in the Anamalai area are either Data Deficient or Not Assessed, while 35 % are considered critically endangered or endangered (IUCN, 2017). Many species are known only from original descriptions or anecdotal records (Ishwar, 2000; Biju & Bossuyt, 2009). Lack of fine-scale information on species distribution and abundance within the landscape hampers reliable conservation status assessments of most of these species.

Surveys conducted between 1995 and 2003 in tea plantations and 14 rainforest fragments in the Valparai Plateau recorded 40 species of amphibians and 40 species of reptiles (Kumar et al., 2002; Daniels, 2003). The diversity and abundance of amphibians and reptiles were higher in rainforest remnants compared to modified habitats such as plantations (Murali & Raman, 2012; Venugopal, 2010). In the last decade, there have been significant changes in

our understanding of the diversity of herpetofauna in the Western Ghats. Many species previously considered as widespread, with better taxonomic understanding, are now considered local endemics with small, restricted distribution ranges (Garg & Biju, 2017; Biju & Bossuyt, 2009; Biju et al., 2014b; Garg et al., 2017; Biju et al., 2011; Biju et al., 2014a). The number of frog species known from the Western Ghats have increased from 104 in 2001 to 220 in 2018 (Biju, 2001; Frost, 2018). Many of the new species descriptions were from forest remnants in human-modified landscapes such as tea plantations (Myers, 1942; Biju et al., 2011; Zachariah et al., 2011; Vijayakumar et al., 2014). Further surveys and ecological studies across this region are required to understand the distribution and abundance patterns of herpetofauna. For a period of two months, we undertook herpetofaunal surveys in six rainforest remnants on the Valparai Plateau of the Anamalai Hills, a landscape dominated by tea plantations.

MATERIALS AND METHODS

Valparai is an undulating plateau (area 220 km²) located between 800 – 1500 m above mean sea level (hereafter asl) (10.32° N, 76.95° E), in the Anamalai Hills in Tamil Nadu, India (Fig. 1). This region receives mean annual rainfall of over 2800 mm (Rathod & Aruchamy, 2010). Natural vegetation of Valparai Plateau is mid-elevation tropical wet evergreen forest of the *Cullenia exarillata* – *Mesua ferrea* – *Palaquium ellipticum* type (Pascal, 1988). Valparai has lost over 75 % of the original tropical rainforest to tea, coffee, and other commercial privately owned plantations. The current land-cover in the plateau is a mixture of plantations and forest remnants. There are numerous rainforest remnants in the Valparai Plateau (Kapoor, 2008; Mudappa & Raman, 2007; Mudappa et al., 2014). Anamalai Tiger Reserve surrounds the plateau on its north, east and south, while Vazhachal Reserved Forest occurs to the west. Valparai has several perennial streams, rivers (mainly, Sholayar and Nirar), and a single large reservoir (Upper Sholayar Dam). Though there

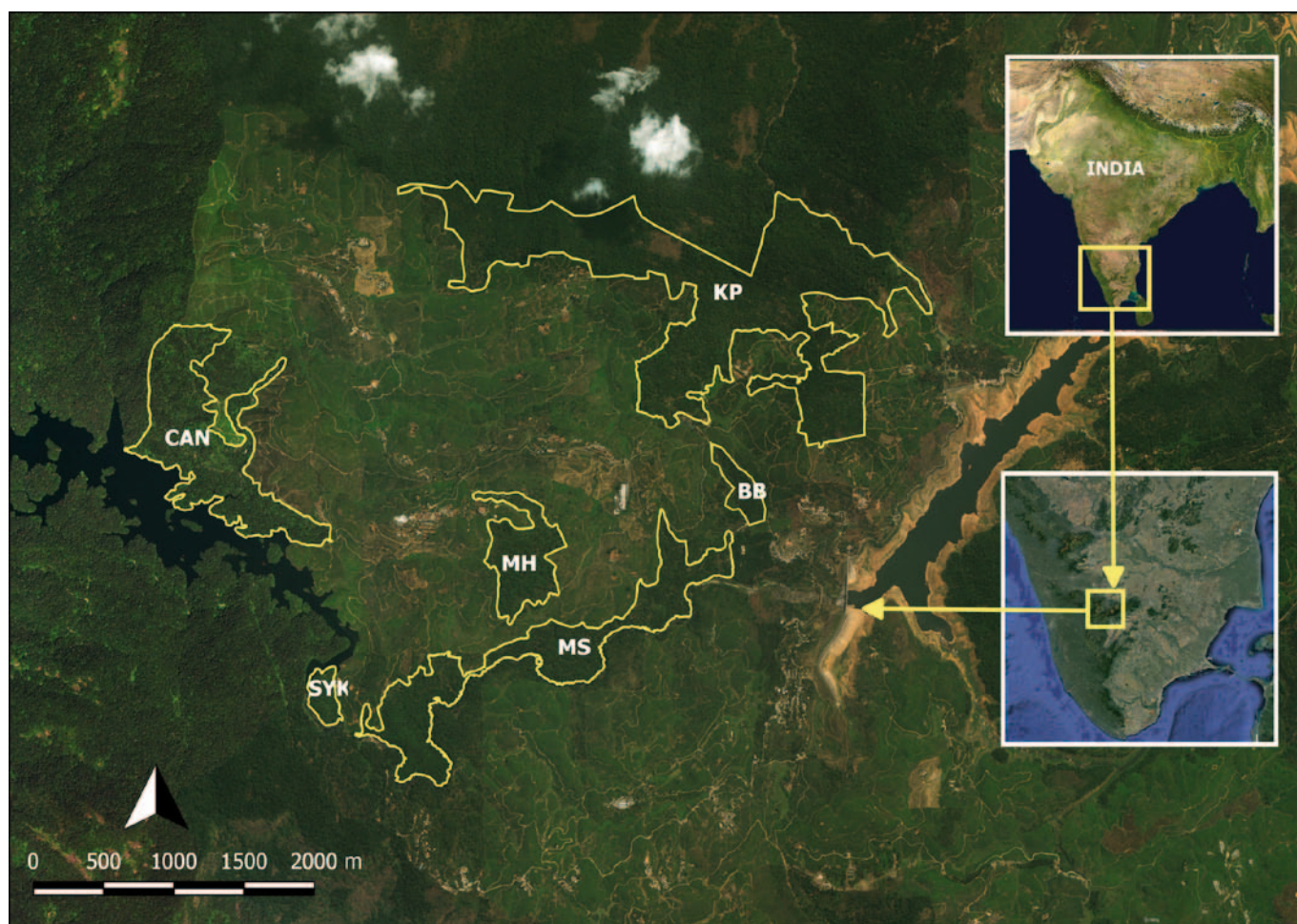


Figure 1. Six rainforest remnants surveyed for herpetofauna in the western part of the Valparai Plateau: KP - Kalyanapandal-Pudukadu, CAN - Candura, MS - Murugalli Sholayar, MH - Murugalli Hospital, BB - Murugalli Black Bridge, and SYK - Murugalli Sykes

are many human settlements spread throughout the plateau, most people live near the Valparai town.

We surveyed six rainforest sites on the western part of the Valparai Plateau (Fig. 1). In order of size, these were Kalyanapandal-Pudukadu (KP, 200 ha, 909-967 m asl), Candura (CAN, 103.3 ha, 845-1019 m asl), Murugalli-Sholayar (MS, 102 ha, 835-893 m asl), Murugalli-Hospital (MH, 38 ha, 929-1028 m asl), Murugalli-Black Bridge (BB, 16 ha, 834-924 m asl), and Murugalli-Sykes (SYK, 9 ha, 826-838 m asl). Two of the larger rainforest remnants, CAN and KP, adjoin contiguous rainforest, separated only by small boundary clearings. The Sholayar River separates the MS and SYK remnants from contiguous rainforest areas. All of these remnants have had human disturbances in the past. MH remnant was the closest to a human settlement and consequently the most disturbed, though we have not quantified the disturbance levels for this preliminary survey. All remnants other than the MH remnant have perennial streams or a river flowing through them or along the edge. The sites surveyed were all between 800 – 1050 m asl.

To record the species richness in the selected rainforest remnants we used time-constrained visual encounter surveys (VES) (Crump & Scott Jr, 1994; Doan, 2003). Each survey was of 1 h duration, during which two observers walked a trail through the sites, actively searching all potential habitats (e.g., leaf-litter, tree buttresses, fallen logs etc.) and recording all specimens sighted. We selected these trails to represent the general habitats and microhabitats present,

Table 1. Visual encounter survey effort (excluding three opportunistic records) and observation rates for six rainforest remnants on the Valparai Plateau in the Anamalai Hills. For site name abbreviations, see Figure 1.

| Site | No. surveys (Day/Night) | Person- hours | No. specimens | No. species |
|-----------|----------------------------|------------------|------------------|----------------|
| KP | 7 (5/2) | 14 | 64 | 17 |
| CAN | 12 (6/6) | 24 | 145 | 17 |
| MS | 8 (8/-) | 16 | 70 | 19 |
| MH | 4 (2/2) | 8 | 23 | 10 |
| BB | 5 (2/3) | 10 | 70 | 15 |
| SYK | 4 (4/-) | 8 | 33 | 10 |
| All sites | 40 (27/13) | 80 | 405 | 33 |

maximise detections, while taking into account logistics and accessibility. To avoid pseudo-replication, we chose a new trail for each survey. We conducted surveys during day (07:00-12:00 h) and night (18:00-19:00 h) but due to logistical difficulties there were no night-time surveys in Murugalli-Sholayar and Murugalli-Sykes fragments. All surveys were undertaken from 9 September to 29 October 2017, coinciding with the retreating south-west monsoon and early north-east monsoon rains.

The survey effort varied between remnants (Table 1). We

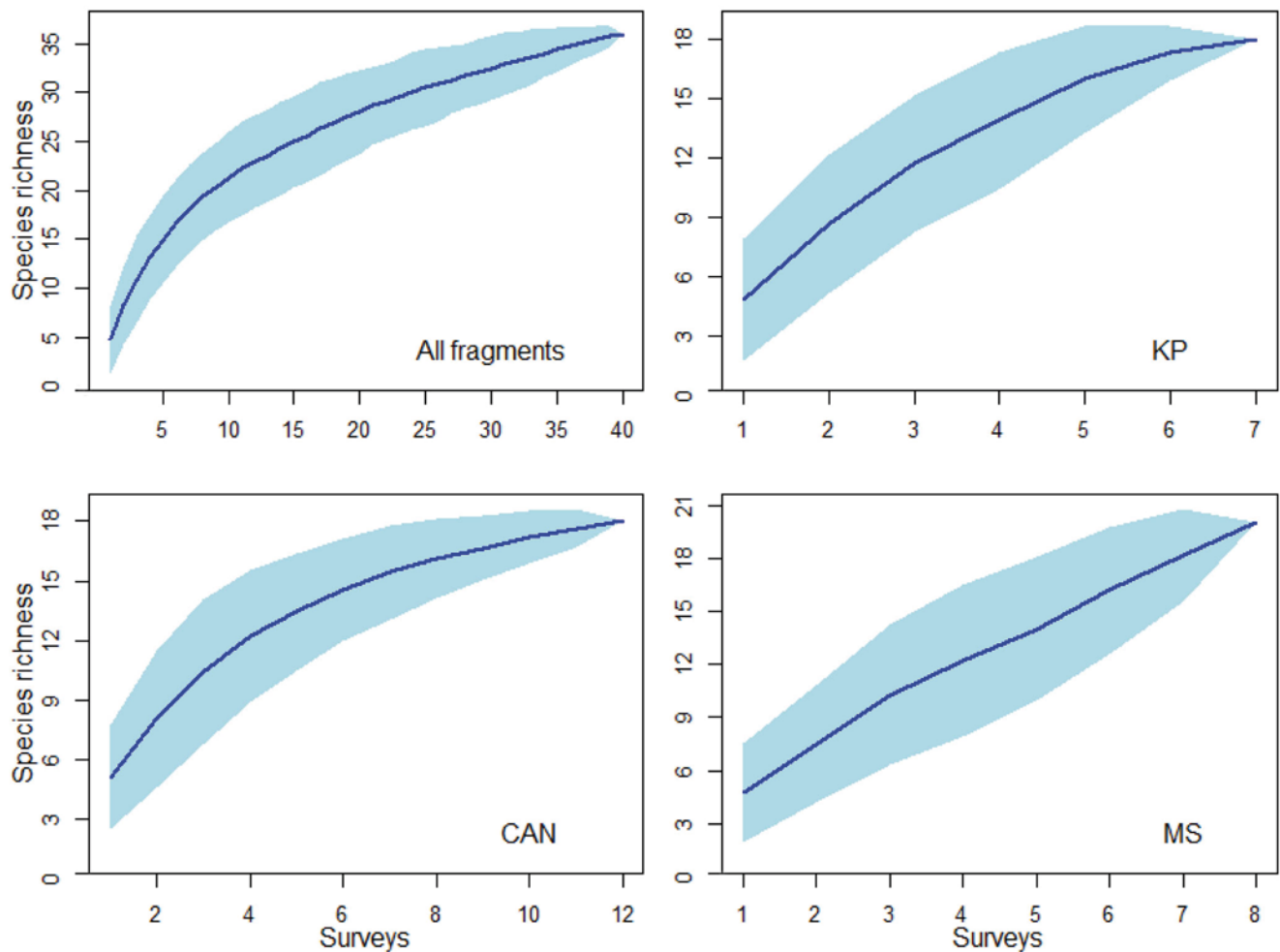


Figure 2. Species accumulation curves for all remnants combined and three largest remnants (KP, CAN, and MS). The feather area indicates 95 % confidence intervals.

calculated encounter rates as number of specimens recorded per person-hour of survey effort. To compare the efficacy of day and night time surveys, we compared mean (expressed as mean \pm standard deviation) and median numbers of specimens and species recorded per survey during day and night surveys. Since the data was not-normally distributed, we used the non-parametric Wilcoxon rank sum tests for these comparisons. To examine the extent of survey completeness, we plotted species accumulation curves using a random addition of samples (number of species against number of surveys) for each remnant surveyed, using the VEGAN package in program R (R Core Team, 2017).

We identified most specimens based on examination of diagnostic morphological characters as described in relevant taxonomic literature. These were mainly Biju et al. (2011) for frogs of the genus *Nyctibatrachus*; Biju et al. (2014a) for frogs of the genus *Micrixalus*; Biju et al. (2014b) for frogs of the genus *Indosylvirana*; Biju & Bossuyt (2005 & 2009) and Bossuyt (2002) for Rhacophorid frogs currently in the genera *Raorchestes* and *Pseudophilautus*; Dahanukar et al. (2016) and Garg & Biju (2016) for frogs of the genus *Indirana*; Garg & Biju (2017) for frogs of the genus *Minervarya*; Manamendra-Arachchi et al. (2007) for geckos of the genus *Cnemaspis*; and Smith (1935 & 1943) in general for lizards and snakes. The nomenclature used

follows Frost (2018) and Uetz et al. (2018) for amphibians and reptiles respectively. We identified specimens that could not be closely examined, some juveniles, and populations whose taxonomic status was unclear only to genus level. Unidentified specimens were not included in estimations of species richness and relative abundance. We did not collect voucher specimens during this survey, as our research permit from the state Forest Department did not include permission to collect biological specimens. In addition, we wanted to avoid invasive methods given the sensitivity and rarity of many species and the already disturbed status of these forest remnants. Field identification based on diagnostic characters and photo-documentation was sufficient for the purposes of this survey. Voucher photos of all species recorded were deposited in the biodiversity database India Biodiversity Portal (Vattakaven et al., 2016).

RESULTS

We undertook 40, one-hour-long visual encounter surveys (VES) in the six rainforest remnants (Table 1). Species accumulation curves reached asymptote only in the CAN remnant (Fig. 2). Other remnants and data pooled across all sites did not reach an asymptote, indicating that additional sampling may reveal the presence of more species in these

Table 2. Number of specimens recorded (encounter rates as specimens/ person-hour in brackets) of amphibians and reptiles in six rainforest remnants on the Valparai Plateau, Anamalai Hills. Species names are followed by their IUCN conservation status: NA – Not Assessed, DD – Data Deficient, LC – Least Concern, VU – Vulnerable, NT – Near Threatened, EN – Endangered, CE – Critically Endangered. For site name abbreviations, see Figure 1. * indicates opportunistic encounters not part of VES.

| Species | All sites | MS | CAN | KP | BB | SYK | MH |
|--|-----------|-----------|-----------|----------|-----------|----------|----------|
| Amphibians | | | | | | | |
| <i>Duttaphrynus melanostictus</i> (LC) | 19 (0.24) | 1 | 4 (0.17) | 3 (0.25) | 3 (0.30) | 7 (0.88) | 1 |
| <i>D. microtypanum</i> (VU) | 3 (0.03) | - | - | - | 3 (0.20) | - | - |
| <i>M. keralensis</i> (LC) | 40 (0.50) | 8 (0.50) | 11 (0.46) | 3 (0.25) | 13 (1.30) | 5 (0.63) | - |
| <i>Minervarya</i> sp. | 18 (0.23) | 1 | 6 (0.25) | 6 (0.50) | 1 | - | 4 (0.50) |
| <i>I. brachytarsus</i> (EN) | 7 (0.09) | - | 4 (0.17) | 2 (0.17) | 1 | - | - |
| <i>I. semipalmata</i> (LC) | 1 | - | - | - | 1 | - | - |
| <i>Clinotarsus curtipes</i> (NT) | 24 (0.30) | 6 (0.38) | - | - | 13 (1.30) | 5 (0.63) | - |
| <i>M. nelliampathi</i> (NA) | 19 (0.24) | 4 (0.25) | 14 (0.58) | - | 1 | - | - |
| <i>N. acanthoderms</i> (NA) | 28 (0.35) | - | 18 (0.75) | 8 (0.67) | 2 (0.20) | - | - |
| <i>N. anamallaiensis</i> (NA) | 1 | 1 | - | - | - | - | - |
| <i>I. sreeni</i> (NA) | 71 (0.89) | 20 (1.25) | 25 (1.04) | 5 (0.42) | 13 (1.30) | 6 (0.75) | 2 (0.25) |
| <i>I. doni</i> (NA) | 14 (0.18) | 1 | 5 (0.21) | 7 (0.58) | - | - | 1 |
| <i>R. ponmudi</i> (CE) | 16 (0.20) | - | 11 (0.46) | - | 3 (0.30) | - | 2 (0.25) |
| <i>R. akroparallagi</i> (LC) | 1 | - | 1 | - | - | - | - |
| <i>R. anili</i> (LC) | 8 (0.10) | - | - | - | 8 (0.80) | - | - |
| <i>P. wynaadensis</i> (EN) | 66 (0.81) | 9 (0.50) | 37 (1.54) | 7 (0.58) | 3 (0.30) | 1 | 9 (1.13) |
| <i>Polypedates pseudocruciger</i> (LC) | 1 | - | - | 1 | - | - | - |
| <i>R. calcadensis</i> (EN) | 6 (0.08) | - | - | 6 (0.50) | - | - | - |
| <i>R. pseudomalabaricus</i> (CE) | 4 (0.50) | - | - | 4 (0.33) | - | - | - |
| <i>U. cf. oxyurus</i> (DD) | 1 | - | 1 | - | - | - | - |
| Reptilia | | | | | | | |
| <i>Cnemaspis</i> sp. | 14 (0.18) | 10 (0.63) | 2 (0.08) | 1 | 1 | - | - |
| <i>C. wynadensis</i> (EN) | 9 (0.11) | 1 | - | 1 | 5 | 1 | 1 |
| <i>E. macularia</i> (NA) | 10 (0.13) | 1 | 1 | 3 (0.25) | - | 5 (0.63) | - |
| <i>E. carinata</i> (LC) | 1 | * | * | * | - | 1 | - |
| <i>R. cf. travancorica</i> (DD) | 1 | - | - | - | - | 1 | - |
| <i>Draco dussumieri</i> (LC) | 5 (0.06) | 1 | 2 (0.08) | 2 (0.17) | - | - | - |
| <i>Monilesaurus ellioti</i> (LC) | | | 2 (0.08) | 3 (0.25) | - | - | 1 |
| <i>C. nemoricola</i> (LC) | * | * | - | - | - | - | - |
| <i>U. cf. nitida</i> (DD) | - | - | - | - | * | - | - |
| <i>U. cf. ocellata</i> (LC) | - | - | - | - | * | - | - |
| <i>L. travancoricus</i> (LC) | 1 | 1 | - | - | - | - | - |
| <i>Dendrelaphis ashoki</i> (LC) | 1 | 1 | - | - | - | - | - |
| <i>A. nasuta</i> (NA) | 3 (0.04) | 1 | - | 1 | - | - | 1 |
| <i>Hebius beddomei</i> (LC) | 1 | - | - | - | - | - | 1 |
| <i>H. monticola</i> (LC) | 2 (0.03) | 1 | * | - | - | 1 | - |
| <i>X. piscator</i> (NA) | 2 (0.03) | 1 | 1 | - | - | - | - |

remnants (Fig. 2). We recorded 405 specimens and 33 amphibian and reptile species in the VES surveys (Table 1, Figs. 3-7). This included 12 families and 22 genera. We recorded 1 species of caecilian, 19 frog species, 7 lizard species, and 6 species of snakes (Table 2). Additionally, we had opportunistic encounters of the Nilgiri forest lizard

Calotes nemoricola in MS remnant, and road-kills of two shield-tailed snakes (*Uropeltis* cf. *ocellata* and *U. cf. nitida*) on the road bordering BB remnant (Table 2).

The mean species richness per survey was higher in night-time surveys (5.6 ± 1.7 species/survey, median = 4, N = 13 surveys) compared to daytime surveys (4.3 ± 1.6 species/



survey, median = 6, N = 27 surveys, Wilcoxon rank sum test $W = 100$, $p = 0.027$). Significantly more specimens were recorded during night-time surveys (15.6 ± 9.4 specimens/survey, median = 14, N = 13 surveys) than during daytime surveys (7.7 ± 4.4 specimens/survey, median = 6, N = 27 surveys, Wilcoxon rank sum test $W = 73$, $p = 0.003$).

The most species-rich group was the largely arboreal amphibian family Rhacophoridae (seven species), followed by the snake family Colubridae (five species). Despite differences in survey effort, species richness was similar in the three larger sites (Table 1). Across all sites, *Indosylvirana sreeni* (Fig. 3h) was the most commonly encountered amphibian species, averaging 0.89 specimens/person-hour of survey, while a diminutive diurnal gecko *Cnemaspis* sp. (Fig. 5f) was the most commonly encountered reptile species (Table 2). Single specimens represented six species of amphibian and five species of reptile. Several species were unique to single remnants, such as the tree frog *Rhacophorus pseudomalabaricus* (Fig. 5c), located only in KP remnant. The MS remnant had the highest number of such unique species despite not having night surveys. Even the smallest remnant (SYK) supported a unique species, *Ristella* cf. *travancorica* (Fig. 5g).

We recorded two amphibian species that are listed as critically endangered—*Raorchestes ponmudi* (Fig. 4h) and *R. pseudomalabaricus* (Fig. 5c), and three as endangered—*Indirana brachytarsus* (Fig. 3c), *Pseudophilautus wynaadensis* (Fig. 4e), and *R. calcadensis* (Fig. 5b). There were five species of amphibian that were ‘Not Assessed’ for threat levels—*Micrixalus nelliampathi* (Fig. 3g), *I. sreeni*, *I. doni* (Fig. 4a), *Nyctibatrachus acanthodermis* (Fig. 4c), *N. anamallaiensis* (Fig. 4d), and one Data Deficient species—*Uraeotyphlus* cf. *oxyurus* (Fig. 5d) (IUCN Redlist, 2017). Among reptiles there was one species listed as endangered—*Cnemaspis wynadensis* (Fig. 5e), three species that have not been assessed for their conservation status—*Eutropis macularia* (Fig. 5h), *Ahaetulla nasuta* (Fig. 6g), and *Xenochrophis piscator* (Fig. 6h), and two Data Deficient species—*R. cf. travancorica* (Fig. 5g) and *U. cf. nitida* (IUCN Redlist, 2017).

Comments on identity of some species

Minervarya sp. – Daniels (2003) reported both *Fejervarya limnocharis* (Gravenhorst, 1829) and *Minervarya nilagirica* (Jerdon, 1854) (as *Limnonectes limnocharis* and *L. nilagirica* respectively) from Valparai. *Fejervarya limnocharis* is currently considered a South-east Asian species (type locality probably in Java). *Minervarya nilagirica* was described by Jerdon from the Nilgiris, north of Palghat gap (Frost, 2018). While the specimens from Valparai, located mostly near swamps and streams in fairly open habitats, resembled *M. nilagirica*, it was impossible to assign these specimens to that or any other known species with certainty.

Nyctibatrachus acanthodermis – This species was described from Kaikatti in Nelliampathi, Palakkad District, Kerala (Biju et al., 2011). This current record is the first report of this species outside of the vicinity of its type locality. The straight-line distance between the sampled locations in Valparai and the type locality is not more than 27 km. We used the following key diagnostic features to identify the species: medium to large sized frogs; skin on dorsum strongly wrinkled with spiny projections which are more prominent on posterior body; an inverted ‘Y’ shaped ridge on snout; toes almost fully webbed with the webbing reaching the base of the fourth toe disc; finger discs without

dorso-terminal groove; toe discs with dorso-terminal grooves and rounded dorsal skin flaps.

Nyctibatrachus anamallaiensis – This species was originally described from ‘Puthuthottam Estate’, a small, privately owned rainforest fragment in Valparai (approximate elevation 1100 m asl) (Myers, 1942). All known specimens (published records) are from the Anamalais. We found a single specimen in leaf litter in MS remnant.

Uraeotyphlus cf. *oxyurus* – The specimen was tentatively identified as *U. cf. oxyurus*. The taxonomy of this group is unclear, but the specimen definitely belongs to the ‘oxyurus’ group (Gower & Wilkinson 2007): eye distinct, surrounded by a pale ring; tentacles below the nostrils; middle groove between two nuchal collars only visible laterally and ventrally; 190 annuli on the body; seven distinct secondary annuli on neck, but primary and secondary annuli indistinguishable throughout the rest of the body; four annuli on tail after the posterior end of cloaca. Gower et al. (2008) also mentioned specimens from Valparai, which they placed in *U. cf. oxyurus*.

Cnemaspis sp. – This small, slender, semi-arboreal species was common in most sites. In possessing prominent spine like tubercles on the flanks, it belongs to group II in Smith (1935). However, this species has keeled ventral scales (thoracic region, abdomen, and underside of thighs) and postmentals separated by a single scale, resembling the description of *C. australis* Manamendra-Arachchi, Batuwita & Pethiyagoda, 2007. However, it differs from that species in having smooth subcaudal scales and presence of spine-like tubercles on flanks.

DISCUSSION

The species accumulation curves (Fig. 2) suggest that the 36 amphibian and reptile species recorded from the six rainforest remnants in this survey are almost certainly an underestimate. This in part may be due to the use of the VES methodology which is inherently biased towards the detection of species that are more easily seen (Doan, 2003). This probably explains the absence/non-detection of several groups such as the amphibian genera *Ichthyophis*, *Uperodon*, *Sphaerothera*, *Nasikabatrachus* and *Melanobatrachus*, alongside reptiles belonging to the families *Typhlopidae*, *Gerrhopilidae*, and more several species of *Uropeltidae*. All these groups are burrowing species that spend most of their life underground and are known to be present in the Anamalai Hills (Smith, 1943; Rajendran, 1985; Ishwar, 2000; Biju, 2001; Kumar et al., 2002; Biju & Bossuyt 2003; Dutta et al., 2004; Subramanian et al., 2013; Pyron, 2016; Garg et al., 2018). Additionally, we conducted most surveys during daytime, and there were no night-time surveys at two sites. The faster species accumulation during night-time surveys, higher encounter rates, and lack of asymptotes in species accumulation curves suggest that additional night-time surveys may reveal more species in these remnants. Some species encountered during daytime surveys were nocturnal species that had taken refuge under rocks, fallen logs, and leaf litter (e.g. *Lycodon travancoricus*, *P. wynaadensis*). Locating these species during the night when they are active will also allow better observations of natural history and behaviour.

Seasonal activity of amphibians and reptiles is a factor that could have influenced the results of this survey. The surveys were conducted during the retreating monsoon and early north-east monsoon rains (September-October).

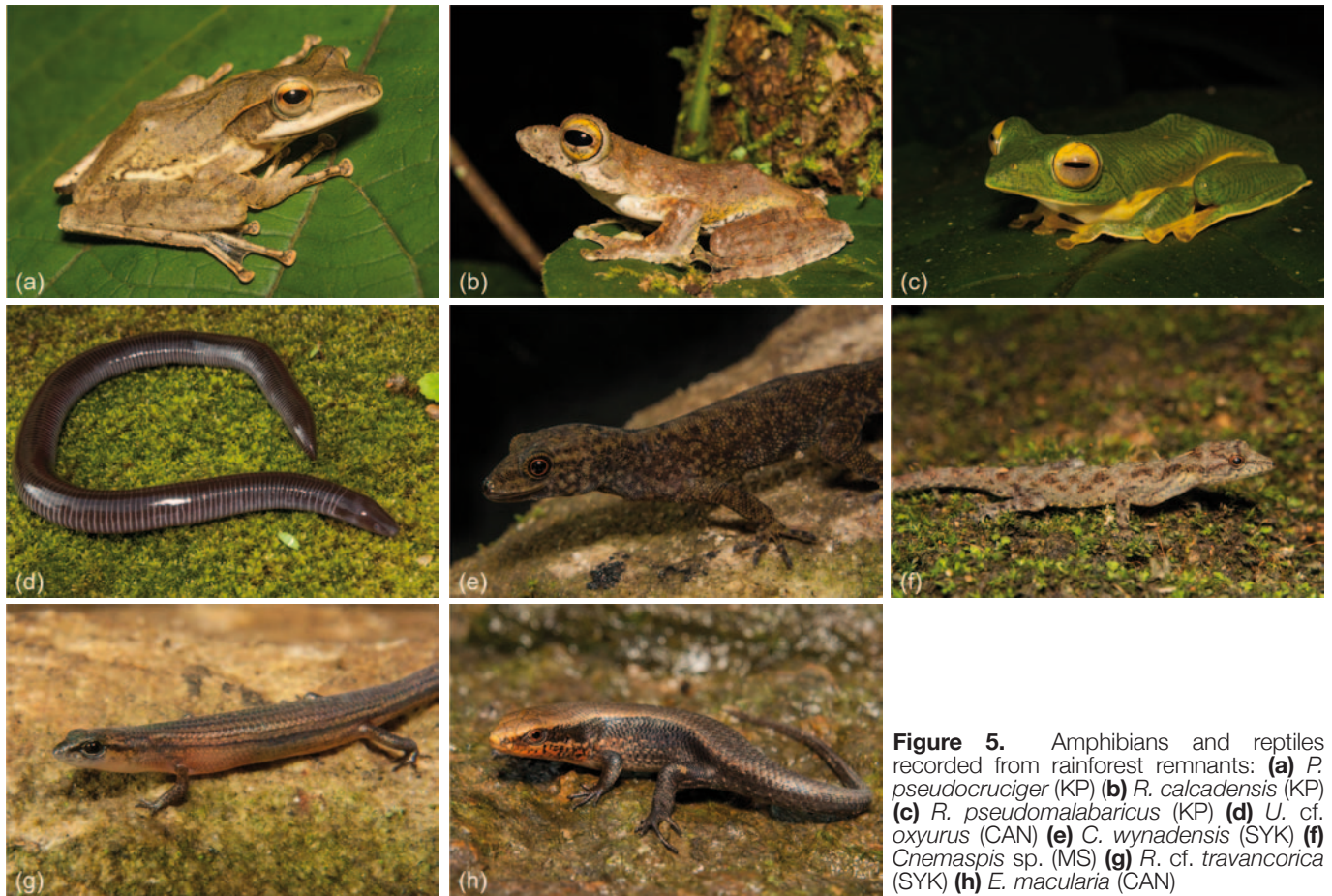




Figure 7. Reptiles recorded from rainforest remnants: (a) *H. beddomei* (MH) (b) *H. monticola* (MS)

Surveys at the beginning of south-west monsoon may add several more species from these forest remnants. Yet another factor that could affect abundance and species richness of herpetofauna is past management practices in these forest remnants, although this survey was not designed to assess these effects.

Although not quantified systematically, observations suggest that the presence of perennial streams and ponds increased the abundance and species richness of herpetofauna. The MH remnant, the only site without either of these, had the lowest encounter rates of amphibians and reptiles (3 specimens/person-hour). Amphibian abundance was generally higher around water bodies (streams, ponds, marshes). In the Anamalai Hills, most amphibians other than members of the genus *Raorchestes* are dependent on specific microhabitats associated with water bodies for breeding. We recorded *M. nelliampathi* and *I. sreeni* males on rocks in perennial streams. We found *R. pseudomalabaricus* and *R. calcadensis* breeding in dammed pools with over-hanging vegetation. *I. doni* was mostly located in swamps associated with streams, and *N. acanthodermis* in rock pools in fast flowing streams. Tadpoles of *Indirana* spp. occurred on wet rock faces and boulders. We also recorded *Minervarya* sp. and *M. keralensis* males calling from puddles created by elephant footprints. Identifying these breeding habitats and associated species is important for long-term monitoring of populations of threatened species.

Of the 36 species recorded in this survey, 32 are endemic to the Western Ghats, and a quarter of the amphibian species recorded are listed as critically endangered or endangered (IUCN, 2017). The Anamalai Hills exhibit high endemism, species richness, and turnover of amphibians among sites, drainages, and elevations (Vasudevan et al., 2006; Biju & Bossuyt 2009; Vijayakumar et al., 2014). Past surveys conducted in other rainforest remnants in the Valparai Plateau have provided baseline information on species presence and distribution (Vijayakumar et al., 2001; Kumar et al., 2002). However, for an improved understanding of the diversity and taxonomy of species from this region, further surveys and ecological studies are required, especially in forest remnants outside protected areas.

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The first assessment of *Batrachochytrium dendrobatidis* in amphibian populations in the Kanuku Mountains Protected Area of Guyana

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ABSTRACT - *Batrachochytrium dendrobatidis* (*Bd*) is a fungal pathogen threatening hundreds of amphibian species with extinction across the globe, especially in Latin America. Extensive investigations have revealed the presence of *Bd* in many South American countries, but there has been a lack of such research conducted in Guyana. We assessed the presence of *Bd* in the amphibian populations of the Kanuku Mountains Protected Area, in the south-west of the country. We swabbed two hundred and fifty anurans and processed the samples using standard Polymerase Chain Reaction analysis to identify cutaneous presence of *Bd*, making this the most comprehensive investigation into the existence of *Bd* in Guyana. All samples were negative for the presence of *Bd* DNA. Given the presence of *Bd* in countries neighbouring Guyana, and the severe declines it has caused in amphibian populations, we consider Guyana to be under severe threat. We advocate further surveillance in Guyana to fully determine the presence or absence of *Bd*, and we emphasise the importance of biosecurity and monitoring in mitigating a potential outbreak of this fungal pathogen.

INTRODUCTION

Across the globe an array of amphibian populations are experiencing severe declines, with threats attributed to habitat loss and exploitation, but many declines are described as “enigmatic” (Stuart et al., 2004). Research suggests that many of these enigmatic declines are caused by infectious disease, such as chytridiomycosis (Lips et al., 2006; Lötters et al., 2009). Chytridiomycosis has contributed to the extinction of species such as the golden toad (*Incilius periglenes*) and both species of gastric brooding frogs (*Rheobatrachus silus* and *R. vitellinus*; Hero et al., 2004; Meyer et al., 2004). Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) and results in high morbidity and mortality in susceptible species and individuals. The fungus infects the permeable skin of amphibians causing hyperkeratosis (Van Rooij et al., 2015), which disrupts respiration, osmoregulation and electrolyte exchange across the skin, ultimately leading to cardiac arrest (Campbell et al., 2012). This fungal pathogen can be spread by direct contact between individuals or by contact with water sources infected with waterborne fungal zoospores (Van Rooij et al., 2015).

Chytridiomycosis is associated with declines in at least 43 species in South America, a region which harbours half of the World’s amphibian species richness (Young et al., 2001; Lips et al., 2005; Lips et al., 2006). Numerous reports from countries including French Guiana (Curtois et al., 2012; Curtois et al., 2015), Venezuela (Hanselmann et al., 2004; Sánchez et al., 2008), Brazil (Valencia-Aguilar et al., 2015; Jenkinson et al., 2016) and Peru (Catenazzi et al., 2011) find evidence of *Bd* in local amphibian populations, demonstrating the extent to which this pathogen has spread

across the continent. However, the presence of *Bd* in Guyana remains undetermined (Olson et al., 2013; Olson & Ronnenberg, 2014). There are 148 documented amphibian species in Guyana, 27 % of which are endemic (Cole et al., 2013) with more species likely to be added to this inventory in the future (Gower et al., 2010; Kok et al., 2006; MacCulloch & Lathrop, 2002). The majority of species in Guyana are under-studied (Jarvis, 2018) with many species known from only a handful of specimens (Cole et al., 2013). This lack of knowledge leads to uncertainty about the threats facing these species. There have been only two previous *Bd* surveys including data from Guyana, and both found no evidence of the fungus (Gower et al., 2013; Luger et al., 2008). However, considering the devastating effects *Bd* has had on endemic populations in countries neighbouring Guyana, it is essential to further investigate *Bd* in this region’s amphibian fauna.

There is an increasing body of evidence indicating the complexity of *Bd* epidemiology; a population’s susceptibility is variable as some species are not necessarily threatened by the presence of *Bd*, and may even act as a disease reservoir for others (Searle et al., 2011; Gervasi et al., 2017; Reeder et al., 2012). In addition, Hanselmann et al. (2004) describe the absence of significant clinical disease or mortality, despite high prevalence of infection in *Lithobates catesbeianus* (formerly *Rana catesbeiana*) in an introduced population in Venezuela. Therefore, the threat of *Bd* to amphibian populations is not fully understood. Conducting surveys for *Bd* in Guyana will shed light on the local disease status, broaden the epidemiological knowledge of this pathogen and potentially aid mitigation efforts (Rödger et al., 2009).

The aim of this work was to carry out a community-wide

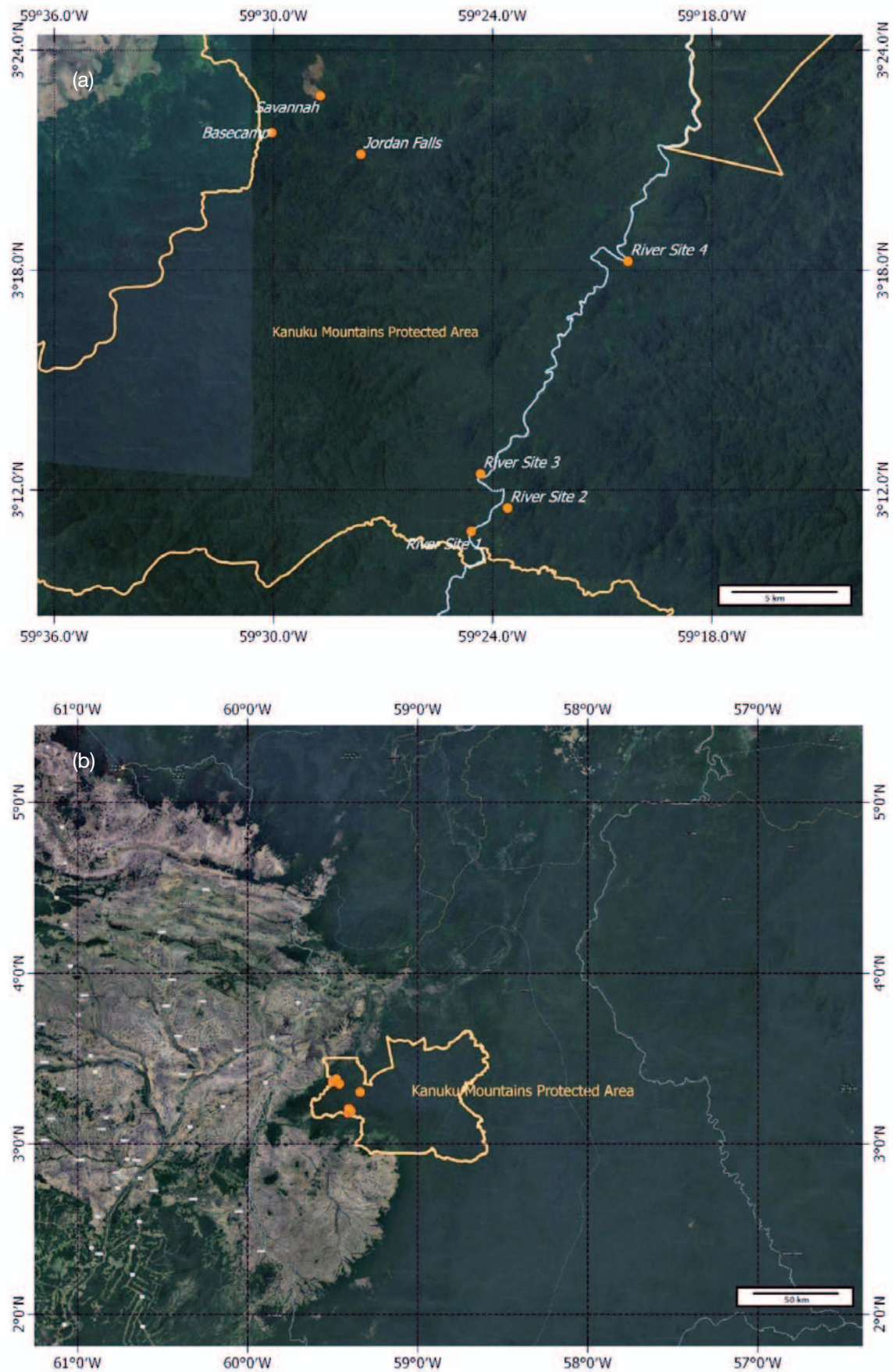


Figure 1. (a) Map of locations in the KMPA where the amphibians were sampled. Orange points represent main areas in which sampling occurred; the boundary of the KMPA is marked in yellow. (b) View of the KMPA within south-west Guyana.

survey to investigate the presence of *Bd* in the amphibian population of the western Kanuku Mountains Protected Area (KMPA), a remote area of rainforest in the south of the country. We see this as a first step towards *Bd* surveillance and monitoring in the Protected Area, as well as an important contribution to the limited body of evidence regarding *Bd* in the Guiana Shield.

METHODS

The KMPA is a national protected area, established in 2011, located in the Rupununi region of south-western Guyana, and one of five protected areas in the country. The KMPA is divided into its east and west ranges by the Rupununi River, and the predominant ecosystem of the KMPA is pristine rainforest, considered the most ecologically diverse in Guyana (Conservation International, 2016). The biodiversity of the KMPA is understudied, and the most recent rapid assessment of this area did not include an investigation of the amphibian community (Montambault & Missa, 2002). Our amphibian survey in the KMPA was conducted over four weeks in July - August 2017. From 14-July to 31-July surveys (*n*=26) took place at the Nappi base camp (3°22'9.57" N, 59°29'56.74" W, Fig. 1a), and the nearby sites of "Jordan falls" (3°21'9.68"N, 59°27'36.55"W) and "Savannah" (3°22'41.19"N, 59°28'46.41"W). From 4-Aug to 8-Aug we surveyed at sites along the Rupununi River (River sites 1-4, surveys *n*=8; Fig. 1a). Surveys consisted of diurnal and nocturnal searches for amphibians in the surrounding area. A visual search technique, aided by auditory signals, was used to locate amphibians in leaf litter, shrubbery and understorey, stream sides and swamp habitats. Each location was visited maximally twice, once at night and once during the day, to minimise recapture given that no marking protocol was followed. On most occasions, amphibians from the nocturnal search were released after the diurnal search the next morning, avoiding recapture.

Amphibians were captured by hand in clean inside-out sealable bags, to avoid potential pathogen transmission between specimens and surveyors. Once caught, Ziploc bags were reversed, inflated and closed. Amphibians were brought back to camp for identification and swabbing. Surveyors used clean gloves for every amphibian swabbed. Individuals were swabbed following standard procedure guidelines (Brem et al., 2007), using clinical grade sterile Deltalab single-packed swabs directed to the ventral surfaces of the individuals. Ziploc bags and gloves were used up to three times and disinfected and dried between surveys using 0.5 % sodium hypochlorite bleach solution. Individuals were identified to species level where possible (Table 1), using Cole et al. (2013). Individuals were released back to the site in which they were initially found within 12 hours of capture. Swabs were stored dry in eppendorf tubes and kept at ambient temperature in the field until return to the UK, after which they were stored at -20 °C until analysis.

DNA extraction and Polymerase Chain Reaction analysis
DNA was extracted from swabs using phenol-chloroform

Table 1. Amphibians swabbed for *Batrachochytrium dendrobatidis*, ranked by frequency of encounter

| Rank | Identification | No. individuals |
|-------|---|-----------------|
| 1 | <i>Leptodactylus mystaceus</i> | 62 |
| 2 | <i>Leptodactylus sp</i> | 29 |
| 3 | <i>Adenomera hylaedactyla</i> | 28 |
| 4 | <i>Bufonidae</i> | 23 |
| 5 | <i>Rhinella martyi</i> | 21 |
| 6 | <i>Physalaemus cuvieri</i> | 13 |
| 7-8 | <i>Allobates spumaponens</i> | 12 |
| 7-8 | <i>Unidentified</i> | 12 |
| 9-10 | <i>Allophryne ruthveni</i> | 7 |
| 9-10 | <i>Ameerega trivittata</i> | 7 |
| 11 | <i>Anomaloglossus kaiei</i> | 6 |
| 12 | <i>Aromabatidae</i> | 9 |
| 13-14 | <i>Leptodactylus guianensis</i> | 4 |
| 13-14 | <i>Leptodactylus knudseni</i> | 4 |
| 15 | <i>Ceuthomantidae/Craugastoridae/ Eleutherodactylidae</i> | 3 |
| 16-18 | <i>Adenomera andreae</i> | 2 |
| 16-18 | <i>Hylidae</i> | 2 |
| 16-18 | <i>Boana xerophylla</i> | 2 |
| 19-22 | <i>Boana boans</i> | 1 |
| 19-22 | <i>Leptodactylus leptodactyloides</i> | 1 |
| 19-22 | <i>Leptodactylus petersii</i> | 1 |
| 19-22 | <i>Pristimantis sp</i> | 1 |
| Total | | 250 |

extraction (Sambrook & Russell, 2001). The presence or absence of *Bd* in each sample was identified using a standardised Polymerase Chain Reaction (PCR), with ITS/5.8S primers (Boyle et al., 2004; ITS Chytr 5' CCTTGATATAATACAGTGTGCCATATGTC-3' and 5.8S Chytr 5'-AGCCAAGAGATCCGTTGTCAAA-3'). Each sample of extracted DNA was subjected to replicate PCR analysis (25 µl reaction volume), with a positive control (Greener et al., 2017; Shepherd et al., 2016). As in previous studies (Greener et al., 2017; Shepherd et al., 2016) an additional control, to demonstrate that PCR quality DNA had been obtained from the extraction, a PCR was performed using universal 16S rDNA primers (Palumbi, 1996), on 12 randomly selected samples.

RESULTS

A total of 250 anurans were swabbed successfully, of 22 taxa (15 confirmed nominal species with seven taxa to generic or family level; Table 1). No individuals of Gymnophiona or Caudata were encountered. No anurans observed displayed obvious disease. Of the 247 swabs for which DNA was successfully extracted (three swabs were discarded due to labelling ambiguity), all were negative for *Bd* DNA.

DISCUSSION

This study is the first survey for *Bd* in the KMPA, and one of only three *Bd* surveys, to our knowledge, to have been conducted on amphibians in Guyana. The first survey consisted of 22 caecilians swabbed in the Iwokrama Forest Reserve, over 130 km from the KMPA. All caecilians samples were negative for *Bd* (Gower et al., 2013). Luger et al. (2008) sampled 202 harlequin frogs (*Atelopus hoogmoedi*) for *Bd* in the Mabura Hill Forest Reserve, more than 200 km from the KMPA, and found negative results. Our sample size was considerably larger than in the aforementioned studies and we sampled the broad amphibian community rather than a specific taxonomic group. Thus, our survey provides further evidence for the current absence of *Bd* from southern Guyana. The KMPA is over 500 km from the closest *Bd* surveys in Surinam (Luger et al., 2008) and over 700 km from the study sites of Courtois et al. (2012) in French Guiana.

The methodology established for swabbing amphibians was based upon previous field studies, and investigations on the presence of *Bd* in Trinidad and Tobago utilised a similar methodology for PCR analysis by the same laboratory (Strathclyde University, Shepherd et al., 2016; Thomson et al., 2018). Although conventional PCR assay has been considered less sensitive compared to real time assays for the detection of *Bd* DNA (Boyle et al., 2004; Hyatt et al., 2007), an experimental study comparing both methods of detection for *Bd* DNA found conventional PCR and gel-based detection to be as sensitive, and a cost-effective alternative, to real time assays when performing prevalence studies (Garland et al., 2011). Confidence in the *Bd*-negative status of the KMPA is additionally supported by the lack of any overt cutaneous lesions or behaviours associated with chytridiomycosis observed in the field. These include: excessive exfoliation of the skin, ulceration, erythema, abnormal posture, absence of flight response and neurological signs (Van Rooij et al., 2015). It should be noted, however, that chytridiomycosis can be asymptomatic and cause sudden death, and basing disease status on clinical findings alone is an insensitive approach to disease surveillance. Additionally, there is likely to be temporal variability in the infection status of populations, and some populations have the ability to recover from infection (Shepherd et al., 2016; Thomson et al., 2018; Alemu et al., 2008; Alemu et al., 2013). Based on Thursfield (1995), with a sample size of 247, we are 95 % confident of detecting *Bd* in the amphibian population given a prevalence of infection >2 % (as found in South American rainforests by Courtois et al., 2015; James et al., 2015; von May et al., 2018; Mccracken & Forstner, 2009). A larger sample size would be required to detect *Bd* if prevalence is <2 %. Such low levels of infection should be considered as an explanation for why this survey did not identify the presence of *Bd*. Therefore, continued surveillance in the KMPA should be directed to new areas as well as previously monitored sites, and include large sample sizes, to maintain an up-to-date knowledge on the *Bd*- status of the area. Additionally, we would greatly

encourage future researchers and the PAC to address why *Bd* has not reached the KMPA, or if it is existing at very low levels of infection, as this could shed light on important epidemiological characteristics of this infectious disease.

Species dispersal may be limited by geographical barriers such as mountain ranges or oceans. To the west between the KMPA and the nearest forests of Brazil (*Bd*-positive) lies a vast expanse of savannah (Fig. 1b). The average daily temperatures in the savannahs of south-western Guyana and northern Brazil are >25 °C, and in the dry seasons ≥ 27 °C (Bovolenta et al., 2012). *Bd* survival is limited by environmental variables; temperatures above 25 °C limit growth of *Bd* in culture, and sustained periods above 30 °C are incompatible with survival (Piotrowski et al., 2004). There is evidence to suggest amphibians adapted to higher thermal environment can clear *Bd* infections (Woodhams et al., 2003). Therefore, the climate of the savannahs may have a barrier effect on the range expansion of *Bd* from Brazilian rainforest into south-western Guyana. Other reasons behind the lack of *Bd* in the KMPA could be the absence of potential non-amphibian hosts from the area, and the minimal human movement in and out of the KMPA. While further research is necessary to understand non-amphibian hosts and their role in transmission of *Bd* (McMahon et al., 2013), it is reasonable to hypothesize upon their influence on the distribution of *Bd*. Human movement and the pet trade have resulted in *Bd* becoming a global pandemic since its emergence during the early 20th century in the Korean Peninsula (O'Hanlon et al., 2018). Human movement is considered one of the ongoing routes of transmission of *Bd* to new areas (Kriger & Hero, 2009). The low level of human movement in southern Guyana should be considered as a potential limiting factor to the spread of *Bd* into the KMPA. Further investigations should focus on the risk of anthropogenic introduction of *Bd* into the KMPA.

Constant surveillance and strictly implemented biosecurity measures are essential to mitigate the alarmingly rapid spread of *Bd* across Latin America. Human movement and activity has played a key role in the rapid geographical spread of *Bd* (Kriger & Hero, 2009; O'Hanlon et al., 2018), and therefore we hope that the PAC may act as a platform for raising awareness of this risk and promoting good hygiene during field research. A priority should be developing Standard Operating Procedures for the rangers, visiting researchers and field technicians, to include specific instructions regarding hygiene and biosecurity during amphibian work. Basic bio-exclusion principles could be extrapolated from other areas of research such as food production systems (Leibler et al., 2009), human populations (Meyerson et al., 2002) or emerging wildlife diseases (Msami, 2008), though not all possibilities are appropriate for chytridiomycosis; for instance, the use of antifungals in the event of a *Bd* incursion would not be viable in the long term. Phillott et al. (2010) provide a comprehensive review of biosecurity measures recommended for *Bd*.

The combination of high diversity and endemism of amphibians in Guyana make this area a priority for

conservation. Our survey showing the absence of evidence for *Bd* in the KMPA gives an opportunity for the pre-emptive conservation of amphibians in the pristine rainforest of southern Guyana, but it remains true that an outbreak of chytridiomycosis in this area could be catastrophic. PAC personnel, KMPA rangers and local field guides have gained a new awareness of chytridiomycosis as a result of this field work. This survey serves as a first step towards continued monitoring and surveillance of *Bd* in Guyana to encourage implementation of measures to prevent an outbreak. It is recommended that the prevention of chytridiomycosis should be paramount in the conservation agenda of Guyana's Protected Areas Commission.

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Population structure, size and morphometry of the white-lipped mud turtle *Kinosternon leucostomum postinguinale* in pond systems in Quindío, Central Andes of Colombia

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ABSTRACT - We analyzed the population structure, size and morphometry of *Kinosternon leucostomum postinguinale* present in ponds of three localities of La Vieja River basin in the Department of Quindío: National Park of Coffee (NPC, Montenegro municipality), La Palmita village (La Tebaida municipality) and Roble River low area (Quimbaya municipality). Twenty-three days of sampling were conducted from May to October 2017. To locate turtles, we implemented an active search method by walking. Each manually captured individual was measured, weighed and sexed in the field. In total, 61 turtles were captured, most of them in the National Park of Coffee (n = 44). The populations in all three locations were dominated by adults with a 1:1 ratio of females to males. Juveniles (three) were only found in the National Park of Coffee, suggesting that this locality is a refuge for the species. Turtle linear dimensions to body mass relationship was allometric; juveniles grew faster than adults. Preanal and postanal length differed between males and females, allowing the efficient determination in mature turtles. Sexual maturity in both males and females is estimated to be reached at a straight maximum carapace length of 9.5 cm.

INTRODUCTION

Colombia has the seventh highest number of turtles and ranks second after Brazil in the Neotropics (Páez, 2012a; Turtle Taxonomy Working Group, 2017; Forero-Medina et al., 2016) with 34 species (six marine and 28 freshwater). However, little is known about the biology, natural history and distribution of many species. In addition, turtles are considered one of the most threatened vertebrate groups (Gibbons et al., 2000; Páez, 2012a; Turtle Taxonomy Working Group, 2017; Vargas-Ramírez et al., 2017), with more than half of the species in a threat category (Páez, 2012a).

It has been recognised that environmental stressors can alter the structure of animal populations and reduce their size, leading to local extinction (Dodd, 1990; Gibbons et al., 2000; Rueda-Almonacid et al., 2007; Morales-Betancourt et al., 2012). In Colombia, the risks and threats to turtle populations are diverse. These include degradation and loss of their ecosystems, such as ponds and wetlands; the introduction of foreign and exotic species that compete directly and indirectly for resources; indiscriminate hunting and illegal trade including the pet trade and as magic-religious objects (Rueda-Almonacid et al., 2007; Morales-Betancourt et al., 2012; Páez, 2012a). These factors in addition to late sexual maturity and low juvenile survival, means that recovery of turtle populations in their natural environment even under optimum conditions, will be very slow (Rueda-Almonacid et al., 2007; Páez, 2012a). One of the species is *Kinosternon leucostomum*, but most information on population and reproductive aspects are

derived from the subspecies *K. l. leucostomum*, which is distributed from the south-eastern Mexico, to Honduras and Nicaragua (Turtle Taxonomy Working Group, 2017). Although the subspecies *K. l. postinguinale* has a wide distribution from southern Nicaragua to Peru, few studies have been carried out on its ecology (Medem, 1962; Berry & Iverson, 2001; Rueda-Almonacid et al., 2007; Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014). Populations of *K. l. postinguinale* in Colombia are affected by: (1) illegal trafficking in the south-west, due to the pet trade (Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014); (2) transformation of the forest, as in the inter-Andean valleys (Valle del Cauca River and Magdalena River Valley) and the Sinú River (Córdoba), where their habitat has been transformed for pastures, agricultural crops and sugarcane plantations; and (3) the drying of wetlands and water pollution, where freshwater reservoirs have high accumulation of industrial and agricultural pollutants, which could drastically affect the viability of populations (Castaño-Mora et al., 2005; Rodríguez-Murcia et al., 2014). Recently *K. l. postinguinale* has been recorded in the Department of Quindío (Arango-Lozano et al., 2018). However, there are no data on the species' population ecology in this Andean Department, which makes research critical for assessing population status and developing management strategies. The objectives of this study were to evaluate the structure of population, size and shell morphometry of *K. l. postinguinale* in ponds at three locations in the Department of Quindío.



Figure 1. Location of the study area and each of the locations in the Department of Quindío, Colombia; 1 = Roble River low area; 2 = National Park of Coffee; 3 = La Palmita, Pisamal

MATERIALS AND METHODS

The study area included three locations in the La Vieja River basin, which covers the whole of Quindío and part of the Departments of Risaralda and Valle del Cauca, in western central Colombia. The landscape of the basin is dominated by an agriculture (livestock and crops) with numerous tributaries, and small areas of native vegetation, mainly *Guadua angustifolia* forest (Botero-Botero et al.,

2016). The region has two seasons of low rainfall (June-September and January-March) and two of high rainfall (October-December and April-May, > 2000 mm per year) (Román-Valencia, 1993; Ramírez et al., 2014). Our three sites were selected where individuals of *K. l. postinguinale* had previously been observed: (1) Roble River low area (4° 34'34.38" N, 75° 52'5.94" W, 975-1100 m, Roble River sub-basin; (2) National Park of Coffee (NPC, 4°32'22.08" N, 75° 46'6.28" W, 970-1250 m, Espejo River sub-basin),

and (3) La Palmita village, Valle de Pisamal (4° 24'53.05" N, 75° 52'34.43" W, 1000-1200 m, Quindío River sub-basin; Fig. 1).

Sampling was carried out monthly from May to October 2017 at each of the study locations. To locate turtles, we used the method of free-search in the field, using visual and manual inspection, actively looking for turtles in wetlands, swamps and temporary ponds with abundant vegetation (Rueda-Almonacid et al., 2007; Giraldo et al., 2012b; Rodríguez-Murcia et al., 2014). Two people, one on each side of a pond or wetland, inspected the perimeter by touch, feeling for similar shapes to the shell of the turtle (Arango-Lozano et al., 2017). Once detected, each individual was hand-captured and uniquely marked with notches on the marginal shields following Cagle (1939).

For each individual, we recorded the straight maximum carapace length (SCL), straight maximum plastron length (SPL), preanal length (from last edge of plastron to cloaca, PRL), postanal length (from cloaca to edge of tail, POSL), carapace maximum height (CH), straight maximum carapace width (SCW) and body mass (MASS). After measuring, each turtle was released where it was captured in the shortest time possible (to minimise the stress of its handling). Sex was determined based on the preanal and postanal length of the tail, which are greater in males. Adults were sexed if $SCL \geq 10$ cm, since at this size they can be considered mature sexually (Rueda-Almonacid et al., 2007; Rodríguez-Murcia et al., 2014), although we found smaller turtles, which were thought to be juveniles, and after results, we determined them as adult males.

The size and sexual structure of the populations in each of the sampled localities was established, and differences in the presence of adults (males and females) and juveniles among localities were evaluated using an independence test, and the proportions were compared using a goodness-of-fit test (Rodríguez-Murcia et al., 2014; Arango-Lozano et al., 2017). The SCL-body mass relationships for the combined populations were determined by a least squares linear regression after transformation to logarithms. Geometric similarity (retention of shape) was indicated when the regression coefficient was in agreement with 3.0 or when mass was the dependent variable 3.0 (e.g. Meek, 1982; Meek & Avery, 1988).

Based on the adults, the morphometric measurements of males and females between locations were compared using a Kruskal-Wallis multiple comparison test and a subsequent Dunn test (Giraldo et al., 2012a; Arango-Lozano et al., 2017). In addition, the mean morphometric measurements were calculated, and a student's *t*-test was used to determine if there were differences between sexes (Giraldo et al., 2012a; Arango-Lozano et al., 2017). Finally, the relationships of SPL to PRL and PRL to POSL by sex were evaluated with an analysis of covariance (Arango-Lozano et al., 2017). The statistical analyses were carried out in the free use software R version 3.4.2 (R Development Core Team, 2017).

RESULTS

During 504 man-hours of sampling effort (168 man-hours

per site), 61 individuals were captured, 44 in NPC, seven in La Palmita and ten in the Roble River low area (Table 1). There were no significant differences in the sex ratio among study locations (χ^2 independence test = 0.88; df = 2; $P = 0.64$). However, significant differences were found in the adult to juvenile ratio among localities. Each population of *Kinosternon leucostomum postinguinale* was dominated by adults; with juveniles only found in NPC (Table 1).

Table 1. Sex ratios of adults *K. l. postinguinale* and adult to juvenile ratios at the different study locations in Quindío; M = male, F = female, A = adult, J = juvenile. The empty cells indicate samples sizes that are too small for analysis.

| Locality | M | F | J | M:F | χ^2 | P | A:J | χ^2 | P |
|-------------|----|----|---|---------|----------|------|--------|----------|-------|
| NPC | 18 | 23 | 3 | 1:1.27 | 0.609 | 0.43 | 13.6:1 | 32.80 | 0.01 |
| La Palmita | 3 | 4 | - | 1:1.30 | 0.14 | 0.70 | - | - | - |
| Roble River | 6 | 4 | - | 1.5:1.0 | 0.40 | 0.52 | - | - | - |
| Total | 27 | 31 | 3 | 1:1.15 | 0.27 | 0.59 | 19.3:1 | 49.59 | <0.01 |

The straight maximum carapace length to body mass was allometric in *K. l. postinguinale* ($M = 0.1143L^{3.06}$; $r^2 = 0.967$; $P < 0.01$; Fig. 2). Also, when performing the multiple comparison analysis between localities, significant differences were found in PRL sizes for females of Roble River low area and NPC ($Q = 1.94$; $P = 0.02$), for CH in both females of La Palmita and Roble River ($Q = -2.3$; $P = 0.01$), and of Roble River and NPC ($Q = 2.03$; $P = 0.02$). In each of the measures, the sizes were greater for females of Roble River low area. On the other hand, for males no significant differences were found for any of the morphometric variables among the locations (Tables 2 and 4).

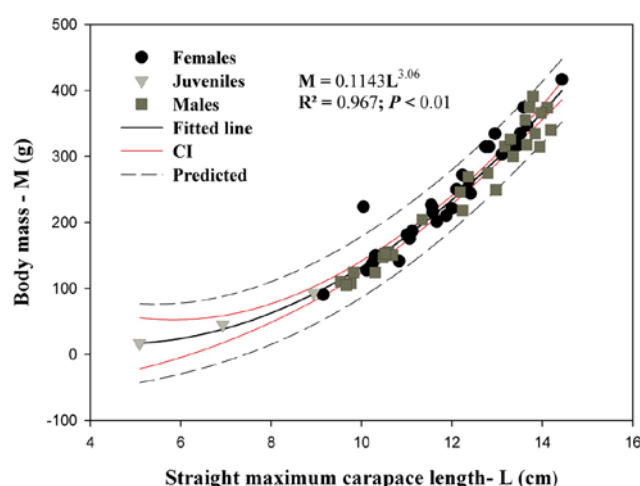


Figure 2. Relationship of the straight maximum carapace length (L) and body mass (M) variables in 61 individuals of *K. l. postinguinale*

From the analysis of comparison of morphometric variables only preanal length ($t = 14.20$; $P < 0.01$) and postanal length ($t = 14.6$; $P < 0.01$), differed by sex, being larger in males (Tables 3 and 4). The preanal region was greater in males than in females of similar sizes. The relationship

Table 2. Multiple comparison of size of the morphometric variables for males and females of *K. l. postinguinale* among the study localities. Q = Dunn test. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass. Values in bold type indicate a difference in greater size (e.g PRL) between two compared populations (e.g Roble River – NPC).

| Variable | Comparison | Males | | Females | |
|-----------|--------------------------|-------|------|---------|-------------|
| | | Q | P | Q | P |
| SCL (cm) | La Palmita – Roble River | 0.56 | 0.28 | -1.04 | 0.14 |
| | La Palmita - NPC | 1.30 | 0.09 | 0.03 | 0.48 |
| | Roble River - NPC | 0.87 | 0.19 | 1.40 | 0.08 |
| SPL (cm) | La Palmita - Roble River | 0.03 | 0.48 | -0.70 | 0.24 |
| | La Palmita - NPC | 0.49 | 0.31 | 0.43 | 0.33 |
| | Roble River - NPC | 0.60 | 0.27 | 1.34 | 0.08 |
| PRL (cm) | La Palmita - Roble River | -0.23 | 0.40 | -1.36 | 0.08 |
| | La Palmita - NPC | -0.21 | 0.41 | 0.17 | 0.40 |
| | Roble River - NPC | 0.07 | 0.47 | 1.94 | 0.02 |
| POSL (cm) | La Palmita - Roble River | 0.16 | 0.43 | -0.56 | 0.28 |
| | La Palmita - NPC | -0.51 | 0.30 | -0.80 | 0.20 |
| | Roble River - NPC | -0.92 | 0.17 | -0.09 | 0.46 |
| CH (cm) | La Palmita - Roble River | 1.00 | 0.15 | -2.30 | 0.01 |
| | La Palmita - NPC | 1.33 | 0.09 | -1.03 | 0.14 |
| | Roble River - NPC | 0.25 | 0.40 | 2.03 | 0.02 |
| SCW (cm) | La Palmita - Roble River | 0.80 | 0.21 | -0.66 | 0.25 |
| | La Palmita - NPC | 1.10 | 0.13 | 0.42 | 0.33 |
| | Roble River - NPC | 0.26 | 0.39 | 1.28 | 0.09 |
| MASS (g) | La Palmita - Roble River | 0.47 | 0.31 | -0.38 | 0.34 |
| | La Palmita - NPC | 1.13 | 0.12 | 0.87 | 0.19 |
| | Roble River - NPC | 0.78 | 0.21 | 1.38 | 0.08 |

Table 3. Comparison of size of morphometric variables between sexes of *K. l. postinguinale*; n = Number of samples; “ \bar{x} ” = Mean; t = value of the Student's t -test. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass. Values in bold type indicate significant differences of size (e.g SCL) between sexes.

| Variable | Males | | Females | | t | P |
|-----------|-------|-------|---------|--------|-------|------------------|
| | n | | n | | | |
| SCL (cm) | 27 | 12.2 | 31 | 11.95 | 0.78 | 0.43 |
| SPL (cm) | 27 | 10.61 | 31 | 10.73 | 0.34 | 0.73 |
| PRL (cm) | 27 | 1.68 | 31 | 0.80 | 14.20 | < 0.01 |
| POSL (cm) | 27 | 1.97 | 31 | 0.99 | 14.60 | < 0.01 |
| CH (cm) | 27 | 4.43 | 31 | 4.18 | 1.21 | 0.22 |
| SCW (cm) | 27 | 7.85 | 31 | 7.77 | 0.30 | 0.76 |
| MASS (g) | 27 | 249.9 | 31 | 241.80 | 0.34 | 0.73 |

between SPL and LPR in adults, differed significantly by sex (Ancova; $F = 273.3$; $P < 0.001$, Fig. 3). Both the influence of the covariate SPL ($F = 5.7$; $P = 0.02$) and the interaction of sex and the covariate SPL were significant ($F = 10.7$; $P = 0.0018$). Thus, the length of the preanal region in males is not correlated with SPL ($P1 = 0.003Spl + 1.65$; $r^2 = 0.0002$; $P = 0.93$; $n = 27$), but it is in females ($P1 = 0.14Spl - 0.73$; $r^2 = 0.79$; $P < 0.001$; $n = 31$; Fig. 3).

The relationship of PRL and POSL also differ significantly by sex (Ancova; $F = 375.8$; $P < 0.001$; Fig. 4). Both the influence of the covariate PRL ($F = 16.06$; $P < 0.001$) and the interaction of sex and the covariate PRL were significant ($F = 18.3$; $P < 0.001$). Length of the postanal region in males is correlated with PRL ($Posl = 0.94Prl + 0.37$; $r^2 = 0.53$; $P < 0.001$; $n = 27$), but not in females ($Posl = -0.03Prl + 1.02$; $r^2 = 0.003$; $P = 0.75$; $n = 31$; Fig. 4).

DISCUSSION

Most of turtles were captured in the NPC, possibly due to the protection offered by the National Park of Coffee Foundation; where fishing and hunting are prohibited and there is 24 hour surveillance, which favours both *Kinosternon leucostomum postinguinale* and *Chelydra acutirostris* as reported by Arango-Lozano et al. (2017). In addition, according to Rodríguez-Murcia et al. (2014), a factor that affects the population size of *K. l. postinguinale* is hunting for human consumption and by the pet trade. Therefore, it is possible that the low number of encounters in La Palmita and Roble River low area is due to free access by fishermen and hunters. The greater incidence of adults with respect to juveniles captured in this study may reflect to the search methodology, which might be biased against smaller individuals, as indicated by Rodríguez-Murcia et al. (2014). Also, the presence of juveniles only in NPC may be due to its refuge status but possibly the white-lipped mud turtle has some different habitat features not found elsewhere.

All adults in the study populations of *K. l. postinguinale* in Quindío had a sex ratio close to parity. Similar information to that reported in populations of white-lipped mud turtles in the Department of Caldas by Rodríguez-Murcia et al. (2014) where although the proportion in some ponds was skewed to males, the general population was 1:1; all the populations studied in Valle del Cauca were 1:1 Vallejo (2013). Since *K. leucostomum* exhibits a pattern of temperature-dependent sex determination (Yntema, 1979), with clutches incubated at constant temperatures above 28 °C producing only females (Ewert & Nelson, 1991; Vogt & Flores-Villela, 1992), it is possible that temperature in the clutches of the Department of Quindío follow a pivotal parameter, where it is stable enough not to bias the sex of neonates and to a certain extent, be reflected in the adult population (Valenzuela & Ceballos, 2012, Rodríguez-Murcia et al., 2014). Also, skewed sex ratios in adults may simply be a product of mortality rates in juveniles (Páez, 2012a, b; Ewert & Nelson, 1991; Arango-Lozano et al., 2017).

Relationship of linear dimension size to body

Table 4. Mean, minimum (Min) and maximum (Max) values of morphometric variables of adults and juveniles of *K. l. postinguinale*, captured in the study localities. SCL = straight maximum carapace length; SPL = straight maximum plastron length; PRL = preanal length; POSL = postanal length; CH = carapace maximum height; SCW = straight maximum carapace width; MASS = body mass

| NPC | Variable | N | Mean | Min | Max |
|------------------------|-----------|----|--------|--------|--------|
| Males | SCL (cm) | 18 | 11.94 | 9.53 | 14.19 |
| | SPL (cm) | 18 | 10.44 | 8.66 | 13.30 |
| | PRL (cm) | 18 | 1.71 | 1.34 | 2.79 |
| | POSL (cm) | 18 | 2.02 | 1.37 | 2.70 |
| | CH (cm) | 18 | 4.40 | 3.25 | 6.65 |
| | SCW (cm) | 18 | 7.76 | 6.36 | 9.69 |
| | MASS (g) | 18 | 233.11 | 107.72 | 391.20 |
| Females | SCL (cm) | 23 | 11.76 | 9.15 | 13.66 |
| | SPL (cm) | 23 | 10.58 | 8.19 | 12.56 |
| | PRL (cm) | 23 | 0.77 | 0.45 | 1.04 |
| | POSL (cm) | 23 | 1.00 | 0.67 | 1.17 |
| | CH (cm) | 23 | 4.12 | 2.56 | 5.06 |
| | SCW (cm) | 23 | 7.67 | 5.56 | 9.34 |
| | MASS (g) | 23 | 228.71 | 90.70 | 374.20 |
| Juveniles | SCL (cm) | 3 | 6.99 | 5.09 | 8.94 |
| | SPL (cm) | 3 | 5.96 | 4.30 | 7.81 |
| | PRL (cm) | 3 | 0.45 | 0.40 | 0.50 |
| | POSL (cm) | 3 | 0.47 | 0.40 | 0.50 |
| | CH (cm) | 3 | 2.65 | 1.71 | 3.68 |
| | SCW (cm) | 3 | 4.91 | 3.69 | 6.17 |
| | MASS (g) | 3 | 51.93 | 17.00 | 93.50 |
| La Palmita | Variable | N | Mean | Min | Max |
| Males | SCL (cm) | 3 | 13.27 | 12.35 | 14.11 |
| | SPL (cm) | 3 | 11.25 | 10.76 | 11.51 |
| | PRL (cm) | 3 | 1.61 | 1.38 | 1.83 |
| | POSL (cm) | 3 | 2.01 | 1.84 | 2.11 |
| | CH (cm) | 3 | 4.78 | 4.42 | 5.30 |
| | SCW (cm) | 3 | 8.36 | 7.59 | 8.82 |
| | MASS (g) | 3 | 314.33 | 269.00 | 374.00 |
| Females | SCL (cm) | 4 | 11.82 | 10.04 | 12.82 |
| | SPL (cm) | 4 | 10.75 | 10.45 | 11.04 |
| | PRL (cm) | 4 | 0.77 | 0.75 | 0.79 |
| | POSL (cm) | 4 | 0.98 | 0.91 | 1.12 |
| | CH (cm) | 4 | 3.76 | 3.40 | 4.42 |
| | SCW (cm) | 4 | 7.81 | 7.69 | 7.99 |
| | MASS (g) | 4 | 259.63 | 223.90 | 314.60 |
| Roble River (low area) | Variable | N | Mean | Min | Max |
| Males | SCL (cm) | 6 | 12.51 | 9.66 | 13.94 |
| | SPL (cm) | 6 | 10.83 | 8.54 | 12.03 |
| | PRL (cm) | 6 | 1.68 | 1.46 | 1.83 |
| | POSL (cm) | 6 | 1.81 | 1.19 | 2.15 |
| | CH (cm) | 6 | 10.83 | 8.54 | 12.03 |
| | SCW (cm) | 6 | 7.85 | 6.36 | 8.73 |
| | MASS (g) | 6 | 268.18 | 104.80 | 374.20 |
| Females | SCL (cm) | 4 | 12.89 | 10.83 | 14.43 |
| | SPL (cm) | 4 | 11.62 | 9.01 | 13.23 |
| | PRL (cm) | 4 | 1.03 | 0.71 | 1.21 |
| | POSL (cm) | 4 | 0.99 | 0.85 | 1.18 |
| | CH (cm) | 4 | 5.01 | 4.00 | 5.47 |
| | SCW (cm) | 4 | 8.30 | 6.87 | 9.32 |
| | MASS (g) | 4 | 299.75 | 141.70 | 416.70 |

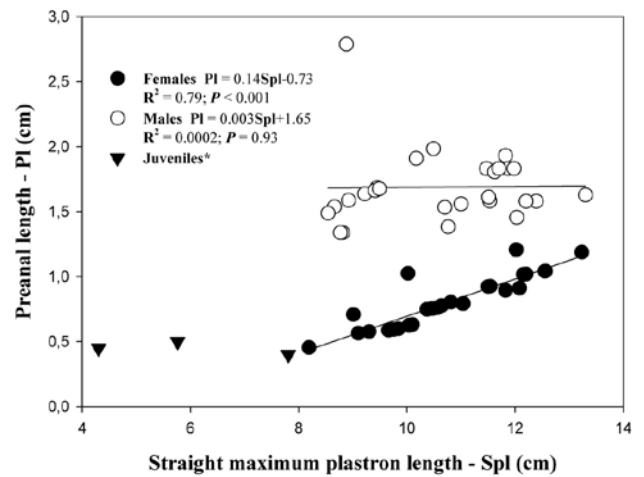


Figure 3. Relationship of straight maximum plastron length and preanal length in 27 males and 31 adult females of *K. l. postinguinale*. *Juveniles, were not include in the statistical analysis.

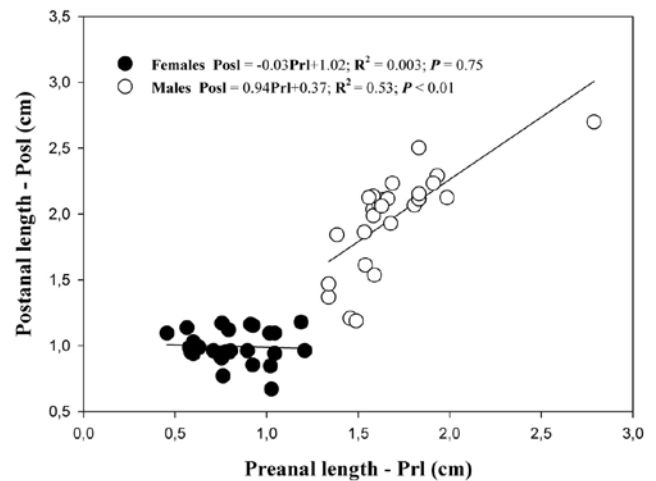


Figure 4. Relationship of the preanal length and postanal length in 27 males and 31 adult females of *K. l. postinguinale*

mass, showed that growth of the 61 individuals of *K. l. postinguinale* was allometric but between linear shell measurements was isometric. When reaching approximately SCL = 10 cm growth slows. Thus, while juveniles invest more energy in growth, adults invest more in reproduction (Congdon & Gibbons, 1990; Páez, 2012b; Arango-Lozano et al., 2017). On the other hand, multiple comparison of morphometric variables between the study localities did not record differences in the size of males, whereas, in females, only The Río Roble low area had larger measures of PRL and CH than those in La Palmita and NPC. However, the small sample size ($n = 4$) for females from La Palmita and Roble River limited our ability to detect differences between localities.

PRL and POSL were strongly sexually dimorphic in *K. l. postinguinale*, with males having larger preanal and postanal lengths than females. Rodríguez-Murcia et al. (2014), reported the same pattern in the Department of Caldas. The longer tail in the adult male allows it to grasp

the female during copulation by means of a pronounced corneal nail at the end of the tail (Giraldo et al., 2012b). The larger preanal region in males reflects the location of the penis (Miller & Dinkelacker, 2007).

Between sexes, there is a significant difference in the growth of preanal length, which is greater in males, presumably since they reach the maturity size (8 cm SPL), PRL remains at a relatively constant value as they grow, while in females, it grows scaled to body size. However, when the PRL is very small (Juveniles, Fig. 3), there is no evidence of its growth to scale in the males, which could be explained by both; very few data of juveniles in this study, and the difficult measurement of small live animals in the field, since the tail of the turtle can be stretched inwards or outwards, which makes accuracy difficult.

Rueda-Almonacid et al. (2007) reported the size at maturity is 10 cm SCL and 8 cm SPL for females and 12 cm SCL and 10 cm SPL for males. However, based on our data for PRL and POSL (Figs. 3, 4), we suggest that sexual maturity may be reached by 9.5 cm SCL and 8.5 cm SPL, at least in males in Quindío, although this should be confirmed by anatomical studies. Similarly, further research on population density, habitat use, and reproductive ecology in *K. l. postinguinale* are needed in order to generate management strategies for this species at least at the local level.

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The origin of common wall lizards (*Podarcis muralis*) in Wales

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Common wall lizards (*Podarcis muralis*) are not native to Britain, but the species has been repeatedly introduced (Michaelides et al., 2013). A genetic study of 23 populations, which represents the large majority of extant populations, revealed at least nine primary introductions from France and Italy (Michaelides et al., 2015). The remaining populations are more likely to be translocations of animals from already established non-native populations or repeated introductions from the same captive stock. All except for one of the known wall lizard populations in Britain are found in England. The sole Welsh population exists on a private estate on Gower peninsula, and is of unknown origin.

The Welsh population was not included in the original study of wall lizard origins in Britain due to access restriction. Recently, lizards have been encountered outside of the estate. We took opportunity of this to sequence a region of the cytochrome b (cyt-b) gene in the mitochondrial genome of two adult individuals. A male and a female (Fig. 1) were captured and the outer tip (ca 1 cm)

of the tails was removed by gently squeezing with a pair of tweezers. DNA was extracted and approximately a 700 base pair (bp) region of the cyt-b gene was amplified using the same protocol as in our previous work (Michaelides et al., 2015; Michaelides et al., 2013). The two sequences were corrected by eye, aligned and trimmed to a uniform length of 655 bp in GENEIOUS R8.1.9 (Kearse et al., 2012). We constructed a parsimonious phylogenetic network using a median-joining algorithm in NETWORK V.4.6.1.1 (Bandelt et al., 1999) and with an extensive coverage of mitochondrial haplotypes (see Michaelides et al., 2015), we could assign the genetic affinity of the population to other non-native and native populations. New sequences used in this study were submitted to GenBank under the accession numbers MK124973-MK124976.

The two individuals from Wales shared the same haplotype (WALES1). The haplotype network revealed that the Welsh haplotype belongs to the Romagna clade in the native range and is identical to the ROM1 haplotype (Fig. 1). Although the Romagna clade occurs in at least

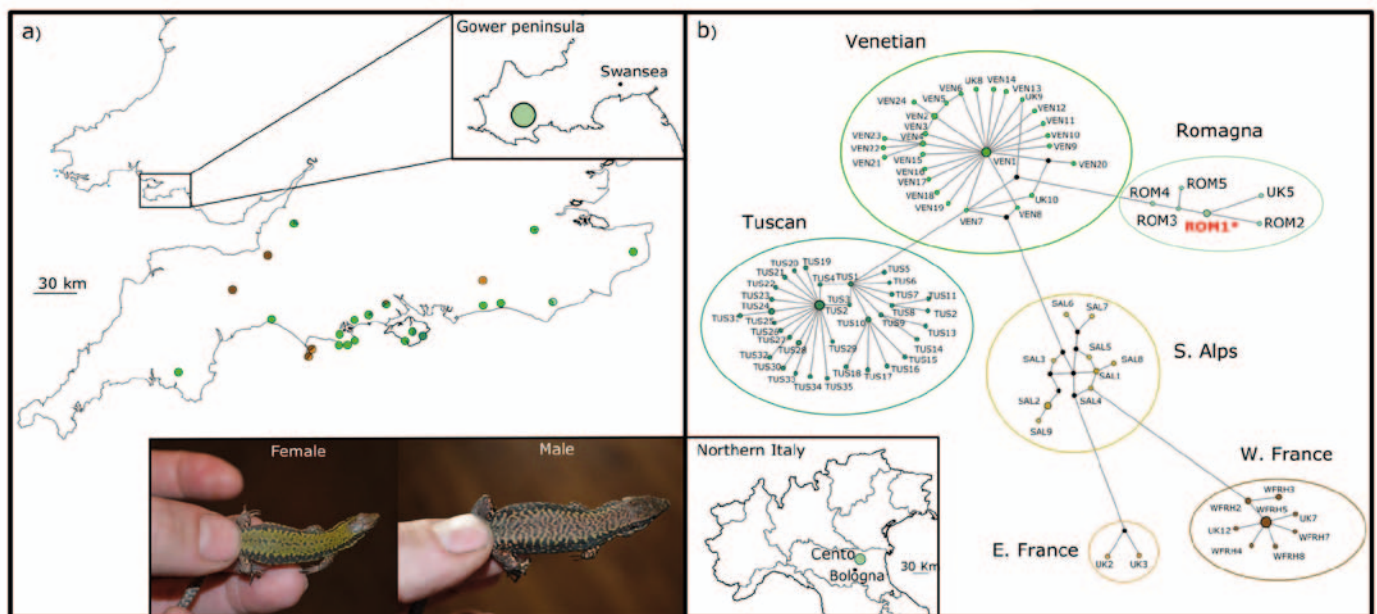


Figure 1. (A) Distribution of non-native populations of *P. muralis* in southern England and the sole Welsh population on Gower peninsula with coloration corresponding to mtDNA lineage origin (see Michaelides et al. 2013 for details). Photographs of the two adults (male and female) used in this study. **(B)** Median-joining network of mitochondrial haplotypes forming six haplogroups of native-range lineages (adapted from Michaelides et al., 2015, with three additional haplotypes; ROM3-ROM5, GenBank accession numbers MK124973, MK124974, MK124976). The haplotype found in Wales belongs to the Romagna lineage and is identical to ROM1 (marked in red) that is found in animals from Cento, northern Italy.

two populations in England, the Welsh haplotype has not been encountered in non-native populations in England (Michaelides et al., 2013; Fig. 1a). This suggests that the Gower population represents a primary introduction. The likely geographic origin is near Cento, Italy, close to the Bologna - Modena - Ferrara region, not very distant from many of the other Italian source populations (Michaelides et al., 2013; Michaelides et al., 2015). The persistence of lizards from substantially warmer climate on the Gower peninsula is further evidence of the highly adaptable nature of common wall lizards (While et al., 2015).

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Reproductive biology of *Ptychozoon lionotum* Annandale, 1905 (Squamata: Gekkonidae) from Northern Peninsular Malaysia

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Lizards from genus *Ptychozoon* are highly arboreal, having the ability to glide from one location to another. Three species of gecko from the genus *Ptychozoon*, including *Ptychozoon horsfieldii*, *P. kuhli* and *P. lionotum*, can be found in the rainforest of Peninsular Malaysia (Grismer, 2011a). Another species, *P. rhacophorus* is endemic to Borneo, East Malaysia (Min & Das, 2012). *Ptychozoon lionotum* is a medium-sized gecko, with a snout-vent length of 89 mm and 81 mm; males and females respectively (Grismer, 2011a). This nocturnal species ranges from India (Pawar & Biswas, 2001), Myanmar, Thailand and Cambodia, south through the Peninsular Malaysia (Das & Vijayakumar, 2009). In Peninsula Malaysia, this arboreal species can be encountered living in primary and old secondary lowland dipterocarp forests up to 300 m a.s.l. (Dring, 1979; Grismer, 2011b).

Data on the reproductive biology of lizards from the genus *Ptychozoon* has been documented by several researchers. Das (2007), recorded the egg diameter of *P. horsfieldii* and *P. kuhli* from Brunei, which was 13.7 x 11.9 mm and 11 - 15 mm respectively. Two eggs of *P. horsfieldii* were found attached to a branch on the floor of dipterocarp forest. The hatchlings measured snout-vent length (SVL) was 34 mm (Das, 2007). Two eggs of *P. kuhli* were found attached to a tree trunk, hatching in 73 days (Das, 2007). Grismer (2011a), reported pairs of *P. kuhli* eggs stuck together on tree trunks, 2 - 4 m above ground in shaded areas. Wood (2007), also found a pair of *P. kuhli* eggs on the shaded underside of a granite rock, approximately one meter above ground. Min & Das (2012) had collected two eggs of *P. rhacophorus* from the forest floor in Gunung Penrissen, Sarawak. The eggs were spherical in shape, fused together and deposited on the surface of a dry leaf. The eggs measured 10 x 10.8 mm and 9.99 x 10.3 mm, while the total length hatched geckos was 50.6 mm and 52.0 mm. However, to the best of my knowledge, there is no information on the reproductive biology of *P. lionotum*.

On 6 July 2017, between 2100 and 2200 hours (MST), a gravid *P. lionotum* female was captured at Sungai Sedim Recreational Forest, Kedah, Peninsular Malaysia (05° 25'N, 100° 46'E; < 150 m a.s.l.) (Fig.1). The specimen was collected while perched on the wall of wooden chalet, approximately 0.5 m above ground, after intermediate rain. The area was shaded, surrounded by lowland dipterocarp forest, and located about 10 m from the main river. Two individuals of *Hemidactylus frenatus* were also sighted



Figure 1. An adult female of *P. lionotum* from Sungai Sedim, Kedah, Peninsular Malaysia

around this area. Air temperature and relative humidity were 29 °C and 62 % respectively (measured by Thermo-hygro meter, Fisher Scientific). The lizard was captured by hand and taken to the laboratory for further inspections. The SVL=78 mm, tail length (TaL=82 mm), total length (ToL=160 mm) and head width (HW=23 mm) of the gecko was measured using a digital calliper.

In the laboratory, the female gecko was placed in a terrarium (60 x 30 x 30 cm). Tree bark, branches and leaf litter were placed in the terrarium as shelter. The gecko was

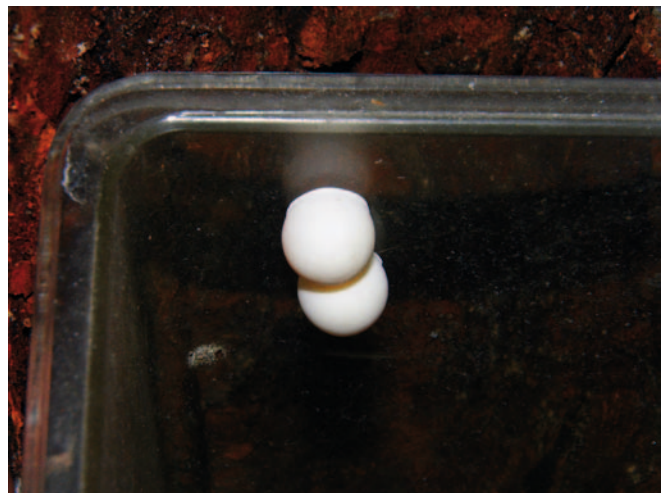


Figure 2. The egg clutch of *P. lionotum*



Figure 3. A newly hatched and unhatched egg of *P. lionotum*



Figure 4. A hatchling *P. lionotum*

fed on small crickets once a day (at night). On 16 July 2017 (10 days after capture) a clutch of two eggs were found. The two eggs were attached, whitish in colour, spherical in shaped and having a flattened bottom. The eggs were glued tightly to the wall of the terrarium (Fig. 2). The length and diameter of the eggs were 9 x 9 mm and 10 x 9 mm, respectively (measured by using digital calliper). After four days, the female gecko was found dead. The specimen (17USM-SS-PL01) was fixed with 10 % formalin, preserved in 70 % ethanol, and deposited at the School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM) for reference.

The gecko eggs were reared in the terrarium. After 45 days (on 31 August 2017) one of the eggs was hatched (Fig. 3 and 4). The other was hatched on the following day (1 September 2017). The interval between hatching of the two eggs was approximately 30 hours. The SVL, TaL, ToL and HW of the newly hatched geckos were 26, 27, 53 and 7 mm for the first specimen, and 25, 27, 52 and 7 mm for the second specimen. After 10 days, the SVL, TaL, ToL and HW of the geckos became 30, 32, 62 and 7 mm for the first specimen and 30, 33, 63 and 8 mm for the second specimen. Later, both lizards were released back at the capture point.

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Axanthism in *Emys orbicularis hellenica* (Valenciennes, 1832) (Testudines: Emydidae) from Piedmont, northern Italy

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The European pond Turtle *Emys orbicularis* (Linnaeus, 1758) is a polytypic and polymorphic species with a western palearctic distribution for which several subspecies were described based on molecular and morphological differences (Lenk et al., 1999; Fritz, 2003). In Italy, three subspecies are currently recognised: *E. o. ingauna* Jesu, Piombo, Salvidio, Lamagni, Ortale & Genta, 2004 in Liguria, *E. o. galloitalica* Fritz, 1995 along the Tyrrhenian coast and *E. o. hellenica* (Valenciennes, 1832) on the Adriatic one (Zuffi et al., 2011).

In the latter, the coloration is typically characterised by a black carapace with yellow elements (spots in males and lines organised in radial pattern in females) and a yellow plastron. Yellow areas are also present on soft parts like specks on limbs and also on the head in females. Iris coloration is usually white or yellow in males (Zuffi et al., 2011), sometimes reddish (R. Cavalcante pers. obs.), while females typically possess a yellow iris (R. Cavalcante pers. obs.). As in other Mediterranean subspecies, *E. o. hellenica* hatchlings present a well-defined yellow pattern on the marginal scutes of the carapace and the plastron is covered for at least two-thirds by a black central blotch, leaving yellow coloration only on the lateral rim (Fritz et al., 2006).

Given that *E. o. hellenica* is at risk of local extinction in Piedmont (Zuffi et al., 2011, Seglie & Cavalcante, 2016), the Conservation Centre “Centro Emys Piemonte” has been implemented in 2016, provided with an outdoor breeding facility. The main activity of the Centre was to rescue adult individuals in the Po and Orba natural reserves to create breeding groups, which would then provide 3-4 years old individuals to be reintroduced in restored habitats. All of these operations were authorised by the Italian Ministry of Environment, Land and Sea (Protocol number 0015025/PNM-28/07/2015). A molecular characterisation was carried on rescued individuals by Ana Rodriguez Prieto at Museo delle Scienze (MUSE) in Trento, which ascribed all the turtles to the *hellenica* subspecies. The outdoor breeding facility is designed to allow the collection of the eggs right after the deposition for artificial incubation. However, not all the nests are easy to spot, and some litters are discovered only at the time of hatching.

On 3 September 2017 during an inspection of these nests, in an area where three females and one male were kept, two out of twenty-nine hatchlings were found to display an anomalous coloration. In these individuals,

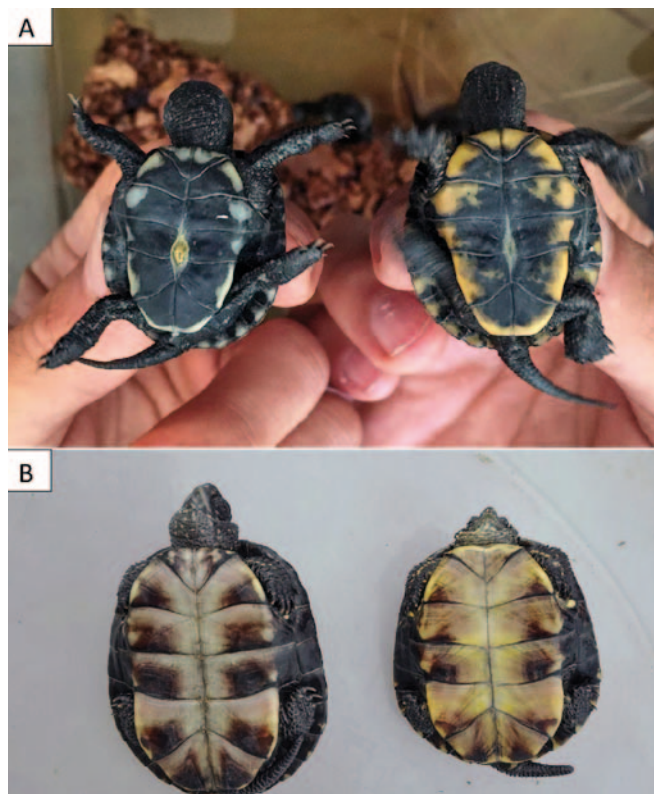


Figure 1. Comparison of the plastron of an axanthic individual (left) with the one of a normal coloured individual (right): **(A)** hatchlings in September 2017; **(B)** one-year old juveniles in September 2018

portions of the carapace, plastron and soft parts that are typically yellow present a whitish-greyish coloration (Fig. 1A), and the iris is dark brown (Fig. 2A). This particular coloration was never observed before in wild individuals or in other hatchlings born in the Centre (10 in 2016, 38 in 2017 and 44 in 2018).

Yellow, orange and red coloration in reptiles are produced via the interaction between xanthophores and the underlying iridophores (Cooper & Greenberg, 1992; Morrison et al., 1995; Steffen & McGraw, 2009). Xanthophores contain pteridines like xanthopterin, sepiapterin and riboflavin inside pterinosomes and carotenoids like carotenes and xanthophylls in carotenoid vesicles (Obika & Bagnara, 1964; Watt, 1964; Bagnara & Hadley, 1973; Morrison et al., 1995). Iridophores contain guanine crystals responsible for structural coloration given

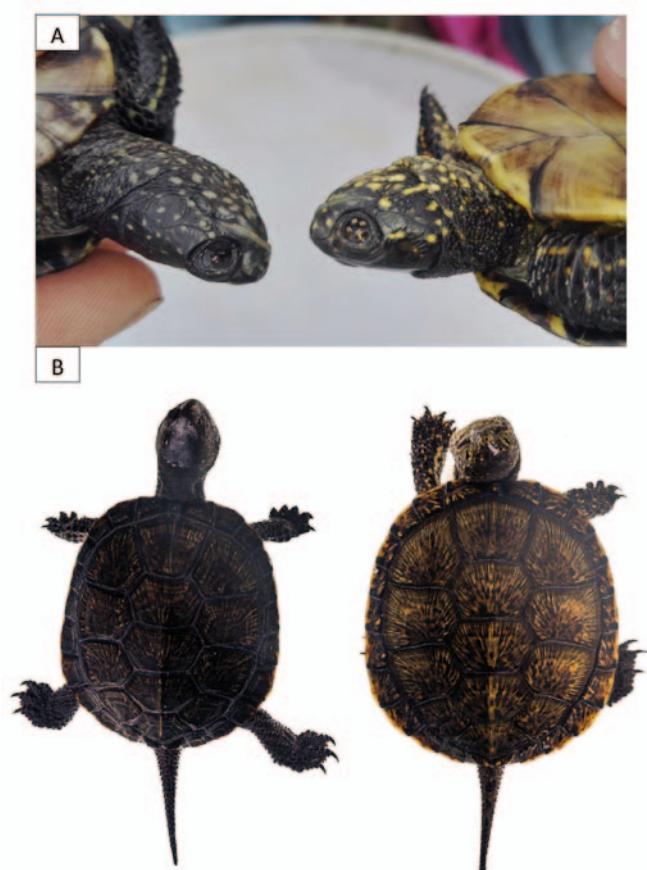


Figure 2. Comparison between one-year old axanthic (left) and normal coloured (right) individuals: **(A)** coloration of head and eyes; **(B)** coloration of the carapace

by light reflecting properties which depends on platelets' size and disposition (Bagnara, 1966). Regarding pigments within xanthophores, pteridine pigments have a major role in reptiles, while carotenoids seem to be less important (Olsson et al., 2008; Steffen & McGraw, 2009) and sometimes to be even absent (Kikuchi & Pfennig, 2012; Olsson et al., 2013). While carotenoids are assimilated from the diet (Olson & Owens, 1998), pteridine pigments are endogenously synthesised from purines (Watt, 1967). Colour aberration for which a reduction or absence of yellow occurs is known as axanthism, and it can be generated through a lack or non-functionality of xanthophores and iridophores (Jablonski et al., 2014).

Considering that the pale coloration was maintained by both individuals in their first year of life, whilst becoming slightly yellow (Figs. 1B and 2B), we assume that xanthophores are present and that this mild "yellowing" may be caused through carotenoids sequestered from the diet, since they have been also fed with KOI beauty First (Tetra®), food pellets which contain β -carotene. Therefore, it is probable that the observed axanthism involves primarily iridophores or some metabolic defects in pteridine production (Olsson et al., 2013).

In literature, axanthism and other chromatic anomalies were never reported before in the European pond Turtle. Axanthism seems also rare in general in testudines, since only cases of albinism (e.g. Türkozan & Durmuş, 2001; Sönmez & Özdilek, 2011), leucism (e.g. Erickson &

Kaefer, 2015), melanism (e.g. Yabe, 1994; Gronke et al., 2006), hypomelanism (e.g. Turner, 2011; Di Giuseppe et al., 2014) and amelanism (e.g. Martínez Silvestre & Soler, 2001) are currently published.

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Observation of limb development in the Oita salamander, *Hynobius dunni*

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The Oita salamander, *Hynobius dunni* is endemic to eastern Kyushu, and distributed mainly in the central area of Kyushu island (Sugawara et al., 2018). The species has a number of interesting characteristics that have attracted the attention of biologists, including cannibalism and development (Michaels, 2015, 2017). The breeding season commences in December until the end of April, with peak activity in February (Sparreboom, 2014). We had an opportunity to observe embryos from Kunisaki Peninsula, Kyushu on February 15, 2018 (Fig. 1A) in an artificial environment for comparative study with the northern species of *Hynobius*, Hokkaido salamander, *H. retardatus* and southern species, Oita salamander, *H. dunni*. An aquarium for the developing embryos was deposited inside a wine cellar used as a cool environment (Foresta Japan, ST-SV140G), which maintained water temperature at 13° C. We observed and analyzed several developmental stages from both embryos and larvae of *H. dunni* to after metamorphosis according to normal development of *H. nigrescens* (Fig. 1 B, D) (Iwasawa & Yamashita, 1991).

Pond-type (larval adaptation to quiet stream), hynobiid salamanders have an interdigital membrane (IM), which is a fin-like structure that forms between digits 1 and 2 during early limb development and disappears as limb development proceeds (Iizuka et al., 2005; Iizuka, 2009). Only the pond adapted species of hynobiid salamanders stand out among hynobiid salamanders in having IM as vestigial organ. A phylogenetic analysis, including using our observations along with that available for other hynobiid and non-hynobiid salamanders indicated the presence of vestigial IM during limb development of the forelimbs on 51 stages of larva (Fig. 1C). This leads us to conclude that 1) an IM is probably characteristic of cryptobranchoid salamanders, 2) the IM has some functional significance in pond larva habitat, a function that is absent in fast stream larva habitat, and 3) the IM has been recognised or reduced two or three times independently in cryptobranchoid lineages (Iizuka et al., 2005; Iizuka, 2009). However, the evolutionary implication of IM require discussion within the genera *Hynobius* and *Salamandrella* as sister taxa. We previously

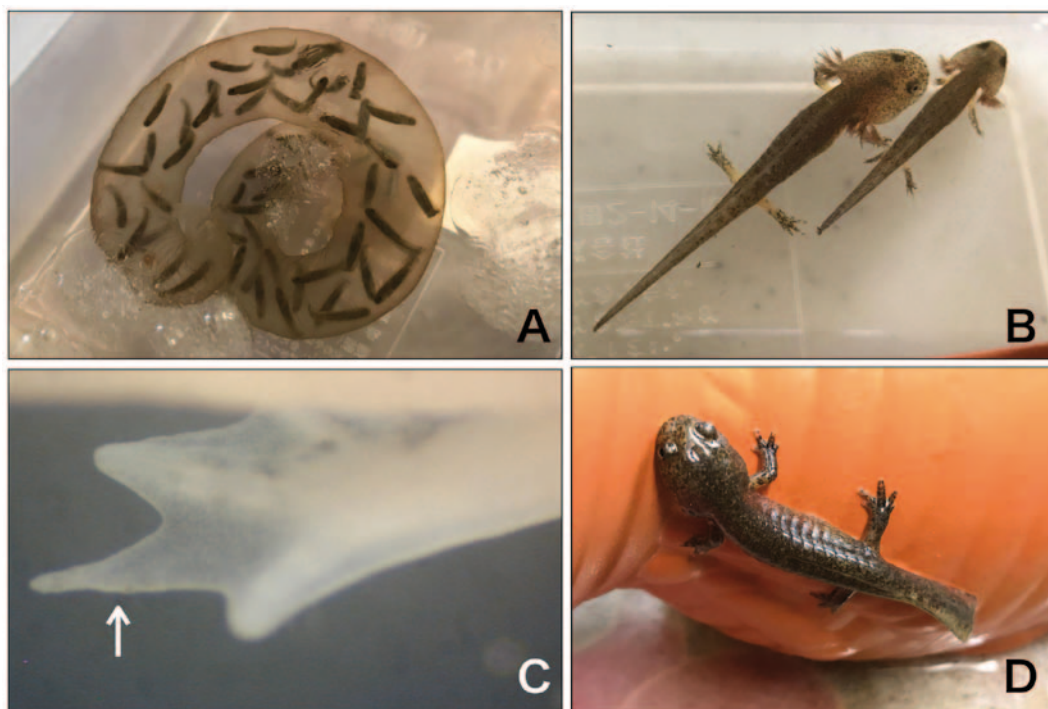


Figure 1. (A) eggs, (B) two different sized larvae, (C) white arrow indicates vestigial organ, interdigital membrane of left forelimb, (D) specimen just after metamorphosis but with lost tail tip.

published an integrative analysis of hynobiid salamanders with IM for adaptation of larvae to an aquatic environment (Iizuka et al., 2016). However there is currently no information about IM in hynobiids endemic to Kyushu and this paper presents the first observation of vestigial IM based on limb development in *Hynobius* from this area (Iwasawa & Yamashita, 1991).

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Report of red-eared slider turtle (*Trachemys scripta elegans*) from a wetland near Kolkata, West Bengal, India

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The natural distribution range of the red-eared slider turtle, *Trachemys scripta elegans* (Wied-Neuwied, 1839), includes south-eastern USA and north-eastern Mexico (Van Dijk et al., 2012). Historical records date back to early 1950s when the species was reported to be introduced by the pet trade into Ontario, Canada (Lamond, 1994). It rapidly became a commonly sold turtle all over the world. Over 52 million *T. scripta elegans* were exported by the pet trade from USA to foreign pet trade markets during 1989-1997 (Telecky, 2001) and more than 23 million during 1998-2002 (Schlaepfer et al., 2005). France alone imported 4,238,809 young turtles between 1985 and 1994, with a maximum of 1,878,809 in 1989-1990 (Warwick, 1991). Owners unable to house them when they reach large size simply released them. This practice has been noted in Europe (Warwick, 1991), Africa (Newberry, 1984), Asia (De Silva & Blasco, 1995; Moll, 1995; Warwick, 1991) and Australia (Ramsay et al., 2007). In 2013, a shipment of as many as 10,000 hatchlings of *T. scripta elegans* was seized at the Kolkata International Airport, West Bengal, India by the Customs department which was being smuggled into India (Mukherjee, 2013), highlighting the magnitude of *T. scripta elegans* being brought into India. Here we report on *T. scripta elegans* from a water body in the Rajarhat wetlands of Kolkata, India.

The Rajarhat wetlands (22°60'32" N, 88°47'38" E, WGS 84, elev. 1 m) is situated in North 24 Parganas district, West Bengal, India. On 19 August, 2015, ca. 07:45 h, an adult *T. scripta elegans*, (Fig. 1), of about 10 inch in total length, was observed basking on a mound of floating dead vegetation in a water body at Rajarhat wetlands. When approached it retreated quickly into water. This is the first photographic evidence of the species having been introduced into the water bodies of West Bengal, India. It is likely to be an abandoned pet rather than an escapee.

Trachemys scripta elegans has already been reported from Gomati Talab, Gujarat (Vyas, 2015) and Goa, India (Jadhav et al., 2018). Pupins (2007) mentions its introduction in India but gives no details of localities. Also in the Rajarhat wetlands the present authors have recorded the Indian flapshell turtle (*Lissemys punctata andersoni*), a species that throughout most of its range is under threat from habitat loss, and human consumption of both eggs and

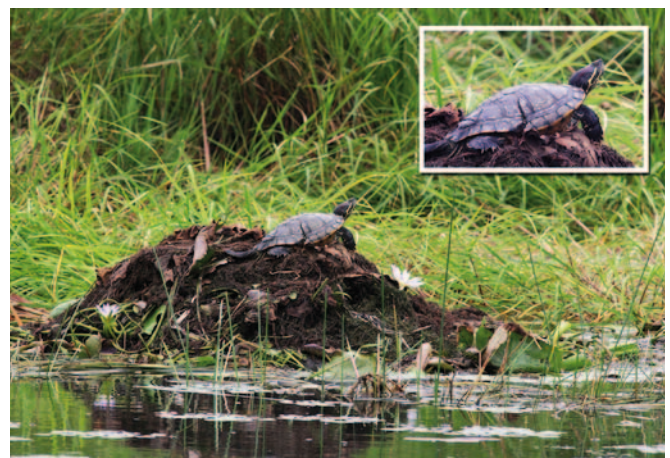


Figure 1. Red-eared slider turtle (*T. s. elegans*) photographed while basking in the Rajarhat Wetlands, Kolkata, West Bengal, India. The specimen appears to be seriously injured with a deep crack in the carapace and the rear left leg oddly distended.

meat (Purkayastha et al., 2015). A further potential threat is that *T. scripta elegans* may compete with it for food, basking sites or breeding habitats as it apparently does with the European pond turtle, *Emys orbicularis* (Cadi & Joly, 2003; Cadi & Joly, 2004; Polo-Cavia et al., 2008). Other than being a threat to native turtle species, introduced *T. scripta elegans* are also known to affect the native aquatic vegetation, arthropods and molluscs (Prévoit-Julliard et al., 2007; Teillac-Deschamps et al., 2009) and transmit diseases (Hidalgo-Vila et al., 2008). We suggest that the Department of Forest, Government of West Bengal should initiate a survey in the Rajarhat wetlands to establish whether action to eliminate slider turtles is needed and if so whether it is practical.

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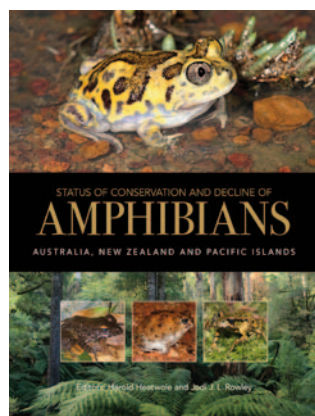
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Status of Conservation and Decline of Amphibians: Australia, New Zealand and Pacific Islands

Editors: Harold Heatwole & Jodi J.L. Rowley
CSIRO Publishing, ISBN 978-1-4863-0838-5, hardback, pp.248



In Australia, there are 240 known native frog species but as this book notes, 'in the past decade alone, 21 new species, representing 9 % of Australia's known frog fauna have been discovered'. In contrast, New Zealand has only four native frog species. The Pacific Islands have 30 known species and more are expected with further research, for example in the Solomon Islands. On first

glance, this is a medium sized, glossy, hardbacked book where a Shoemaker frog *Neobatrachus sutor* catches the eye on the front cover. It is a collaborative work by twenty-nine authors focusing on informing the reader of the conservation status and decline of frogs in the Eastern Hemisphere. It is separated into fourteen chapters with the plates, which include photographs, charts, graphs and maps are placed altogether between Chapters 12 and 13. Tables are embedded in the text in each of the chapters. The aim of the book is to collate data to help plan future research projects and implement successful conservation programmes.

The book is dedicated to Dr Harold Cogger, a devoted, award winning, herpetologist who "...freely shares his knowledge, expertise and enthusiasm for reptiles and amphibians". Following the Introduction, Harold Cogger gives an overview of data gathering methodology, species richness and range centres, the different frog families, conservation legislation and the creation of Action Plans. The chapters that follow encompass threats in the bioregions of Australia including disease, invasive species, climate change, habitat destruction and fragmentation due to logging and agricultural clearing. Chapter six discusses the current status, decline and how these threats are mitigated in south-eastern mainland Australia and Tasmania. One of the major threats being chytrid fungus *Batrachochytrium dendrobatidis*, the key host reservoir of which is the common eastern froglet *Crinia signifera*. Several options to mitigate chytrid disease are described including captive breeding, habitat management, and the monitoring and control of introduced predators, both aquatic and terrestrial. The authors explain the struggle to implement viable conservation programmes that are both consistent and able to achieve long-term goals. The subsequent six chapters echo this content for other areas of Australia, including a chapter devoted to the impacts of the alien cane toad *Rhinella marina* by Richard Shine. An

objective of the book, as noted in the preface, is to focus on mitigating human attributed threats rather than the 'natural forces beyond the control of mankind'. The last two chapters deal in a similar manner with New Zealand and then the Pacific Islands.

In previous books in the series, photographs are embedded in text (Busack & Heatwole, 2013; Heatwole, & Indraneil, 2014; Heatwole & Wilkinson, 2015) which may appeal more to readers rather than having to search for plates. Nevertheless, the plates are filled with diagrams and charts most of which have colour elements. The photographs are good quality showing life stages and habitats. The maps give a great visual representation aiding further understanding of spatial distribution. Comparisons regarding species-richness can be seen indicating the easterly side of Australia having more richness in the temperate zones (Plate 2.3). There is an impressive wealth of knowledge and incorporated literature used in this collaborative work. Harold Heatwole has been the editor and an author throughout the series showing his dedication to the sharing of this knowledge.

This book can be purchased online from CSIRO for AU\$160 (about £93) but there are a few other retailer options where prices may vary. At this price it is not a book that everyone could afford but is a 'must have' if you are a herpetologist concerned with the conservation of amphibians in this part of the world. There aren't any other books encompassing this amount of collective information on this subject.

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THE HERPETOLOGICAL BULLETIN

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