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THE HERPETOLOGICAL BULLETIN

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Front Cover: Purple balloon frog (*Nasikabatrachus sahyadrensis*) from Anamalai Hills, Western Ghats, India, see article on p. 3.
Photographed by Ben Tapley.

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REPORT

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Promoting research, education and conservation: 9th World Congress of Herpetology

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Every 3-4 years, herpetologists from around the world meet at the World Congress of Herpetology. The 1st World Congress was held in England (Canterbury) in 1989 when mysterious amphibian declines were discussed; this was a full decade before one of the main causative agents *Batrachochytrium dendrobatidis* was identified. The World Congress series has since proved very popular so that we recently arrived at the ninth Congress, held this time in New Zealand (5th-10th January 2020). The venue was the University of Otago in Dunedin and even though New Zealand is not the easiest place to reach, 874 delegates from 57 countries attended. It is a pleasure to record that a quarter of attendees were students. For detailed information on the Congress series and its origins, readers should refer to the World Congress of Herpetology Newsletter (2020).

At the Congress opening address, delegates were welcomed by the Mayor of Dunedin and offered a traditional Māori welcome. The schedule thereafter was very busy with a total of 593 presentations, running in up to eight concurrent symposia, covering every aspect of herpetology imaginable from disease ecology, taxonomy, and bioacoustics, to urban ecology and even the life and work of Emeritus Professor Richard 'Rick' Shine from the University of Sydney. The latter included talks from members of his family and former PhD students that have since gone on to start their own labs within various academic institutions around the world. Despite a long list of awards and accolades to his name, Rick is a very modest man and was clearly humbled by the tributes. That evening there was an informal social gathering for the Shine Lab and various offshoots to network and trace academic family trees.

Complimenting the symposia were two equally diverse poster sessions. There were also several workshops exploring emerging techniques and topics. One concerned how to use open-source GPS data loggers for wildlife movement studies. The main issue here is finding a way to attach the loggers to a target species: once this has been established, the process is quite simple and intuitive. Other workshops tackled less technical subjects such as the use of social media to engage with new audiences and amplify the reach of your research. Each day of the Congress began and ended with a plenary talk. These covered a wide range of topics and started with a presentation given by Alison Cree on the problems of studying reptiles (such as tuatara) that have exceptionally long lifespans compared to the length of a researcher's career. Other notable plenaries included Jodi Rowley on amphibian conservation and citizen science and Philippe Kok's journey to study and discover amphibians and reptiles living on the table top mountains (tepuis) of South America. A small number of short films were also available to watch during lunch breaks.

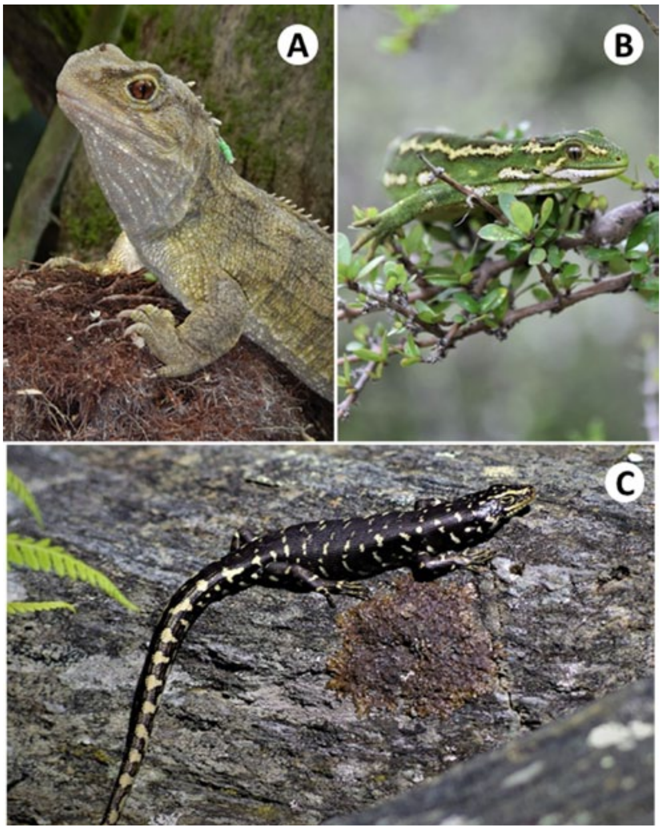

Figure 1. Some of the fascinating New Zealand reptiles seen by Congress participants- **A.** Tuatara *Sphenodon punctatus*, **B.** Otago jewelled gecko *Naultinus gemmeus*, and **C.** Otago skink *Oligosoma otagense*

Student contributions were recognised by an opportunity to win awards for talks and posters. An expert panel selected winners on the basis of a clear message, tackling a difficult problem in a new way, and a meaningful contribution to herpetology. After attending a number of talks and perusing the posters, it was easy to see where herpetology is heading in the future. There are major pushes towards bio-banking and the feasibility of using cryogenically preserved sperm to produce embryos, technological developments to support research such as bioacoustics and morphology, and an increased use of advanced molecular techniques. New tools are becoming available all the time and finding effective ways to use them in a cost effective manner will govern how herpetology advances over the coming decades.

As expected there were many opportunities to socialise and network both during tea and lunch breaks and at the formal social events. These included a dinner at the Dunedin Town Hall and a visit to one of the Congress's sponsors, the Emerson Brewery. There was also ample opportunity to do a spot of herping both before and after the Congress. The ticketed events took delegates to nearby locations such as Orokonui Ecosanctuary and Otago Peninsula. Both of these are important areas for local reptiles, with the Orokonui Ecosanctuary being the largest predator-free cloud forest in New Zealand. If the weather is kind then there is the likelihood of seeing tuatara (*Sphenodon punctatus*, Fig. 1A), Otago jewelled geckos (*Naultinus gemmeus*, Fig. 1B) and Otago skinks (*Oligosoma otagense*, Fig. 1C) among the other rare endemic species. If delegates missed out on any of these field trips, then a small number of local amphibians and reptiles could be seen in a secure lab within the University of Otago. There were also other predator-proof sites open to the public that combined habitat recovery with the eradication of invasive species; these have been designed in a way to bring long-term economic benefits to the local community.

The native fauna of New Zealand has evolved in isolation, but is now threatened by non-native species from elsewhere in the world. Despite its small size, New Zealand accommodates a large diversity of reptiles and amphibians, some of which are endemic to very particular islands. Alongside the tuatara are four species of frogs, 63 skinks and 43 geckos all of which are endemic to the archipelago. With this in mind, there was a specific symposium on 'Improving the interface between research and management for conservation of New Zealand reptiles and frogs' that presented the multiple species programmes led by the Department of Conservation. It was a great opportunity to see New Zealand's commitment to protecting the last refuges of their native fauna, eradicating invasive species, and restoring habitats. Among the non-native species being controlled is the alpine newt (*Ichthyosaura alpestris*) that was first detected in New Zealand in 2013. The climate of Dunedin and the Otago Peninsula is not unlike that of Britain, which makes the diversity of reptiles there even more astounding. Imagine if there were native skinks and geckos present in Britain!

On the subject of alien species, New Zealand has rigorous management of potentially invasive species. In managed reserves and even on remote islands, the Department of Conservation has possum and rat traps placed every 20 m or so in an attempt to control these damaging species; others include such introductions as the European hedgehog (*Erinaceus europaeus*), the blackbird (*Turdus merula*) and the house sparrow (*Passer domesticus*). It was alarming to see how many European mammals were dead on the roads and how frequently this occurred. The customs checks on arrival in New Zealand are just as stringent as you'd expect, looking for every last seed or animal product that could potentially cause further damage to country's already imperilled fauna and flora.

One output of the Congress was the 'Aotearoa Climate Change Declaration' calling upon the international community to acknowledge evidence for global climate change and to take appropriate action. In particular, the Declaration calls for all air travel to future World Congresses to be carbon-mitigated via reputable and certified avenues, with the eventual goal that they should be 100 % carbon-neutral. So the important question is where will the next World Congress be in 2024? There were four candidates bidding for this honour and after much debate and discussion Kuching (Malaysia) won the bid. Malaysia has an impressive assemblage of herpetofauna that will make the 10th World Congress an unforgettable event.

REFERENCE

World Congress of Herpetology Newsletter (2020), 1. 68 pp. https://www.worldcongressofherpetology.org/newsletter (accessed July, 2020)

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RESEARCH ARTICLE

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Are local and traditional ecological knowledge suitable tools for informing the conservation of threatened amphibians in biodiversity hotspots?

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ABSTRACT - Globally, amphibians are declining more rapidly than any other vertebrate group. A general shortage of funding for the support of focused scientific studies led us to investigate local and traditional ecological knowledge as an alternative data source for amphibian conservation. In this context, we undertook a questionnaire-based interview survey with forest-dwelling indigenous and non-indigenous communities across the Anamalai Hills, within the southern Western Ghats of India, to gather ecological knowledge on three cryptic and threatened frog species. Our results suggest that local communities possess ecological knowledge of frogs and that the magnitude of this knowledge is influenced by gender, community type, education, and age. Accuracy of local knowledge was primarily influenced by the morphological distinctiveness of the focal species, but cultural association and utilisation were also important factors especially for the enigmatic purple frog *Nasikabatrachus sahyadrensis*, which has uses in medicine and amulets.

INTRODUCTION

India harbours exceptional diversity and endemism of amphibians, with a total of 459 species (Frost, 2020). Most amphibian studies have focused on taxonomy and systematics (e.g. Van Bocxlaer et al., 2009; Biju et al., 2011, 2014) rather than population status, ecology and threats to these species. In the absence of resources for focused scientific studies, alternative data collection approaches to understand key amphibian conservation parameters need to be identified and tested.

Communities develop an array of knowledge and management strategies to exploit the local natural resources on which they depend (Berkes et al., 2000). The collection and assessment of such knowledge from untrained observers therefore represents a potentially cost-effective approach to understand the biology, distribution, population status, and potential threats for otherwise poorly-known and potentially threatened amphibian species. This approach may be particularly important for species that are difficult to detect using standard scientific survey methods, that have limited activity patterns, and/or are rare or possibly extinct (Anadón et al., 2009; Turvey et al., 2010; Meijaard et al., 2011; Stuart, 2012; Ziembicki et al., 2013).

The knowledge that local communities possess can be classified as either traditional ecological knowledge (TEK), a composite set of beliefs, information and practices that are handed over from one generation to another, or local ecological knowledge (LEK), the observations gathered by an individual over a lifetime (Gadgil et al., 1993; Berkes et al., 2000; Gilchrist et al., 2005). These two knowledge categories differ; TEK often relates to beliefs towards species that can lead to their protection, culling or utilisation (Stacey et al., 2012). Whereas LEK, by being observational in nature, can assist in understanding species occurrence, abundance, habitat use and threats (Gilchrist et al., 2005; Anadón et al., 2010; Lescureux et al., 2011), especially for globally threatened and/or elusive species for which very few data are otherwise available (Turvey et al., 2014, 2015; Pan et al., 2015). LEK is particularly useful for understanding status and threats for large-bodied, morphologically distinct species (Turvey et al., 2014) or economically or culturally important species (Jones et al., 2008; Pan et al., 2015). However, collecting and interpreting data about focal species from untrained respondents to establish baselines for conservation is not straightforward (Gilchrist et al., 2005). TEK and LEK data can be affected by errors around species identification or associated encounter details (e.g. timing recall), negative reporting bias, retrospective bias, exaggeration, and/or varying data breadth and quality depending on species and respondent (Davis & Wagner, 2003; Gilchrist et al., 2005; McKelvey et al., 2008; O'Donnell et al., 2010).

Under the Forest Rights Act of India, 2006, indigenous communities in the Western Ghats are permitted to harvest non-timber forest products and freshwater fish sustainably. Indigenous communities in the Western Ghats region of Kerala have been found to utilise 54 wild animal taxa, primarily freshwater fish, herpetofauna and small mammals, for meat and traditional medicine (Kanagavel et al., 2016). Amphibians are known to be used by indigenous communities across the Western Ghats and in other parts of India for medicinal purposes (Tiwari et al., 2013; Narzary &

Bordoloi, 2014; Thomas & Biju, 2015; Kanagavel et al., 2016) and are also the focus of legends and taboos (Harpalani et al., 2015). The area therefore offers an opportunity to investigate the LEK and TEK of amphibians, within a wider continental context where baseline data on amphibian species diversity and distributions are very limited (Molur, 2008). We focused on three poorly-known threatened frog species from southern India, all identified as EDGE (Evolutionarily Distinct and Globally Endangered) species for conservation (Isaac et al., 2012). We aimed to assess whether LEK and TEK can be effective tools for gathering ecological knowledge to inform future amphibian-based conservation initiatives in the Western Ghats.

MATERIALS AND METHODS

Our study was undertaken in three adjoining areas of the Anamalai Hills (Kerala and Tamil Nadu States), within the southern Western Ghats; Valparai (municipality), Topslip (Forest Range) and Munnar (town). These locations are bordered by several protected areas, reserve forests, and private forest fragments (Fig. 1). The primary vegetation of this area, which historically comprised tropical rainforest, has now been transformed into a mosaic of plantations interspersed with fragmented patches of original evergreen forest (Raman & Mudappa, 2003).

Both indigenous (Kadar, Mudhuvar, Malasar, Malai Malasar, Pulayar) and non-indigenous forest-dwelling communities live in the study area. Indigenous communities are defined by their historical occupancy of the area, geographic isolation, distinctive culture and ancient cultural traits (MTA, 2012). Non-indigenous communities are mostly recent settlers from other regions of India. Both indigenous and non-indigenous communities work with the State Forest Department or as labourers in farms and plantations, and collect non-timber forest products (Chandi, 2008; Surendran & Sekhar, 2011). The population of the area has a higher proportion of non-indigenous than indigenous individuals (>13:1; Chandi, 2008; DCO, 2011).

Three threatened EDGE amphibians, endemic to the study area were selected as focal species: the purple frog (Nasikabatrachidae: *Nasikabatrachus sahyadrensis*, Fig. 2A), the black microhylid frog (Microhylidae: *Melanobatrachus indicus*, Fig. 2B), and the toad-skinned frog (Ranixalidae: *Walkerana phrynoderma*, Fig. 2C) (Table 1). These three species vary in their morphological distinctiveness and in the availability of scientific knowledge about them. This provides a useful framework for assessing correlates of potential LEK and TEK variation and usefulness. *Nasikabatrachus sahyadrensis* was described scientifically in 2003 but was already known by indigenous communities and is relatively well studied (Aggarwal, 2004; Table 2). This fossorial frog is morphologically distinct, and is only active above the soil for the annual two-week breeding season (Biju & Bossuyt, 2003; Thomas et al., 2014). It has been consumed by indigenous communities for decades, and these communities possess considerable knowledge about its behaviour and lifecycle (Thomas & Biju, 2015). *Melanobatrachus indicus* is

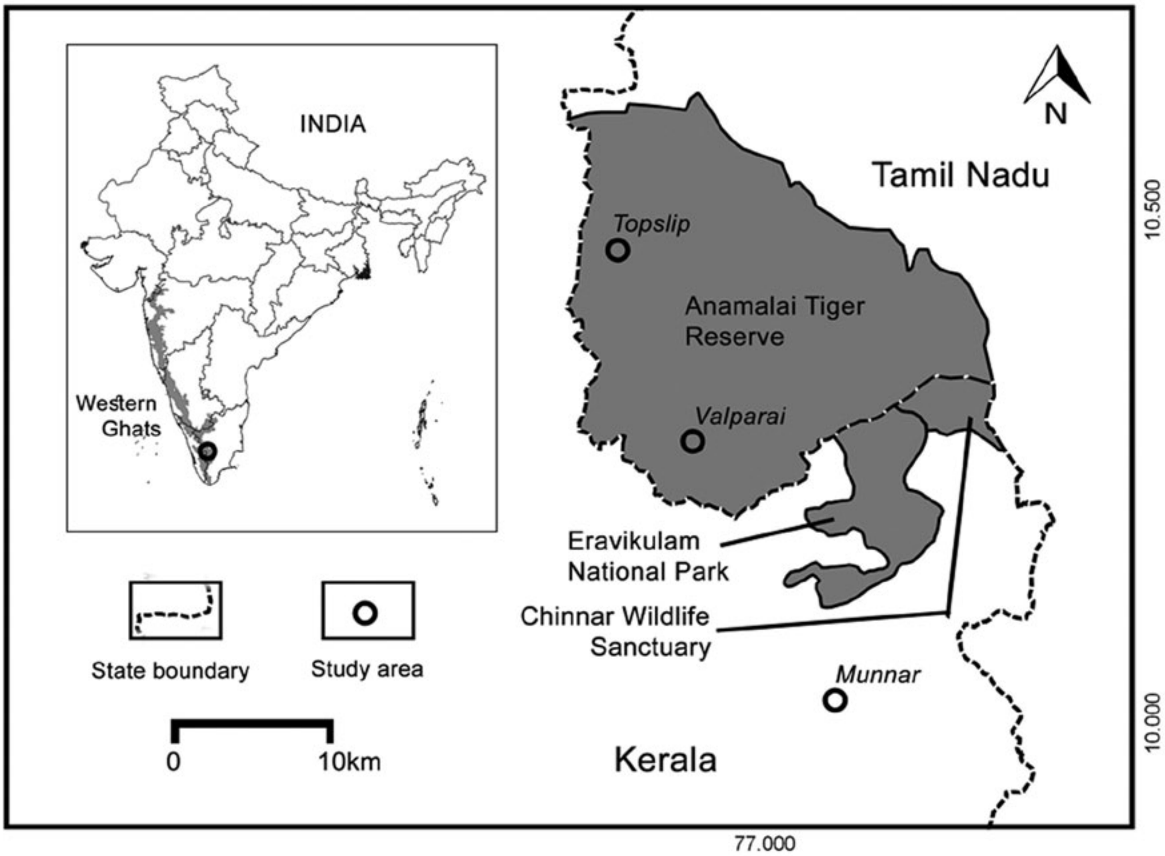

Figure 1. Map of the study area in the southern Western Ghats of India

Figure 2. Focal frog species for the study- **A.** Purple frog *N. sahyadrensis*, **B.** Black microhylid frog *M. indicus*, **C.** Toad-skinned frog *W. phrynoderma*

Table 1. Details of how anuran species are utilised by local communities in the Anamalai Hills, Western Ghats, India

	Scientific Name	Status[k]	Habitat[l]	Community Type & No.[m]	Utilisation Type[n]
1	*Nasikabatrachus sahyadrensis*[a, b, c]	EN	Fossorial, active above the ground only for a few weeks during the monsoon	Kadar (27)	Adults and larvae are consumed specifically by children and infants as medicine for skin-based ailments, cold, cough, throat infection, asthma, measles, chicken pox and stomach pain. They are consumed after cooking or after drying and/or powdered or vapours from burning the dried frog are inhaled. Fat tissue/mucous lining is applied on external wounds. Small-sized individuals, skin, limbs, or digit ends are used as amulets for children to reduce fear, when they are unable to sleep at night or who do not speak or walk well.
2	*Melanobatrachus indicus*[a, d, e]	EN	Fallen bark and leaf litter close to streams	Kadar (1)	For those who have a problem with walking, the frog is tied within a small sack and worn around the individual's neck.
3	*Walkerana phrynoderma*[a, f]	CR	Leaf litter	Kadar (0), Mudhuvan (0)	Since it is confused with *Duttaphrynus* sp., this species and others in the same genus could be used as medicine to cure skin burns and other skin-based body ailments.
4	*Rhacophorus pseudomalabaricus*[g]	CR	Understory of rainforests	Kadar (1)	Used as medicine for coughs and as amulets for children who do not speak or walk well
5	*Raorchestes jayarami*[h]	NE	2m above the ground in forest undergrowth	Kadar (3)	Used as medicine for coughs, as general medicine for children and as amulets for children who do not speak or walk well
6	*Raorchestes beddomii*[h]	NT	Moist forest patches, wayside vegetation and tea plantation	Kadar (3)	Used as medicine for coughs, as general medicine for children and as amulets for children who do not speak or walk well
7	*Duttaphrynus melanostictus*[i]	LC	Wide range of habitats	Kadar (2), Mudhuvan (1), Non-indigenous communities (1)	Used as medicine to cure skin burns and other skin-based body ailments
8	*Indosylvirana* sp.[j]		Leaf litter and streams in open secondary and primary forests	Kadar (3)	Used during the rainy season as bait for fishing
9	*Hoplobatrachus tigerinus*[f]	LC	Close to streams, lakes, pools and farms	Non-indigenous communities (5)	Meat is consumed
10	*Fejervarya* sp.		Close to water bodies, muddy areas	Kadar (1), Mudhuvan (1), Non-indigenous communities (1)	Used as bait to catch fish and crabs
11	*Euphlyctis* sp.		Water bodies	Kadar (1), Mudhuvan (1), Non-indigenous communities (1)	Used as bait to catch fish and crabs

[a]Focal species of this study; [b]Biju, 2004; [c]Gururaja, 2012; [d]Biju et al., 2004b; [e]Kanagavel & Tapley, 2013; [f]Biju et al., 2004a; [g]Vasudevan & Dutta, 2000; [h]Biju & Bossuyt, 2009; [i]van Dijk et al., 2004; [j]Biju et al., 2014; [k]IUCN Red List Category- CR: Critically Endangered, EN: Endangered, NT: Near Threatened, LC: Least Concern, NE: Not Evaluated; [l]Habitat according to published scientific literature; [m]Number of respondents who consumed the species; additionally, five respondents used any frog available as bait for capturing fish and crabs; [n]This information is from the current study

distinctively patterned but is rarely encountered and not well studied; some data are available on habitat associations, but its reproductive biology is unknown (Daltry & Martin, 1997; Kanagavel & Tapley, 2013; Table 2) and there are no published accounts of the species being utilised by people. *Walkerana phrynoderma* is an uncommon, rainforest-dependent, ground-dwelling brown frog that bears a superficial resemblance to several other frogs in the region (Biju et al., 2004a; Kanagavel et al., 2018). It has been the focus of a published scientific study and it is known to occur at mid and high elevations with dense canopy cover (Kanagavel et al., 2018). There are no published accounts of the species being utilised by people.

We conducted questionnaire-based interview surveys from August 2013 to May 2016, where LEK data correspond to species presence, associated habitats and locations while

Table 2. Numbers of responses contributing local ecological knowledge on the three focal anuran species and the reliability of response relative to the scientific literaturea

Scientific name	Vernacular name[b]	Community type[c]	Accurate habitats associated with species occuranced	Unreliable habitats associated with species[d]	Known scientific information
Nasikabatrachus sahyadrensis	Kottraan/Kottaan* (Kadar) = 26 Mannu/Manal tavala* (soil/sand frog; Malasar, Pulayar) = 8 No name = 15 Kunjunni*+ (Mudhuvan) = 11 Makkan/Makachi tavala+ (non-indigenous) = 2 Koku tavala* (beaked frog; Malasar) = 1 Kuyi aamai* (turtle-like; Malasar) = 1	Kadar = 27 Mudhuvan = 19 Malai Malasar, Malasar & Mannan = 11 Pulayar = 4 Non-indigenous = 2	Within the ground and/or found it during digging = 38 First rains, rainy season, rains accompanied by thunder, lightning or hail = 30 Stream & stream bank = 10 Forests = 3 Plantations & Settlement = 4 Water cavity within firewood = 1 On ground = 1 In water = 1	Forests of Kerala = 1 Don't know anything else = 3	Fossorial, active above the ground only for a few weeks during the monsoon, found close to forest streams with rocky pools (Zachariah et al., 2012)
Melanobatrachus indicus	No name = 14 Velladichi tavala* (close to water; *Kadar*) = 4 Thotri tavala* (*Kadar*) = 2 Peckachi tavala* (*Kadar*) = 1 Karin tavala* (black frog; Kadar) = 1 Mara tavala+ (tree frog; Mudhuvan) = 1	Kadar = 13 Mudhuvan = 7 Malai Malasar, Malasar & Mannan = 2 Pulayar = 1 Non-indigenous = 0	Stream and water body = 12 Forest = 6 Rocks & leaf litter = 4 Trees = 3 Bamboo = 2 On rocks and ground = 2 Dry areas = 2 On green plants = 1	Everywhere = 2	Fallen bark close to streams in semi-evergreen forest (Kanagavel & Tapley, 2013)
Walkerana phrynoderma	No name = 2 Porkan tavala* (warty frog; Kadar) = 1 Vadakan tavala+ (Mannan) = 1 Metru tavala* (Kadar) = 1	Kadar = 3 Mudhuvan = 0 Malai Malasar, Malasar & Mannan = 1 Pulayar = 1 Non-indigenous = 0	Evergreen forest = 5 Ground, leaf litter = 3 Close to stream = 2 Mist covered area = 1	Stream, water body & wetland=44 Ground, leaf litter, crevice, rock, grass, bush, bamboo = 18 Forest = 15 Houses, plantations, fields, well = 12 Everywhere = 8 Rainy season = 4 Don't know anything else = 9	Leaf litter in evergreen forest at 1300-1700m asl, where canopy cover is high (Kanagavel et al., 2018)

[a] To safeguard the species, the names of locations have not been mentioned in this table. Researchers and conservationists can apply to the authors for this information; [b] Only those vernacular names associated with accurate LEK data have been mentioned. The meanings for some of the vernacular names were not known by the respondents. The indigenous community that uses the specific name has been mentioned in italics. The names correspond to two local languages – Tamil* and Malayalam+; [c] The total number of respondents belonging to each community type who could accurately identify the specific focal species; [d] The different habitats have been grouped and the total number of respondents for each group has been mentioned

TEK data correspond to the vernacular names of species and to their utilisation. Interviews were held with forest-dwelling indigenous and non-indigenous communities across the three localities. We selected 16 settlements (Valparai=6, Topslip=5, Munnar=5) for surveying using a targeted sampling strategy and then selected the respondents at each settlement using a convenience sampling strategy (Newing, 2011). We undertook a door-to-door survey where each household was visited and conducted interviews face to face in local languages (Tamil or Malayalam). After introducing ourselves, we interviewed any consenting individual over the age of 18. There were both male and female interviewers; to reduce gender-related respondent bias, female respondents were interviewed only by female interviewers (Newing, 2011).

We used a standard questionnaire composed of open ended and closed questions (see Supplementary Material) for all interviews. Interviews took a maximum of 20 minutes to complete. Firstly, we recorded each respondent's socio-economic characteristics. We then showed each respondent un-labelled photographs of the three focal amphibian species, in the same sequence in all interviews. After each photograph was shown, we asked respondents whether they had seen the species, and if they had, about its vernacular name and the associated habitats and locations in which they had seen it. We then asked respondents whether any frogs were utilised, and if so, which species and the type of utilisation. Since we did not know if frog utilisation in general was a sensitive topic or not, we used both direct and indirect questioning approaches, asking both whether respondents utilised frogs themselves and whether they knew of anyone else who utilised them. Finally, we asked respondents whether and why they visited forests, and then asked two separate questions about whether they were interested in protecting forests and frogs in their area.

If respondents were only able to provide very general information (e.g. species is "found everywhere" or is found "in the forests of Kerala"), we considered such data unreliable and excluded them from further analysis. Since *W. phrynoderma* resembles several other frog species, we cross-checked habitat details reported by respondents with the limited scientific information available for this species (Kanagavel et al., 2018), and only retained information from respondents who reported the specific habitat requirements. We calculated the frequency of respondents who had seen

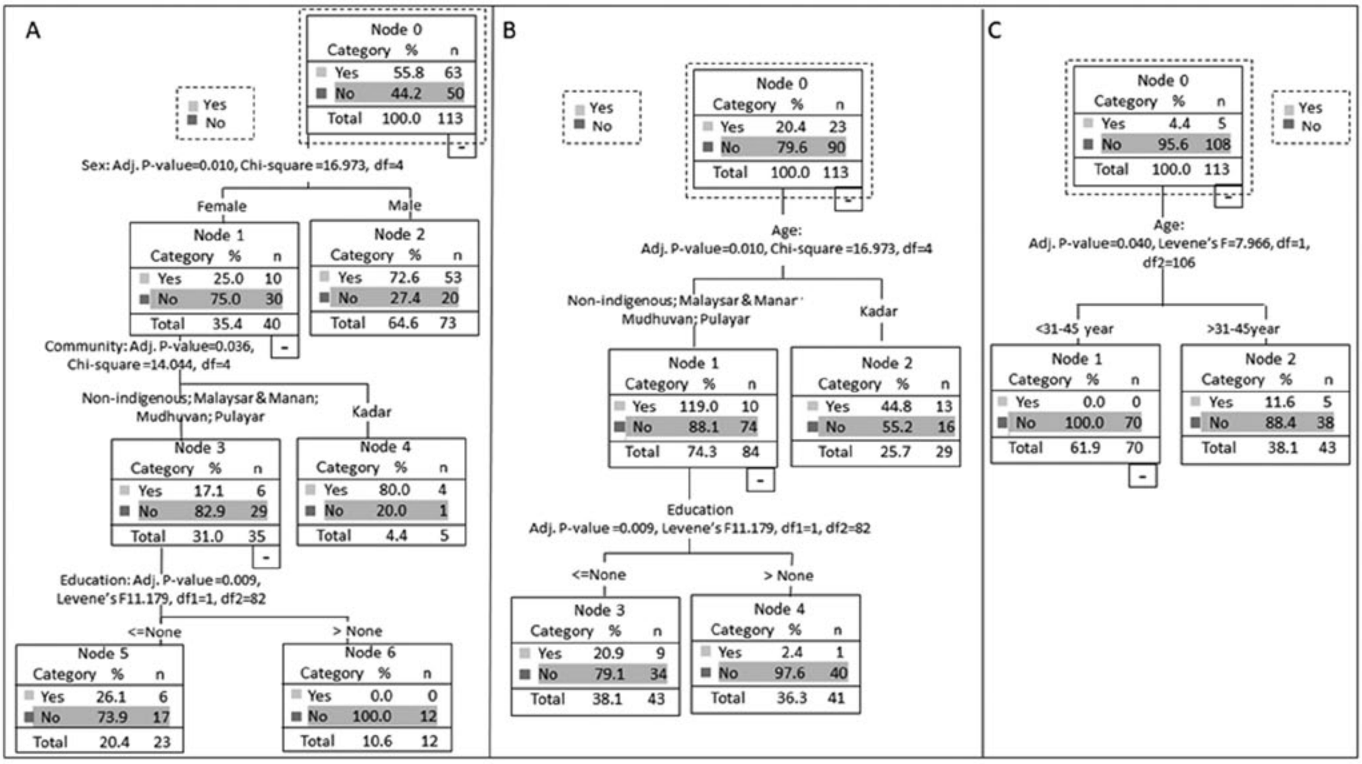

Figure 3. A QUEST decision-tree detailing the socio-economic characteristics that influence whether local individuals have encountered - **A.** Purple frog *N. sahyadrensis*, **B.** Black microhylid frog *M. indicus*, and **C.** Toad-skinned frog *W. phrynoderma*

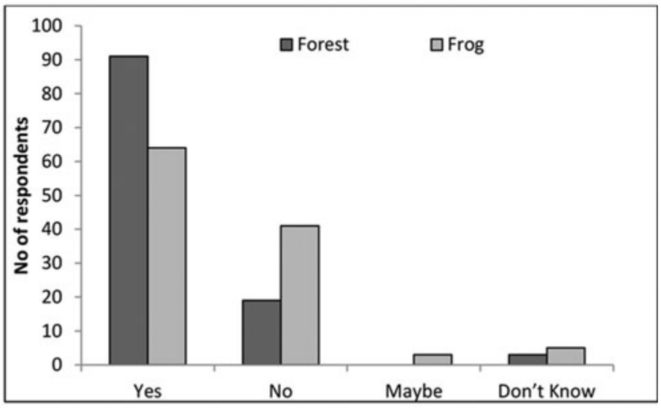

Figure 4. Interest in protecting forests and frogs by local communities in the southern Western Ghats

each focal species in relation to the habitat associations of each species. We assessed reported vernacular names for each species to understand local folk taxonomy of amphibians (cf. Atran et al., 1997).

We explored the relationship between species detection and socio-economic characteristics of respondents using a QUEST decision-tree (Brewer & Rabeni, 2011, Lin & Fan, 2019), to identify appropriate 'experts' for informing future LEK surveys. Decision-tree analyses assist in establishing classifications and QUEST was chosen in this case since it can handle variables with multiple categories. It uses Anova F and Chi-square tests to select variables for splitting and the resultant tree was pruned using the CART algorithm. Then in order to identify appropriate audience groups towards whom future conservation initiatives could be targeted, we calculated the frequency of utilisation of different anuran species by respondents and people they knew. These frequencies were explored using a binomial logistic regression model followed by ad-hoc analysis of deviance (Bond et al., 2017) to show the influence of socio-economic characteristics, experience of seeing focal species, and frequency of forest visits, on amphibian utilisation. Respondent interest in protecting frogs was analysed, also using a binomial logistic regression model, incorporating respondent socio-economic characteristics, experience of seeing focal species and utilising frogs, and interest in protecting forests. The statistical analyses were undertaken using IBM SPSS Statistics 21.0 and R version 3.3.0.

RESULTS

A total of 113 questionnaires were completed with 1 to 15 respondents based at 16 different settlements (Table 3). Of the respondents most were male (65 %, n=73) and most belonged to the Kadar and Mudhuvan indigenous communities (58 %, n=66). Many had no formal education (48 %, n=54) and the majority were involved in non-timber forest product collection, daily-wage labour or farming for their daily livelihood (59 %, n=67). Most visited forests (81 %, n=92) to collect non-timber forest products and fuelwood (76 %, n=86) or for temporary work related to the Forest Department (19 %, n=22).

Local ecological knowledge

Of the three focal amphibian species, *W. phrynoderma* had reportedly been seen by the greatest number of respondents (77.9 %, n=88), followed by *N. sahyadrensis*

Table 3. Description of the socio-economic characteristics and the numbers of questionnaire respondents from local communities in the Anamalai Hills, Western Ghats, India

	Socioeconomic characteristic	Description	No. of respondents by group (n=113)
1	Interview locality	Regions in which respondents were residing	Munnar = 40 Topslip = 19 Valparai = 54
2	Age	Respondent's age in years	18-30 = 35 31-45 = 35 46 & above = 38 Don't know = 5
3	Gender	Male or female	Male = 73 Female = 40
4	Education	Maximum formal education attained	None = 54 Primary Education (1st -5th) = 23 Secondary Education & above = 36
5	Occupation	Main livelihood of the respondent	Labourer/Farmer/NTFP* collection = 67 Other occupations = 10 Forest Department work = 14 Housewife/ Retired/ Not working = 22
6	Community type	Indigenous/non-indigenous community to which the respondent belonged	Kadar = 29 (Topslip = 6, Valparai= 23) Mudhuvan = 37 (Munnar = 24, Valparai = 13) Malai Malasar, Malasar & Mannan = 18 Pulayar = 17 (Munnar = 6, Topslip = 12 Non-indigenous communities = 12 (Munnar = 10, Topslip = 1, Valparai = 1)

*Non-timber forest product

(59.3 %, n=67) and *M. indicus* (22.1 %, n=25). However, following critical verification of *W. phrynoderma* reports with published ecological data, we only considered five reports (4.4 % of respondents) to represent reliably identified records of this species. Conversely, we only excluded four reports of *N. sahyadrensis* and two reports of *M. indicus* on the basis of dubious identification (final reliable species totals: *N. sahyadrensis*, 55.8 %, n=63; *M. indicus*, 20.4 %, n=23). While a greater proportion of respondents from Topslip reported *N. sahyadrensis* (78.9 %, n=15) followed by Valparai (53.7 %, n=29) and Munnar (47.5 %, n=19), *M. indicus* was reported mostly by respondents from Valparai (29.6 %, n=16) followed by Munnar (15.0 %, n=6) and Topslip (5.3 %, n=1). *Walkerana phrynoderma* was mostly reported from Valparai (7.4 %, n=4) followed by Munnar (2.5 %, n=1). Of the three focal species, only *N. sahyadrensis* was identified by respondents belonging to non-indigenous communities (16.7 %, n=2; Table 3). Respondents who were able to identify the focal species frequently provided information on locations where the species occurred in the study area (Table 2). Respondents described *N. sahyadrensis* as being found beneath the ground (n=38) and mostly encountered during the rainy season (n=30), coming out of the ground only to lay eggs and calling by making loud noises beneath the soil. Respondents reported that *M. indicus* was associated with streams and water bodies (n=12) while *W. phrynoderma* was known from evergreen forests (n=5).

Decision tree analysis indicated different demographic predictors associated with increased likelihood of detecting each of the three focal amphibian species. For *N. sahyadrensis*, respondent gender (P<0.01) was the most powerful predictor, with men more likely to have encountered the species than women (Fig. 3A). Within the subset of female respondents, community type (P=0.04) further improved the predictive power of the model, with female Kadar respondents more likely to have encountered the species than female respondents from other communities. Within the subset of female, non-Kadar communities, education (P<0.01) further improved the model, with respondents lacking formal education more likely to have encountered the species. For *M. indicus*, community type (P=0.01) was instead the most powerful predictor, with Kadar respondents again more likely to have encountered the species (Fig. 3B). Within the subset of non-Kadar communities, education (P<0.01) further improved the predictive power of the model, with respondents lacking formal education again more likely to have encountered the species. For *W. phrynoderma*, age was the only significant predictor (P=0.04), with respondents above 45 years of age more likely to have encountered the species (Fig. 3C).

Traditional ecological knowledge

Vernacular names used for *N. sahyadrensis* were either culturally significant or based on morphology or habitat, whereas *M. indicus* mostly did not have a local name, although some respondents referred to it by its habitat or colour (Table 2). Vernacular names used for *W. phrynoderma* were based on its morphology (Table 2), and respondents used the same vernacular name for other common species of the families Ranixalidae and Bufonidae ('chori/pori thavala' n=20), leading to frequent misidentification with such species and inaccurate ecological association with water bodies, wetlands, and habitats close to human settlements.

Thirty-eight respondents (33.6 %) reported that they utilised frogs themselves, mostly *N. sahyadrensis* (n=27). A larger proportion of respondents from Valparai utilised frogs (38.9 %, n=21) followed by Munnar (30.0 %, n=12) and Topslip (26.3 %, n=5). The focal species were utilised only by the Kadar indigenous communities (Table 1). Other non-focal anurans were also mostly utilised by the Kadars and to a small extent by Mudhuvans. Non-indigenous communities depended on common, widely distributed species (Table 1). Frogs were used for general consumption and medicine (n=28), as amulets to reduce fear among children (n=12), and as bait to catch freshwater fish and crabs (n=8) (Table 1). Only indigenous communities utilised amphibians for traditional medicine while non-indigenous communities used them for general consumption and as bait (Table 1). Thirty-two respondents (28.3 %) stated that they knew of other individuals or communities that utilised frogs, including *N. sahyadrensis*, *Hoplobatrachus tigerinus* and *Indosylvirana* sp., which were eaten and used as medicine (n=23), as bait for fishing (n=6), or for other reasons (n=3, perceived export of frog legs). Analysis of deviance performed on the logistic regression model revealed that community type (df = 5, P<0.001) and gender (df = 1, P<0.001) were the most statistically significant factors predicting utilisation of

Table 4. The influence of several explanatory variables on the utilisation of frogs (dependent variable). Analysis of deviance performed on a logistic regression model fitted to explain the effect of explanatory variables listed in the table. The result indicates significant change in deviance (P<0.05) with the addition of the variables 'Gender' and 'Community' type to the model.

	df	Deviance	Residual df	Residual deviance	*P*(>Chi)
Null			96	127.95	
Interview localitya	2	2.57	94	125.38	0.276
Age	2	3.08	92	122.31	0.215
Gender	1	11.45	89	106.08	<0.001
Education	2	4.78	90	117.53	0.092
Community	5	33.58	84	72.49	<0.001
N. sahyadrensis sighting	1	2.47	83	70.02	0.116
M. indicus sighting	1	0.02	82	70.00	0.897
W. phrynoderma sighting	1	1.12	81	68.89	0.291
Forest visit	1	0.97	80	67.91	0.324

Table 5. The influence of several explanatory variables on interest of local communities in protecting frogs (dependent variable). Analysis of deviance performed on a logistic regression model fitted to explain the effect of explanatory variables listed in the table. The result indicates significant change in deviance (P < 0.05) with the addition of the variables 'Interview locality' and interest in 'Protecting forests' to the model.

	df	Deviance	Residual df	Residual deviance	*P*(>Chi)
Null			96	128.97	
Interview localitya	2	8.51	94	120.46	0.014
Age	2	1.16	92	119.30	0.560
Gender	1	3.79	91	115.51	0.051
Education	2	2.20	89	113.31	0.333
Community	5	10.42	84	102.89	0.064
N. sahyadrensis sighting	1	2.79	83	100.10	0.095
M. indicus sighting	1	0.01	82	100.09	0.915
W. phrynoderma sighting	1	0.93	81	99.16	0.335
Forest visit	1	0.12	80	99.04	0.731
Use of frogs	1	2.75	79	96.29	0.098
Protecting forests[e]	1	41.92	78	54.38	<0.001

frogs (Table 4), with utilisation more common in the Kadar community and by men.

Local support for conserving forests and frogs

Respondents were more interested in protecting forests than frogs (Fig. 4). Respondent interest in protecting forests was the most statistically significant factor predicting interest in protecting frogs (df=1, P<0.001) followed by interview locality (Table 5). Respondent interest in protecting frogs was nested within their interest in protecting forests, as all respondents who wanted to protect frogs also wanted to protect forests and not vice versa, and interest in protecting frogs was higher at Topslip (72.2 %) and Valparai (71.4 %) than at Munnar (42.1 %).

DISCUSSION

Local ecological knowledge

LEK has not often been gathered to provide researchers with information about herpetofauna. Community-based surveys have rarely been used to assess the conservation status or to obtain other conservation-relevant data for amphibians, and so far, have only been applied to very large-bodied "charismatic" taxa such as the giant salamander (*Andrias davidianus*) (Pan et al., 2015). Indeed, researchers have sometimes previously ignored LEK of amphibians, as in the case of the enigmatic *N. sahyadrensis*, which was well known to local communities long before its formal scientific description (Aggarwal, 2004). Our study demonstrates that despite this lack of past attention, LEK can be a suitable tool for collecting conservation-relevant information on focal amphibian species, in this case especially for *N. sahyadrensis*. Accurate collection of LEK has been shown in previous studies to be greatly improved if the focal species is morphologically distinct and easily identifiable even to non-trained observers, is non-cryptic, and has an exclusive vernacular name (Anadón et al., 2009; Pillay et al., 2011). Of the three focal species included in our study, *N. sahyadrensis* and *M. indicus* are both morphologically distinct, and each indigenous community had an exclusive vernacular name for *N. sahyadrensis*, which could account for why it was locally the best known of the three focal species. *Nasikabatrachus sahyadrensis* was also the only focal species that was known among non-indigenous communities and this reveals its potential as an effective conservation flagship among local communities (Kanagavel et al., 2017a) who dwell close to forests. Conversely, *W. phrynoderma* resembles many other frogs, and had no consistently used vernacular name, consequently the majority of LEK that we collected for *W. phrynoderma* was considered to be unreliable. We conclude that LEK-based surveys may only provide limited data on morphologically indistinct amphibian species. Accuracy of identification may have been improved if we had used control images of locally occurring amphibian species that are morphologically similar to *W. phrynoderma*, and we encourage further investigation of the ability of local respondents to differentiate between similar species using this approach in future LEK-based amphibian surveys.

Our results help to identify local expert groups who possess greater levels of knowledge about different focal amphibian species. These groups could be preferentially targeted in future studies that aim to collect additional amphibian-related data. In our study, men provided LEK that corresponded more closely with existing knowledge of *N. sahyadrensis* than women, possibly because men are more involved with hunting activities in the Western Ghats (Kanagavel et al., 2016), and likely visit forests more frequently. The Kadar communities had better knowledge of both *N. sahyadrensis* and *M. indicus*, possibly because they have greater cultural associations with *N. sahyadrensis*. Their livelihoods are more forest-dependent and both the species occur in their traditional lands, increasing the possibility of encountering them. The positive relationship between respondent age and level of LEK about *W. phrynoderma* is consistent with the well-known phenomenon seen in many LEK studies where older respondents are more knowledgeable of local environmental conditions (e.g., Papworth et al., 2009; Turvey et al., 2010). The demographic predictors varied for the three amphibians which could be explained by the different local distribution ranges of the species that may not be present within the traditional lands occupied by all the communities. Moreover, the communities also differ in their extent of dependence on forests and the frequency of their visits to surrounding forests. This means that 'expert' groups would vary based on the species concerned and more relevant data could be collected across multiple species by focusing on major predictors, e.g. males, the Kadar community, respondents above 45 years of age etc..

Traditional ecological knowledge

Folk nomenclatures are based on morphology, use of the species, social constructs, economic importance, and ecology (Newmaster et al., 2007; Ulicsni et al., 2013; Berlin, 2014). For large plants and animals, vernacular names are mostly exclusive for a species, since the majority of species within these groups are distinctly identifiable (Atran, 1998; Souza & Begossi, 2007; Ulicsni et al., 2013). However, this is not the case for small vertebrates, many of which appear superficially similar to untrained observers. Hence they are typically grouped together under a single name, making indigenous taxonomy less reliable for species-specific identification (Forth, 2009; Beaudreau et al., 2011). In our study, *N. sahyadrensis* is well recognised among numerous indigenous and non-indigenous communities and has distinct vernacular names that are based on culturally significant attributes and direct awareness of the species by each indigenous community. This distinctive and charismatic amphibian may therefore represent a potential flagship species for building local community interest in amphibians and their conservation (Bowen-Jones & Entwistle, 2002).

Melanobatrachus indicus is distinct and associated with specific vernacular names based on body colour and habitat. However, it is relevant only for the Kadar community who themselves associated with it little, resulting in reduced ecological knowledge of the species. Although *W. phrynoderma* was found to have only a general vernacular name based on body morphology and colour which is shared with many other anuran species, this frog grouping was known to local communities as these species are incorrectly perceived to be pests of cardamom (*Elattaria cardamomum*), a major high-value crop in the region (Kanagavel & Parvathy, 2014; Kanagavel et al., 2017b). The differences between the local communities in how they refer to the three focal species highlight the role of cultural and utilitarian values in shaping TEK as well as LEK (Atran, 1998; Beaudreau et al., 2011).

TEK of amphibian utilisation is better documented than LEK detailing amphibian ecology, since amphibians are utilised by many cultures world-wide and for many different reasons (Adeola, 1992; Alves & Souto, 2011). Our study highlights the traditional and subsistence use of frogs by indigenous communities in the Western Ghats. It also reveals the cultural association of indigenous communities with frogs from their intricate utilisation in traditional medicine. This use is absent in non-indigenous communities who are recent settlers from other parts of the country. Frog utilisation did not appear to be a sensitive issue in the communities investigated in our study, as respondents appeared happy to discuss this subject openly. The rationale for the use of frogs among indigenous communities in this study, especially in treating skin burns, was similar to that reported for other communities from North India (Negi & Palyal, 2007). *Nasikabatrachus sahyadrensis* was the most notable species utilised by indigenous communities in the region, and this should be taken into consideration while formulating conservation plans for this species. Our results identify distinct user groups and rationale for utilisation of this endangered frog, providing an important baseline for further research, stakeholder discussions (Kanagavel et al., 2013), and development of culturally appropriate conservation interventions. We recommend that more research should be focused on the use of amphibians by local communities, to identify more species that may be important to communities and for which useful knowledge may therefore be collected.

Local support for conserving forests and frogs

Although there has been an increase in scientific research on Indian amphibians, there is very little awareness about the status of amphibians among regional forest departments (Kanagavel et al., 2017c) and local communities; two groups of stakeholders that are integral to successful amphibian conservation. Interest in amphibian protection among local communities has, until now, not been well-understood. Our results suggest that for an amphibian-based community conservation initiative to be effective, it must be linked to protection of forests, since a significant proportion of community livelihoods depend directly on the continued presence of forests, and local respondents were only interested in frog conservation within the wider concept of forest protection. Such programmes could be initiated at Topslip and Valparai as determined by our study since the interest of respondents in protecting frogs was greater at these localities. Respondents at Munnar showed a reduced interest in frog conservation, due to the widespread misperception of frogs as pests of cardamom (Kanagavel et al., 2017b). Clearly an educational campaign to improve the profile of frogs among local communities is required in Munnar (Kanagavel et al., 2017a).

Our study demonstrates that the knowledge of local communities can potentially be used to gather reliable information on the ecology and distribution of amphibian species that are morphologically distinctive, have a specific local name, and are associated with specific cultural and/or utilitarian values. We also highlight patterns of folk utilisation of frogs in the southern Western Ghats and provide new insights into respondent typology that can assist in future LEK-related amphibian research.

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SHORT NOTE

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Salamandra algira atlantica in the Tazzeka National Park, Morocco

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North-west Africa has relatively few amphibian species although many of these are endemic, including *Salamandra algira* (Escoriza & Ben Hassine, 2019). The populations of this species are fragmented in the mountain systems of the extreme north of the region, between the Tingitana Peninsula (north-west Morocco) and Djebel Edough (north-east Algeria) (Escoriza & Ben Hassine, 2015). Some of these isolated populations are currently recognised as distinct subspecies based on genetic and morphological differences, and distributional range (Beukema et al., 2013; Hernandez & Escoriza, 2019). *Salamandra algira atlantica* Hernandez & Escoriza, 2019 is a recently described subspecies, completely confined to the Middle Atlas Mountains. However, its habitat requirements and the limits of its distribution are still poorly understood (Hernandez, 2018a,b).

From 2006 to 2020, we surveyed the Tazzeka National Park (Middle Atlas Mountains, Taza province, north-east Morocco) for *S. algira atlantica*, mostly during late autumn to early spring. Habitats were sampled for adults by flipping logs and stones in terrestrial habitats and by inspecting streams and pools for larvae. Caves were selected based on topographic maps provided by speleological expeditions (Association de Spéléologie Marocaine, Randoxygène). Geographical coordinates and elevational data were collected in situ by GPS (Garmin Montana 680; Garmin Ltd., Olahe, KS, USA). The temperature of water was measured in situ using an Expresstech @ LCD PH Medidor Digital Meter (Expresstech; Kingpow Company Limited; Hong-Kong; China).

We recorded 19 localities occupied by *S. algira atlantica* (Fig. 1), (see also Supplementary Materials) four of which were caves (Grotte Izora, Gouffre du Friouato, Grotte de Chaâra and Grotte Lazrak). The salamander was detected mainly in the elevational zone dominated by oak forests, from the thermophilic (*Quercus coccifera*) to the mesophilic (*Quercus canariensis*) formations at 730 to 1679 m asl (Fig. 2). However, in other regions of the Middle Atlas this subspecies has been found as high as 2455 m asl (Hernandez, 2018a; Hernandez & Escoriza, 2019). In general, the habitats used by *S. algira atlantica* show a relatively dense forest cover, but the species can also occupy more open habitats in other regions of the Middle Atlas. These habitats are similar to those described for the other subspecies of

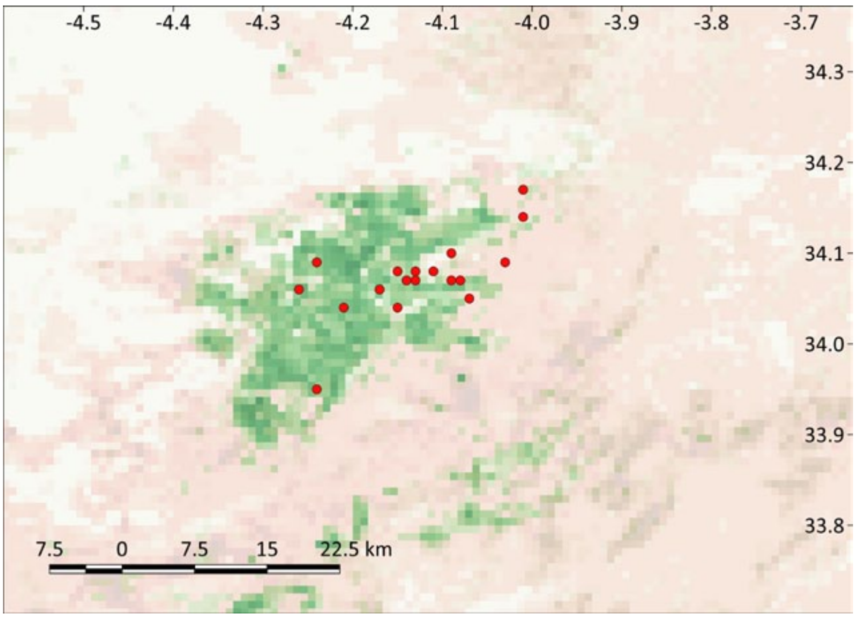

Figure 1. Map of the study region (Jebel Tazzeka National Park and surrounding region), with the localities of *S. algira atlantica* superimposed (red dots). The green gradient showed the degree of forest cover (higher density, greener colour) and the pink gradient showed bush cover (higher density, redder colour). Blank represents cultivated fields, barren terrain and villages (based on Tuanmu & Jetz, 2014).

Figure 2. Example of the typical habitat of *S. algira atlantica*, at the mid elevational strip of its ecological range, a mature forest of Quercus suber, Bab Azhar

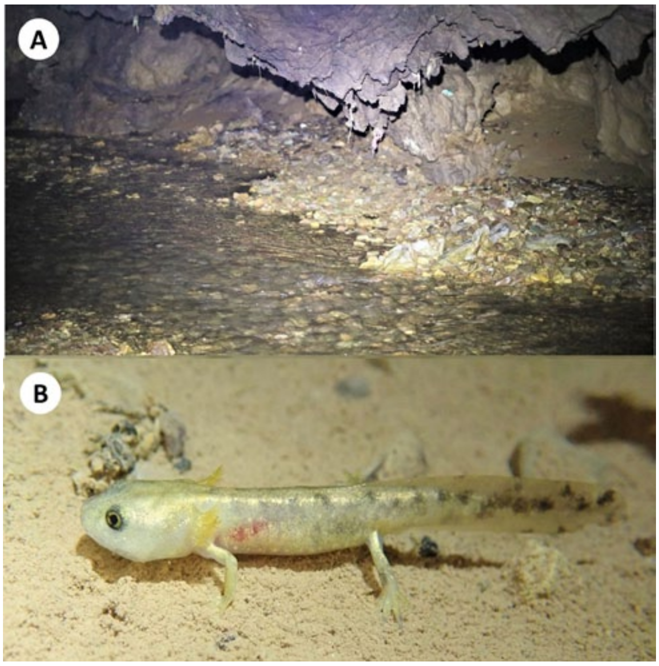

Figure 3. A. Habitat view of the flowing stream inhabited by larvae of the target species at Grotte de Chaâra, Taza province, **B.** Larva of *S. algira atlantica* found in a cave pool fed by water from the stream

S. algira (Escoriza & Ben Hassine, 2017), although *S. algira atlantica* is the only subspecies that is known to occur in the supra-forest grasslands such as alpine meadows (Hernandez, 2018a). In February, at Grotte de Chaâra, the air temperature at midday was 13.1˚ C and water temperature 11.1˚-12.6˚ C. The conditions of stable temperature and high humidity allowed these salamanders to remain active independently of the diel cycle, unlike the populations living outside, whose daytime activity is usually only occasional. Cave specimens were located from the entrance up to 2 km inside the caves. During 2018-2019, larvae were located in rock pools in caves such as Grotte de Chaâra (Fig.3), in complete darkness. The cave dwellers did not show any particular specialisations for life in caves and most were phenotypically identical to those found outside caves. A typical example of *S. algira atlantica* is shown in Figure 4 but alongside these we also found individuals that were hypomelanistic through to full coral red (Hernandez & Escoriza, 2019).

The current study and those of Hernandez (2018b, 2019) confirm that there are several cave populations of *S. algira atlantica* in the Tazzeka National Park. Reproduction has also been observed in at least two caves, so although the subspecies generally lives above ground it would appear to have the capacity to maintain a permanent population in caves (i.e. the species is eutroglophilic). Other sub-species of *S. algira* that inhabit caves (e.g. in the Beni Snassen and Jebel El Haouz) similarly show no particular phenotypes adapted to this environment but do appear to differ in their ability to reproduce in caves. For example, in the Beni Snassen, *S. algira spelaea* occupies systems of small cavities and in Jebel El Haouz, *S. algira tingitana* also occupies small galleries but in neither case has reproduction within the caves been documented. For *S. algira spelaea* at Beni Snassen, reproduction in the cave is unlikely since larvae were only found in a small pond at the entrance to the small cave. It would appear that these other two other subspecies may be rather less well adapted to life in caves and may therefore be considered subtroglophilic.

This study has indicated that *S. algira atlantica* is still widespread and abundant within the Tazzeka National Park area, particularly in some well-preserved patches of oak

Figure 4. An adult male of *S. algira atlantica* found at Grotte de Chaâra, Taza province

forest. However, given the geographical isolation of this population it can be considered vulnerable (Hernandez & Escoriza, 2019). One important conservation problem is the growing demand for specimens in the pet trade because of their beautiful coloration. In addition, visits to caves by collectors and tourists also have an indirect negative effect, as visitors damage the fragile ecosystem (Gamble, 1981). We suggest that measures be implemented as a matter of urgency to conserve these very interesting populations of cave salamanders.

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Are the Mascarene frog (*Ptychadena mascareniensis*) and Brahminy blind snake (*Indotyphlops braminus*) really alien species in the Seychelles?

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INTRODUCTION

Alien species are those introduced by man, accidentally or intentionally, outside of their natural geographic range. Where alien species pose a major threat to native biota through resource competition, predation, and the introduction of disease, they are referred to as invasive alien species (IAS); IAS are especially a problem on remote islands with small and naïve populations (Kraus, 2009; Rocamora & Henriette, 2015; Young et al., 2017). It can be difficult to determine whether species are alien or native within given habitats. Molecular genetic methods offer one approach to testing hypotheses of alien status, and determining putative source and introduction pathways. Alien populations are predicted to vary little genetically from populations in their native range (Tsutsui et al., 2000), and relatively recently introduced populations are also predicted to have low within-population genetic variation if founded by a small number of individuals (i.e. the founder effect) (e.g. Sakai et al., 2014).

The biota of the granitic Seychelles islands have high levels of endemism, largely due to geographic isolation from other landmasses for approximately 65 million years (Davies, 1968; Mart, 1988; Collier et al., 2008; Chatterjee et al., 2013; Ali, 2018). In addition, the Seychelles are considered to be home to alien (introduced) species and IAS (Rocamora & Henriette, 2015), including plants (Fleischmann, 1997), mammals (Merton et al., 2002), reptiles (Nussbaum, 1980, 1984; Vences et al., 2004b), amphibians (Vences et al., 2004a), birds (Canning, 2011) and insects (Gerlach, 2004).

We examined mitochondrial (mt) DNA sequence data to test the hypotheses that the Mascarene frog, *Ptychadena mascareniensis* (Duméril & Bibron, 1841), and Brahminy blind snake, *Indotyphlops braminus* (Daudin, 1803), are introduced species in the Seychelles (Nussbaum, 1980; Vences et al., 2004a). The Mascarene frog has been reported from nine of the Seychelles islands (Labisko et al., 2015) and, based on molecular genetic data for the single Seychelles individual thus far sampled, is hypothesised to have been introduced by humans from Madagascar in the recent past (Vences et al., 2004a). The Brahminy blind snake, sometimes considered native to India (Hedges et al., 2014), has a global distribution including Asia, Europe, the Middle East, Africa, Australia and the Americas (Uetz et al., 2019). It is well equipped to invade new regions because of its small size, tolerance of dry and human-modified habitats, and because it reproduces by parthenogenesis (McDowell, 1974; Nussbaum, 1984; Wynn et al., 1987). It has been reported from seven Seychelles islands (Nussbaum, 1980; Rocha et al., 2009) where it is thought to be non-native (Nussbaum, 1980). Genetic data for the single *I. braminus* from the Seychelles sampled in previously published studies are identical to those from localities in Europe, Africa, the Comoro islands, Asia and Central America (Rato et al., 2015).

To date no studies have assessed intraspecific molecular genetic variation within Seychelles *P. mascareniensis* or *I. braminus*. We address this data gap and provide further tests of the hypotheses that these taxa are alien to the Seychelles.

METHODS

We generated DNA sequence data for a region of the mt 16s rRNA gene (16s), for multiple individuals of each species from the Seychelles and additional samples of *I. braminus* from Sri Lanka (Tables 1, 2). Tissue samples (frog liver or toe tips; snake liver or muscle) were obtained from 10 Seychelles (from the islands of Curieuse, La Digue, Mahé, Praslin and Felicité – the latter a new record for the species taking the known Seychelles range to eight islands) and five Sri Lankan *I. braminus*, and 19 Seychelles *P. mascareniensis* (La Digue, Mahé, North, Praslin, Silhouette). All available published 16s sequence data (16) for *I. braminus* and the single previously published sequence of *P. mascareniensis* from the Seychelles were obtained from Genbank and added to our datasets (Tables 1, 2). The mitochondrial marker 16s was targeted because this locus is the most commonly published for *I. braminus* and had been used in previous Seychelles studies of both *I. braminus* and *P. mascareniensis* (Vences et al., 2004a; Rato et al., 2015).

Genomic DNA was extracted from tissue using a Qiagen DNeasyTM Blood and Tissue Kit following manufacturer's guidelines. Partial fragments of 16s were amplified using the polymerase chain reaction (PCR) and sequenced following protocols reported by Maddock et al. (2014, 2017). Sequences were manually trimmed using Geneious Prime (Biomatters) and aligned using default parameters in Muscle (Edgar 2004).

RESULTS

The *I. braminus* and *P. mascareniensis* 16s datasets comprised a total of 502 and 588 aligned base pairs (bp), respectively. For the *I. braminus* alignment all overlapping sequences from across the globe (Seychelles, Sri Lanka, Thailand, Mexico, Equatorial Guinea, Spain (+Tenerife), Comoro Islands and China) were identical, with the exception of one individual from southern India (JN172940), which differs by 9 bp, including one deletion. All *P. mascareniensis* sampled were identical. Newly generated sequences (Table 1 & 2) have been deposited in GenBank.

DISCUSSION

Our multi-specimen mtDNA sequence data are consistent with (and provide additional support for) the hypotheses that both the Mascarene frog and the Brahminy blind snake are alien species in Seychelles. Our much expanded sampling found no intraspecific molecular variation for either taxon within the Seychelles and is in agreement with results from previous studies of both *P. mascareniensis* (Vences et al., 2004a) and *I. braminus* (Rato et al., 2015).

In the Seychelles, the Mascarene frog occurs in lowland, often human modified landscapes, including habitats much more degraded than those that support Seychelles native frogs (sooglossids and the Seychelles treefrog, *Tachycnemis seychellensis*) (pers. obs.). For sooglossids and Seychelles treefrogs, 16s sequence data vary substantially among the four islands on which they occur (Maddock et al., 2014; Labisko et al., 2019), contrasting strongly with the single 16s haplotype found within *P. mascareniensis* across the five sampled islands. Vences et al. (2004a) found this same 16s haplotype in *P. mascareniensis* from Mauritius, with this differing from a Reunion and Madagascar haplotype by only a single substitution, consistent with a recent introduction of one (or very few) individual lineages of this species in the Seychelles and the Mascarenes. In addition, Zimkus et al. (2017) indicated intra-specific variation of *P. mascareniensis* within Madagascar, supporting a single introduction source to Seychelles.

Published studies of native Seychelles reptile species occurring on multiple islands display a substantial amount of intraspecific mtDNA variation among populations from at least some islands (e.g. Rocha et al., 2013; Valente et al., 2014; Harris et al., 2015), with the only exceptions discovered thus far being the Brahminy blind snake and the terrapins *Pelusios castanoides* and *P. subniger* (Silva et al., 2010). The two terrapin species are considered to be recent (likely human mediated) arrivals in the Seychelles based on haplotype sharing with conspecifics from Madagascar and Africa (Fritz et al., 2013). Interpretation of the biogeographic history of Seychelles Brahminy blind snakes is complicated by scant sampling across this species' global range. Although our data are consistent with an introduction to the Seychelles,

Table 1. Sample information for the Mascarene frog *Ptychadena mascareniensis*. Individuals with a "Sample ID" had 16s sequence data generated for them in this study. GenBank accession numbers are provided (https://www.ncbi.nlm.nih.gov/genbank/).

Species	Sample ID	Locality	Latitude (S)	Longitude (E)	GenBank#
P. mascareniensis	SM086	Mahé, Seychelles	04° 36'21.4"	055° 26'20.8"	MT509738
P. mascareniensis	SM124	Praslin, Seychelles	04° 19'33.24"	055° 45'29.10"	MT509739
P. mascareniensis	SM126	Praslin, Seychelles	04° 19'33.24"	055° 45'29.10"	MT509740
P. mascareniensis	SM127	Praslin, Seychelles	04° 19'33.24"	055° 45'29.10"	MT509737
P. mascareniensis	SM244	Silhouette, Seychelles	04° 29'01.2"	055° 14'56.9"	MT509741
P. mascareniensis	SM253	La Digue, Seychelles	04° 20'57.4"	055° 49'48.6"	MT509742
P. mascareniensis	SM255	La Digue, Seychelles	04° 20'25.38"	055° 50'11.88"	MT509743
P. mascareniensis	SM256	La Digue, Seychelles	04° 20'25.38"	055° 50'11.88"	MT509744
P. mascareniensis	SM258	La Digue, Seychelles	04° 21'43.20"	055° 49'57.84"	MT509745
P. mascareniensis	SM434	North, Seychelles			MT509755
P. mascareniensis	SM435	North, Seychelles			MT509754
P. mascareniensis	SM436	North, Seychelles			MT509753
P. mascareniensis	SM437	North, Seychelles			MT509752
P. mascareniensis	SM438	North, Seychelles			MT509751
P. mascareniensis	SM439	North, Seychelles			MT509750
P. mascareniensis	SM440	North, Seychelles			MT509749
P. mascareniensis	SM441	North, Seychelles			MT509748
P. mascareniensis	SM442	North, Seychelles			MT509746
P. mascareniensis	SM443	North, Seychelles			MT509747
P. mascareniensis		Praslin, Seychelles			AF517589[1]

[1] Vences et al. (2004a)

Table 2. Sample information for the Brahminy blind snake *I. braminus*. Individuals with a "Sample ID" had 16s sequence data generated for them in this study. MW numbers from Sri Lanka are field tags of uncatalogued National Museum of Sri Lanka, Colombo specimens. New island locality records are denoted with a *. GenBank accession numbers are provided (https://www.ncbi.nlm.nih.gov/genbank/).

Species	Sample ID	Locality	Latitude (S)	Longitude (E)	GenBank#
I. braminus	RAN25158	Curieuse, Seychelles			MT509731
I. braminus	SM313	La Digue, Seychelles	04° 21'43.20"S	055° 49'57.84"E	MT509730
I. braminus	MW10438	La Digue, Seychelles	04° 21'43.20"S	055° 49'57.84"E	MT509729
I. braminus	MW10439	La Digue, Seychelles	04° 21'43.20"S	055° 49'57.84"E	MT509728
I. braminus	MW10443	La Digue, Seychelles	04° 21'43.20"S	055° 49'57.84"E	MT509727
I. braminus	MW10240	Mahé, Seychelles	04° 36'24.05"S	055° 26'25.05"E	MT509725
I. braminus	SM455	Mahé, Seychelles	04° 36'24.05"S	055° 26'25.05"E	MT509725
I. braminus	SM481	Praslin, Seychelles	04° 19'54.88"S	055° 44'23.81"E	MT509724
**I. braminus*	SM482	Félicité, Seychelles	04° 19'36.9"S	055° 52'26.5"E	MT509723
**I. braminus*	SM483	Félicité, Seychelles	04° 19'36.9"S	055° 52'26.5"E	MT509722
I. braminus	MW1777	Sri Lanka	06° 7'22.20"N	080° 33'43.80"E	MT509736
I. braminus	MW1778	Sri Lanka	06° 23'26.40"N	080° 14'29.40"E	MT509735
I. braminus	MW1779	Sri Lanka	06° 23'26.40"N	080° 14'29.40"E	MT509734
I. braminus	MW1780	Sri Lanka	06° 24'51.00"N	080° 19'22.20"E	MT509733
I. braminus	MW1781	Sri Lanka	06° 24'51.00"N	080° 19'22.20"E	MT509732
I. braminus		Mahé, Seychelles			KJ783470[1]
I. braminus		Thailand			AF544823[2]
I. braminus		Unknown			DQ343649[3]
I. braminus		Unknown			NC_010196[3]
I. braminus		Mexico			GQ469240[4]
I. braminus		Southern India			JN172940
I. braminus		Equatorial Guinea			KJ783466[1]
I. braminus		Equatorial Guinea			KJ783467[1]
I. braminus		Spain			KJ783468[1]
I. braminus		Tenerife, Spain			KJ783469[1]
I. braminus		Comoro Islands			KJ783471[1]
I. braminus		Comoro Islands			KJ783472[1]
I. braminus		Comoro Islands			KJ783473[1]
I. braminus		Comoro Islands			KJ783474[1]
I. braminus		Equatorial Guinea			KJ783475[1]
I. braminus		China			MK194179

[1] Rato et al. (2015), [2] Vidal & Hedges (2002), [3] Yan et al. (2008), [4] Adalsteinsson et al. (2009)

we cannot rule out that the islands occupy part of its natural range because of the apparent global universal genetic homogeneity of the sampled mtDNA marker. The genetic homogeneity may be, in part, due to a slowly evolving 16s mtDNA marker and due to the species' ability to reproduce by parthenogenesis. Additionally, the high dispersal ability of the species may reduce the opportunity for genetic diversification. The discovery of *I. braminus* on the island of Felicité takes the known Seychelles range of the species to eight islands.

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SHORT NOTE

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Anomalies in amphibians from the eastern Amazon region

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INTRODUCTION

Over the years, a broad range of morphological abnormalities (anomalies) have been documented from the anurans of both wild and urban populations (Ouellet, 2000; Blaustein & Johnson, 2003; Bionda et al., 2012). These anomalies have been recorded as a consequence of factors related to changes in the environment, such as exposure to UV-radiation and environmental pollutants (Cohen, 2001; Lannoo, 2009; Lunde & Johnson, 2012; Agostini et al., 2013) and parasitic infection (Kiesecker, 2002). Anomalies include incomplete or missing digits and limbs, additional digits and limbs, and body deformations, in which limb malformations seems to be the most prevalent (Gardiner & Hoppe, 1999; Blaustein & Johnson, 2003). Records have come from isolated individuals and from populations, and in the latter case they normally occur in about only 5 % of the population (Read, 1997). Thus the rate of occurrence may be considered abnormally high when the value in a population exceeds this mark (Piha et al., 2006; Peltzer et al., 2011; Bionda et al., 2012).

In this study we report anomalies in several anuran species from Amapá state, eastern Amazonia, Brazil, consider an endemic zone for the herpetofauna in the Guiana Shield (Silva et al., 2005), and reinforce the importance of the monitoring and conservation of the habitats for anuran populations in these areas.

MATERIALS AND METHODS

We sampled anurans during fieldwork conducted in the municipalities of Macapá (0° 00’29.9” S, 51° 05’43.1” W), Santana (0° 02’09.2” N, 51° 09’38.8” W) and Serra do Navio (0° 54’50.0” N, 52° 59’59.2” W), all in the State of Amapá, Brazil. Samples were collected between July 2017 to June 2018, with active visual and auditory searches along transects (Crump & Scott Jr., 1994; Heyer et al., 1994) during the night (from 17:00 h to 21:00 h), in temporary lentic water bodies surrounded by arboreal vegetation, wetlands and urban areas. The total search effort amounted to 900 person hours.

Abnormal individuals were examined for malformations which were then identified with reference to a recent publications on the subject (Lannoo, 2009; Peltzer et al., 2011; Henle et al., 2017; Meteyer, 2000), although for terminology and traits we have followed Henle et al. (2017) (see Table 1).

Specimens with anomalies were collected under permit (authorisation #48102-2) from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), killed with 5 % lidocaine, fixed in 10 % formalin and transferred to 70 % ethanol (Heyer et al., 1994). They have been deposited in the Herpetological Collection of the Universidade Federal do Amapá (CECC).

Table 1. Terminology of anuran anomalies and traits adopted in our study, according to Henle et al. (2017)

Type of anomaly	Traits
Amely	Missing limb
Anophthalmy	Missing eye
Brachydactyly	Presence of short toe, but the metatarsal bones remain present, with the number phalanges reduced
Ectrodactyly	Completely missing digit, including metatar-sal bone
Ectromely	Missing limb segments
Ocular anomaly of the eye	Anomalous pigmentation of iris
Polydactyly	Extra digit, including metatarsal bone
Polymely	Duplication of a complete limb or parts thereof
Syndactyly	Fused digits
Schizodactyly	Forked digits

RESULTS

A total of 255 adult anurans were observed, of which 20 had one or more morphological abnormality. These are listed in Table 2 and illustrated in Figure 1. Between habitat types, 97 individuals were recorded in primary forest, of which 6 (6.2 %) had anomalies; 77 individuals were recorded in urban areas, of which 9 (11.7 %) had anomalies; and, 81 individuals were reported in floodplain areas, of which

Table 2. Anuran species reported in our study with anomalies, Amapá State, Brazil

Species	Type of abnormalities	Localities	Voucher specimens (CECC)
Bufonidae			
Rhinella cf. *margaritifera*	Brachydactyly	0° 54'39.0" N 52° 00'42.1" W	2465
Rhinella cf. *margaritifera*	Brachydactyly + Syndactyly	0° 54'38.7" N 52° 00'41.9" W	1275
Rhinella cf. *margaritifera*	Schizodactyly	0° 54'38.8" N 52° 00'41.1" W	1218
Rhinella marina	Ectrodactyly	0° 54'28.9" N 52° 00'51.0" W	1416
Rhinella marina	Ectromely	0° 54'28.8" N 52° 00'50.9" W	1417
Rhinella marina	Brachydactyly + Polydactyly	0° 54'28.5" N 52° 0 0'51.2" W	1418
Rhinella marina	Polydactyly	0° 54'28.4" N 52° 00'51.1" W	2696
Rhinella marina	Ectrodactyly	0° 54'28.2" N 52° 00'51.2" W	2697
Rhinella marina	Brachydactyly + Ectrodactyly	0° 54'28.0" N 52° 00'51.4"W	2698
Dendrobatidae			
Ranitomeya amazonica	Syndactyly	0° 54'45.9" N 52° 00'32.7" W	2699
Hylidae			
Boana boans	Colour anomaly of the eye	0° 54'36.5" N 52° 00'37.5" W	0836
Lysapsus bolivianus	Ectromely	0° 02'09.0" S 51° 09'33.0" W	0696
Scinax cf. *ruber*	Colour anomaly of the eye	0° 02'06.8" S 51° 09'31.5" W	2390
Sphaenorhynchus lacteus	*Amelia* + Colour anomaly of the eye	0° 02'06.8" S 51° 09'31.5" W	0436
Leptodactylidae			
Adenomera hylaedactyla	Ectromely	0° 53'55.1" N 52° 00'05.4" W	0839
Leptodactylus longirostris	Ectromely	0° 54'29.8" N 52° 00'50.1" W	1516
Leptodactylus mystaceus	Anophthalmy	0° 54'23.8" N 52° 00'26.3" W	0368
Leptodactylus cf. *podicipinus*	Polymely	0° 00'30.8" S 51° 05'43.9" W	2628
Leptodactylus petersii	Brachydactyly	0° 02'08.5" S, 51° 09'31.4" W	2403
Leptodactylus sp.	Amely	0° 02'09.4" S 51° 09'32.5" W	2633

with 5 (6.2 %) had anomalies. The species most frequently showing anomalies was *Rhinella marina* (Bufonidae) (n = 6). We identified 10 types of anomalies and their frequencies were as follows: Brachydactyly (25 %; n = 5); Ectromely (20 %; n = 4); Ectrodactyly (15 %; n = 3); Ocular anomaly (15 %; n = 3); Syndactyly (10 %; n = 2); Amely (10 %; n = 2); Polydactyly (10 %; n = 2); Anophthalmy (5 %; n = 1); Polymely (5 %; n = 1); and Schizodactyly (5 %; n = 1). Individuals of the species *Sphaenorhynchus lacteus*, *Rhinella* cf. *margaritifera*, and *R. marina* presented more than one type of abnormality (Table 2).

DISCUSSION

Globally, most accounts of morphological abnormalities in anurans have been reported in species of Bufonidae (e.g., García-Muñoz et al., 2010; Bionda et al., 2012; Correia et al., 2018) and Ranidae (e.g., Haas et al., 2017). For Brazil, there are also reports mentioning other families but with apparently lower frequencies of anomalies, in particular Leptodactylidae (Sousa & Costa-Campos, 2016; Santos el al., 2017), Hylidae (Sousa & Costa-Campos, 2017; Ramalho et al., 2017; Silva-Soares & Mônico, 2017) and Aromobatidae (Santana et al., 2020).

In the literature, brachydactyly has been cited in several cases (e.g., Bionda et al., 2012; Sousa & Costa-Campos, 2016; Oliveira-Souza et al., 2020), being the most common in semi-aquatic and terrestrial species (Agostini et al., 2013), and in our study was observed in the genera *Leptodactylus* and *Rhinella* (Table 2). In addition, it is of interest to note the anomalies of *Leptodactylus* sp., *L.* cf. *podicipinus* (Fig. 1E) and *Sphaenorhynchus lacteus* (Fig. 1K). Previously, in these cases duplication of a complete limb or parts thereof (polymely), and missing limb (amely) have been cited as malformations caused by the trematode *Ribeiroia* (Kiesecker, 2002; Stopper et al., 2002; Schotthoefer et al., 2003; Johnson et al., 2012). However, there is no evidence of the presence of this parasite in Amapá state.

During this study, the urban areas provided the greatest number of specimens with anomalies although differences between habitat types were small. The urban areas of Amapá state have a historical record of environmental impacts. For example, in the 1990s an environmental accident in the port area of the municipality of Santana caused the pollution of

Figure 1. Examples of morphological abnormalities shown by anuran species in this study - Colour anomaly of the eye: **A.** *Boana boans*, **B.** *Scinax* cf. *ruber* and **C.** *Sphaenorhyncus lacteus*; Anophthalmy: **D.** *Leptodactylus mystaceus*; Polymely: **E.** *Leptodactylus* cf. *podicipinus*; Brachydactyly: **F.** *Rhinella* cf. *margaritifera*; Ectromely: **G.** *Rhinella marina*; Polydactyly: **H.** *Rhinella marina*; Ectromely: **I.** *Adenomera hylaedactyla* and **J.** *Lysapsus bolivianus*; Amely: **K.** *Sphaenorhyncus lacteus*

the water table and surface waters with arsenic, barium and manganese in several areas of the region, in which heavy metals from mining tailings were responsible for a biological imbalance (Casara, 2003; Muniz & Oliveira-Filho, 2006); this area is located 3.5 km from the floodplain area and 4 km from the urban area sampled in our study. Also, there are reports of environmental impacts of Serra do Navio mining in and around the Parque Natural Municipal do Cancão (protected conservation unit sampled in our study), resulting in contamination with chemical residues, including arsenic, deforestation and pollution of streams (Drummond, 2000; Queiroz et al., 2008), which might be considered exogenous factors affecting the frog populations (Santana et al., 2020). Future ecotoxicological studies of anuran populations are needed to establish the actual causes of morphological anomalies, especially in areas with a history of environmental impacts.

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SHORT COMMUNICATION

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Dorsal stripe polymorphism of *Vipera berus* in south-east England

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The northern viper or adder (*Vipera berus*) is sexually dimorphic and well known for variations in both colour and dorsal markings. Typical markings are a dorsal stripe composed of mirror waves and zigzags (for convenience referred to as a 'zigzag' stripe) either side of which is a dorso-lateral row of spots/bars that oppose the indentations of the zigzag (Benson, 1999; Steward, 1971). Smith (1951) suggests that variations in dorsal patterning are restricted to about 2 % of individuals and mentions records of specimens from England (Lake District and Yorkshire) and South Wales where the zigzag is instead a smooth stripe and the dorsolateral spots/bars are lacking. Benson (1999) who studied *V. berus* in Yorkshire indicates that the stripe may be solid or have a lighter middle band. According to the Kent County recorder for reptiles and amphibians, there are no previous records of smooth striped northern vipers from Kent (Brady, L. pers. comm.). However, between 2005 and 2020 we recorded several cases in Kent where the dorsal stripe was not the classic 'zigzag'.

Here we report records of nine individuals with varying degrees of divergence from the classic zigzag from four sites in south-east of England, three in the county of Kent and one in the adjacent county of East Sussex (Table 1). In Kent, the first specimen recorded was an adult female (confirmed via cloacal probing) with a partial smooth stripe (Fig. 1A) and vestiges of the dorso-lateral spots/bars. This bears a close resemblance to the individual pictured by Smith (1951), where the zigzag is retained posteriorly. From the second Kent site, four further specimens were observed but with variants of the partial smooth stripe. This stripe had short lateral projections, making it in effect a rough stripe, no sign of the lateral bars/spots, and in all specimens the zigzag pattern was retained posteriorly (Fig. 1B). At the third Kent site, an adult male (Fig. 1C), an adult female (Fig 1D), and a sub-adult male (Fig. 1E) were observed with complete smooth stripes (no zigzag even posteriorly) and with lateral spots/bars completely absent. The adult female was unusual in being only very weakly patterned and with only with a hint of the stripe (Fig. 1D). The sub-adult male (Fig. 1E) had the classic brown colouration of a female *V. berus* but was identified as male based on hemipenal swelling, tail-length and rostral markings (Burghardt, 2005). Most other records of vipers from the third site were of normally patterned *V. berus* but our small sampling effort prevents us from making any justifiable estimate of the proportion of smooth striped individuals present on the third site but potentially this was greater than the 2 % quoted by Smith (1951). In addition to the Kent records, a smooth striped male was observed in April 2020 in East Sussex (Webster, pers. com.).

Table 1. Observations of *V. berus* in south-east England with variations on the classic zigzag dorsal stripe

Site and habitat	Date	Vipers observed	Marking
Kent, Lyminge and Denge Forests, 165 ha of mixed broad-leaf and coniferous woodland plantations and chalk grassland	June 2005	1 adult female	Partial smooth stripe (Fig. 1A)
Kent, Folkestone, 2.5 ha of open chalk grassland and mixed scrub habitat	June - August 2016	2 juvenile females	Partial rough stripe
		1 adult female	Partial rough stripe (Fig. 1B)
		1 adult male	Partial rough stripe
Kent, Ashford, 134 ha of chalk grass-land, woodland and mixed scrub and grassland	April 2019	1 adult male	Complete smooth stripe, lateral spots / bars absent (Fig. 1C)
	June 2019	1 adult female (?)	Very weakly marked with faint hint of a complete smooth stripe, lateral spots / bars absent (Fig. 1D)
	March 2020	1 sub-adult male	Completely smooth stripe, lateral spots / bars absent (Fig. 1E)
East Sussex, 15 ha of chalk grassland	April 2020	1 adult male	Complete smooth stripe, presence of lateral spots/bars unknown

The 'striped' morph of *V. berus* is described as being extremely geographically confined, occurring alongside vipers with the zigzag stripe but not melanistic individuals (Wolf & Werner, 1994). This is consistent with the third site in Kent where no melanistic individuals have yet been recorded, which can be compared to another viper population, about 9.5 km away from the third site, where melanistic individuals occur but to date only the zigzag morph has been observed

Figure 1. Northern vipers with unusual dorsal stripes - **A.** Adult female with a partial smooth stripe that becomes a typical zigzag posteriorly, **B.** Adult females with a partial rough edged stripe that becomes a typical zigzag posteriorly, **C.** Adult male with a complete smooth stripe, i.e. extending the whole length of the body, **D.** Adult female (?) with only very faint body markings including a complete smooth stripe, and **E.** Sub-adult male with a complete smooth stripe (despite showing classic female brown colouration this specimen was confirmed as a male)

(Griffiths, pers. comm.). No evidence either way can be offered from the other sites.

We observed that the snakes with variants of the zigzag stripe often lacked lateral spots/bars. This may offer some clues as to how the marking pattern is formed during embryological development with perhaps the spots/bars cleaving from a stripe to create the zigzag. The zigzag stripe is common to eight of the European viper species (Speybroeck et al., 2016) and much research has focussed on identifying its role in behavioural ecology. It is suggested that it is cryptic (camouflage), and/or aposematic (a warning that the vipers are venomous), and particularly in the case of the males may facilitate 'flicker fusion' to disorientate predators (Santos et al., 2014; Valkonen et al., 2011; Lindell & Forsman, 1996). Such benefits may be lost by other phenotypes. For example, it has been demonstrated that there are differences in reproductive success and predation between zigzag and melanistic phenotypes, where the latter had a higher risk of predation but greater reproductive success than the former (Andren & Nilson, 1981).

Declines in northern viper populations have been a matter of conservation concern for some years. One possible factor in these declines emerged in Sweden where an isolated population of northern viper was found to suffer from inbreeding that resulted in still born or deformed young (Madsen et al., 1996); the problem was resolved by a conservation translocation of adult males into the inbred population. The genetic health of northern viper populations is therefore a matter of concern but it would appear that presence of a range of phenotypes within a single population may be an indicator that the population is genetically robust (Madsen, pers. comm.). The occurrence of variously striped individuals in our populations may therefore be considered a positive feature in that regard. Furthermore, although genetic sampling in southern England has found striking levels of relatedness amongst northern viper populations, it is more likely that demographic factors rather than genetic ones are the cause of declines in small British populations (Ball et al., 2020; Gardner et al., 2019). The advisability of conservation translocations of adders for genetic or demographic enhancements still requires detailed consideration while habitat management and public engagement are more firmly established conservation options (Julian & Hodges, 2019).

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SHORT COMMUNICATION

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Defensive strategies in three Amazonian hylids (Anura: Hylidae)

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Anurans may adopt different defensive strategies to prevent predation (Duellman & Trueb, 1994; Wells, 2007). A variety of these strategies, either on their own or in combination, have been reported from hylid species and include eye-protection, crouching down, puffing up the body, body raising and feigning death (thanatosis) (Azevedo-Ramos, 1995; Angulo & Funk, 2006; Angulo et al., 2007; Toledo et al., 2011). These behaviours are considered 'defensive strategies' when they are effective in reducing predator attacks, and are a common and diverse survival mechanism employed by many anurans (Toledo & Haddad, 2009; Toledo et al., 2010 & 2011; Humphreys & Ruxton, 2018; Ferreira et al., 2019). In this study we describe defensive strategies of three hylids, *Boana geographica* (Spix, 1824), *Boana lanciformis* (Cope, 1871) and *Boana calcarata* (Troschel, 1848), from the eastern Amazonia region. These species were identified morphologically as detailed in previous studies, *B. geographica* and *B. calcarata* (Silva e Silva & Costa-Campos, 2018) and *B. laciformes* (Costa-Campos et al., 2014).

On 21 February 2013, at 20.34 h, an adult male *B. geographica* (Fig. 1A) was recorded in the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá State, Brazil (0.9138° N, 52.9997° W). When handled it displayed eye-protection, and its head remained in an upright position for a few minutes. After its release, it remained in a lower than the habitual sitting posture, exhibiting the 'crouching down' defensive behaviour (Toledo et al., 2011, Fig. 1B).

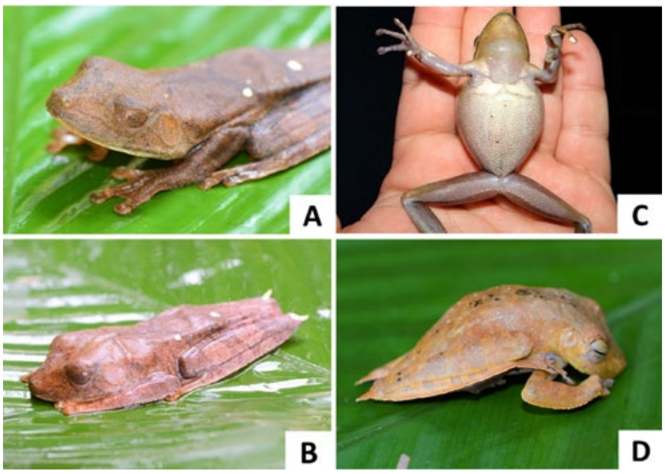

Figure 1. Defensive strategies of Amazonian hylids - **A.** and **B.** *B. geographica* displaying eye-protection and 'crouching down', respectively; **C.** *B. lanciformis* displaying puffing up the body with thanatosis; **D.** *B. calcarata* exhibiting 'crouching down'

On 4 October 2013, at 22.14 h, an adult male *B. lanciformis* was recorded calling on branches above temporary lentic water bodies surrounded by arboreal vegetation inside secondary forest in Santana Island (0.0802° N, 51.1821° W), municipality of Santana, Amapá State, Brazil. When handled it puffed up the body by inflating its lungs, this was followed by thanatosis with limbs outstretched (Fig. 1C).

On 13 October 2017, at 21.53 h, an adult *B. calcarata* was collected in the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state, Brazil (0.9138° N, 52.9997° W). When handled, the individual immediately exhibited 'crouching down' behaviour, remaining motionless for three minutes with body arched, arms and legs bent and kept close to the body, and with its eyes partially open (Fig. 1D). After release the individual resumed its initial position, moving slowly.

According to Toledo et al. (2011), eye-protection and crouching are generally associated with puffing up the body and body raising, however, we did not observe this association. Puffing up the body behaviour has been reported in anurans with the accompaniment of other defensive behaviours, such as thanatosis, as was the case for *B. lanciformis* in this study.

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SHORT COMMUNICATION

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Egg attendance behaviour of an Amazonian poison frog *Ameerega hahneli* (Anura: Dendrobatidae)

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The neotropical family Dendrobatidae includes nearly 200 species known as 'poison frogs' (Frost, 2020). Poison frogs lay their eggs in arboreal nests, e.g. leaves on bushes, leaf litter and phytotelmata (Grant et al., 2006; Lötters et al., 2007; Stynoski et al., 2015). After oviposition, the parents attend and take care of the eggs during development until the tadpoles hatch (Weygoldt, 1987; Grant et al., 2006). Once they hatch, the adult male or female will typically transport the tadpoles on their backs to a location where each tadpole will complete metamorphosis (Grant et al., 2006; Pyron & Wiens, 2011; Hime et al., 2020). For several dendrobatid species, information on phylogenetic relationships (e.g. Pyron & Wiens, 2011; Grant et al., 2017), ecology (Toft, 1995), natural history (Haddad & Martins, 1994), and behaviour (Hödl et al., 2004; Pašukonis et al., 2014) has been well-documented. However, detailed information on egg attendance and parental care is still scarce for most species. Here we describe our observations on egg attendance of an Amazonian species of poison frog, *Ameerega hahneli* (Boulenger, 1884).

Between 14 and 19 May 2020, we monitored the egg attendance behaviour of a single male *A. hahneli* in a relictual forest in the municipality San José del Fragua, Caquetá, Colombia (1.352876, -75.971376, WGS84; 466 m a.s.l; Fig.1). We observed clutch attendance during the night using red light to reduce disturbance. On 14 May 2020 at 19:17 h, we observed a male of *A. hahneli* attending an egg mass, which was composed of 22 eggs containing tadpoles (Fig. 2A), deposited on the surface of a leaf at 50 cm above the ground. We captured the male to measure its body length

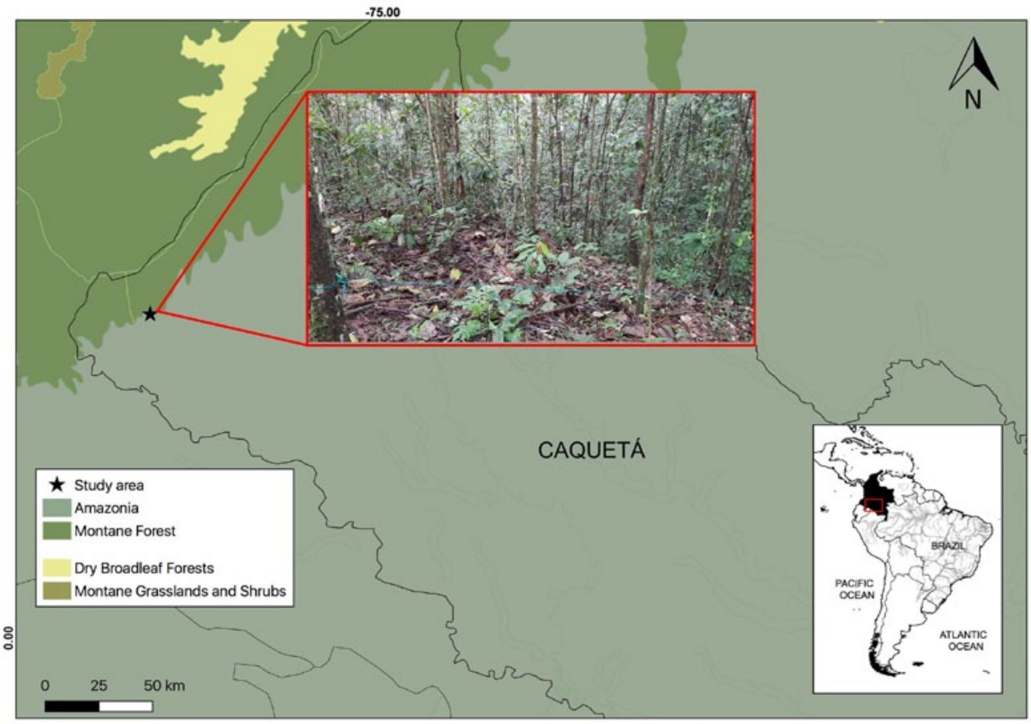

Figure 1. Ecoregions of Colombia, adapted from Dinerstein et al. (2017), showing the location of the study area (black star)

Table 1. A detailed description of egg attendance behaviour of a male of *A. hahneli* over a five-day period

Date	Behaviour	Time	Figure	Observation
15 May	The male was attending the clutch.	07:00 h	2A	-
	The male left the clutch and withdrew from the site.	10:47 h		-
	The male returned to the base of the plant where the clutch was located and remained for 41 minutes.	13:14 h	2B	-
	The male left the clutch again and returned at 16:56 h, perching on a leaf one meter away from the clutch.	14:15 – 16:56 h		During that time, it emitted a territorial call composed of five notes (see Rodríguez & Duellman, 1994). Other individuals of *A. hahneli* also began to vocalise. Afterwards, the male jumped three times and stopped calling.
	The male positioned itself at the base of the plant where the clutch was located and stopped calling.	17:52 h		-
	The male was quiet for five minutes on another leaf near the clutch and called back for four minutes.	18:12 h		The male began to climb the plant (see video, YouTube, 2020).
	The male moved several times, touched the clutch, and the tadpoles began to move quickly for approximately one minute and male turned again.	18:22 h		We continued monitoring until 22:00 h.
16 May	The male was not attending the clutch.	09:00 h	-	We also did not see the male or other individuals of this species around the study site.
	The same individual of *A. hahneli* approached the base of the plant where the clutch was located.	14:45 – 15:46 h	-	-
	The male started to climb another branch and made several movements as it climbed.	15:48 h	-	-
	It started to rain and the male took refuge at the base of a leaf.	16:02 – 16:40 h	-	-
	The male started calling.	16:41 h	-	-
	The male emitted a courtship call composed of three notes (see Rodríguez & Duellman, 1994).	16:49 h	-	-
	The male started calling again at intervals.	16:59 – 18:05 h	-	-
	The male continued climbing towards the clutch.	18:07 h	-	-
	The male jumped to the leaf where the clutch was located.	18:10 h	-	The male turned its body in several directions but always stayed next to the clutch.
	The male placed its belly on the clutch and the tadpoles began to move quickly.	18:25 h	-	Three minutes later, we captured the male and corroborated with the photo marking that it was the same individual that was attending the clutch the previous day.
	The male continued attending the eggs.	22:00 h	-	We continued monitoring.
17-19 May	The egg attendance behaviour was repetitive for three more days until the tadpoles hatched.		2C	Only on 17 May did the male not attend the clutch site.

with a digital caliper (SVL = 24 mm). Also, we took ventral and dorsolateral photographs as a non-invasive method for individual identification (Díaz-Ricaurte et al., 2019). After measurement, the male was released next to the clutch where he remained and we monitored egg attendance behaviour for five days until tadpoles hatched from the eggs (Table 1). Our observations suggest that the male left the clutch at dawn to forage during the day and returned to attend the eggs in the afternoon and during the night. When the male returned to attend the eggs, it emitted territorial and courtship calls composed of five and three notes, respectively (see Rodríguez & Duellman, 1994; Twomey & Brown, 2008); and most calls were emitted at sunset.

Egg attendance is one of the modes of amphibian parental care that enhances the chances of survival (Trivers, 1972). This behaviour benefits offspring by protecting them from predation and dehydration (Weygoldt, 1987), and has been documented in other lineages (e.g. Kluge, 1981; Juncá, 1996; Bickford, 2004; Delia et al., 2013 & 2020). For poison frogs who attend their eggs during development, the final step is the transportation of tadpoles to water shortly after hatching (Weygoldt, 1987). Unfortunately, we did not observe the moment of hatching nor the male taking the tadpoles to water. We think that the male transported them on his back as previously observed in other individuals of the same species (Fouquet, 2003; Lötters et al., 2007).

Figure 2. Egg attendance behaviour by a male of *A. hahneli* - **A.** Male in attendance of the egg mass, **B.** Male returning to the plant where the clutch was located; **C.** Remains of the egg mass, tadpoles hatched and male departed

We offer a description of a detailed observation of egg attendance in *A. hahneli* showing that males of this species offer partial egg attendance until hatching, corroborating previous findings of Fouquet (2003) and Lötters et al., (2007). An experimental evaluation of the survival of embryos during development with or without parental attendance would be of interest in determining the value of this behaviour.

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Parasitic infestation and site preference of the tick *Amblyomma gervaisi* on the monitor lizard *Varanus bengalensis* in India

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Ticks are the most common arthropod group that parasitise reptiles and are particularly abundant in warm and humid climates (Auffenberg & Auffenberg, 1990). They are well-known as a cause of dermatological problems in reptiles (Cooper, 2006) and the organisms spread by ticks are a major constraint to animal health and productivity by causing morbidity and mortality (Ghosh & Nagar, 2014). Of 102 species of the tick genus *Amblyomma* (previously Aponomma), 37 species feed exclusively on reptiles (Buczek et al., 2006). Here we describe tick infestation of the Bengal monitor *Varanus bengalensis*.

From 4 September 2017 to 31 July 2019, 11 free- living *V. bengalensis* from different locations of the union territory of Delhi were examined for ectoparasites. The monitor lizard specimens studied included road-kills, live rescued animals from the local area, and animals that had died naturally in the wild. All visible ticks were collected using sterile forceps and were transferred to vials containing 70 % ethanol. The ticks collected were examined under a light microscope and identified using the key of Sen & Fletcher (1962). After collection of the ticks, the rescued monitor lizards were released back into the wild.

A total of 337 ticks were recorded from 9 of the 11 specimens of *V. bengalensis* (Table 1). These ticks were all identified as *Amblyomma (Aponomma) gervaisi* (Lucas, 1847) and were mostly concentrated in the axilla, inguinal and perianal regions. Female ticks (Fig. 1A) were recorded exclusively on anterior parts, i.e. neck region, axilla and inguinal (fore and hind limbs), while male ticks were recorded exclusively near the cloaca and posterior tail regions (Fig. 1B & C). Male ticks were generally present in much greater numbers than females (Table 1). There were two instances where a few males ticks were copulating with females on the inguinal regions.

Ticks of the genus *Amblyomma* have often been recorded in south India from captive and wild snakes such as the rat snake *Ptyas mucosus*, Indian cobras *Naja naja*, king cobra *Ophiophagus hannah*, Indian rock python *Python molurus* and reticulated python *Malayopython reticulatus* (Rajesh et al., 2015; Pandit et al., 2011) and there is a single previous record of a monitor lizard infested with *A. gervaisi*, from the Delhi region (Nagar et al., 1977). The tick *Amblyomma gibsoni* has also been recorded on *V. bengalensis* from Maharashtra (Harkare et al., 2007). But to date, there are apparently no reports that male and female *A. gervaisi* occupy different

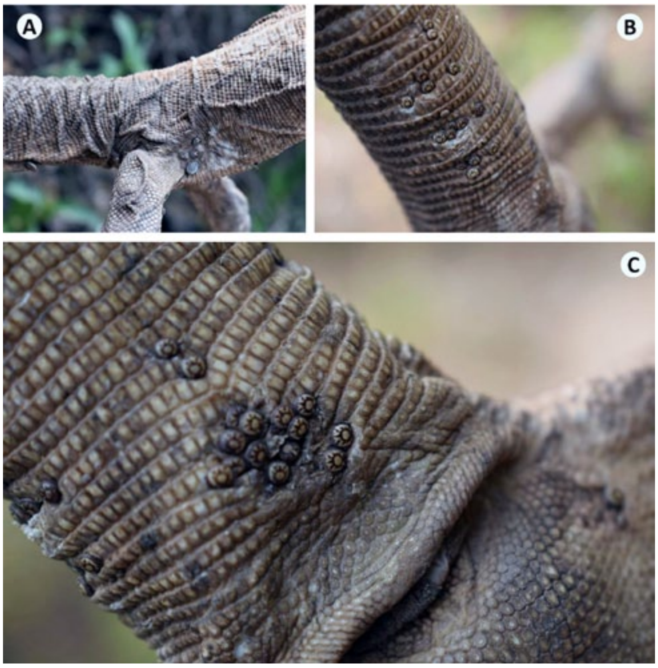

Figure 1. Ticks (*A. gervaisi*) attached to a monitor lizard (*V. bengalensis*)- **A.** Female ticks attached to the neck and axilla region, **B.** Clusters of male ticks on the posterior part of the tail, **C.** Male ticks attached near the cloaca

Table 1. The number of the ticks (*A. gervaisi*) recorded from specimens of monitor lizards (*V. bengalensis*)

Date of observation	Lizard details		Tick numbers			
	Snout vent length (cm)	Gender	Adult males	Adult females	Nymphs	Total ticks
04-Sep-17	96	Male	14	5	7	26
31-Oct-17	84	Female	22	12	6	40
24-Feb-18	43	Male	0	0	0	0
19-Mar-18	41	Male	34	17	0	51
30-Mar-18	59	Male	17	4	6	27
02-Apr-18	39	Female	9	4	1	14
12-Jun-18	56	Male	36	19	14	69
08-Oct-18	49	Male	0	0	0	0
20-Oct-18	82	Female	38	11	9	58
15-Dec-18	54	Male	19	3	2	24
07-Jan-19	66	Female	16	9	3	28
		Totals	205	84	48	337

locations on the bodies of monitor lizards. It would be of interest to undertake further research to elucidate why this is the case. There are two hypotheses that could be tested to explain the effect 1) Males and females may have different dietary preferences such that females may need easy access to the protein in blood so favour areas of thin skin overlying vascular tissue (armpits, necks, etc.) whereas males may be less dependent on protein and so may target the fat stored in the tail region, and/or 2) Female ticks may be more mobile than males, as unlike males they have been recorded from the burrows of monitor lizards, consequently the transfer of female ticks possibly occurs directly between hosts, especially during combat. For host to host transfer, females benefit from positioning on the main body as it is more favourable than on the tail; female ticks may also reach the host by launching themselves from the tips of grasses and herbs. But this is not the case for the less mobile male ticks that may be restricted to the underside of the tail and cloaca region and may generally be transferred only during copulation.

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Observations on the diet of the nose-horned viper (*Vipera ammodytes*) in Greece

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The nose-horned viper *Vipera ammodytes* (L., 1758) is a widespread southern European species and the only one to possess a distinguishable soft nose-horn (Arnold & Ovenden, 2004). Among European snakes, it is notable in having the most potent venom (Latinović et al., 2016) and in south-east Europe is responsible for the majority of envenomations (e.g. Frangides et al., 2006).

Vipera ammodytes inhabits a variety of habitats but shows preference for rocky slopes and embankments where it preys on a range of animals. Prey preference has been found to change as these vipers grow. Luiselli (1996) found that small vipers (0-2 years old) preyed upon small lizards (100 %, *n*=24), larger and older specimens also consumed lizards, but their diet was predominantly passerine birds and mammals (65 %, *n*=80). Arthropods such as large insects and centipedes have regularly been found in the stomach contents of *V. ammodytes*, albeit more frequently in juveniles that eat small centipedes (e.g. Arnold & Ovenden, 2004; Heckes et al., 2005; Stille & Stille, 2017).

Two confirmed and one putative record of predation by two juvenile and one adult *V. ammodytes* are described herein. Precise collection data are not mentioned here as *V. ammodytes* is subject to poaching, especially for the pet-trade. However, locality data is available on reasonable request to the author.

Figure 1. Three specimens of *V. ammodytes* with various prey items – **A.** Juvenile adjacent to a regurgitated *O. elegans*, **B.** Close-up of the regurgitated *O. elegans* from Fig. 1A showing a folded tail, **C.** Adult roadkill found next to a dead passerine bird, **D.** Juvenile with a regurgitated centipede *S. cingulata*

Predation on the lizard *Ophisops elegans*

On 20 April 2017 at 16:45 h in Thrace, Greece (28 m asl), a juvenile *V. ammodytes* was found under a rock and was placed on a white cloth to enable photography. The snake proceeded to regurgitate an *Ophisops elegans*, identified from the distinguishing keeled dorsal scales and dorso-lateral stripes (Fig. 1A). The lizard's tail was folded and compressed (Fig. 1B), likely a result of the contracting intestinal muscles. No measurements were taken. This is the first record of this predator-prey interaction and additionally the folded tail of the lizard is an interesting observation (Fig. 1B). This compression of the food-item would appear to ensure an efficient fit in the gastrointestinal tract. Furthermore, many Mediterranean lizards have long tails, which if not somehow folded or compressed during ingestion could hinder the mobility of the predating snake.

Putative predation on a passerine bird *Passer* sp.

On 12 June 2019 at 12:32 h in Macedonia, Greece (62m asl) an adult roadkill *V. ammodytes* was found adjacent to a small dead passerine bird, *Passer* sp. (Fig. 1C). Both animals are estimated to have died within 2 h of their discovery. It appears that the adult snake had bitten and then pursued the bird on to the road where it was subsequently hit by a vehicle; passerine birds are well known prey of *V. ammodytes* (e.g. Luisella, 1996). The author has encountered an estimated 300 snakes, with a total of 101 confirmed roadkills, on roads in 10 countries over 4 years, but this is the first observation of one adjacent to a possible prey item.

Predation on a centipede *Scolopendra cingulata*

On 24 April 2017 at 08:59 h in Macedonia, Greece (62 m asl), a juvenile *V. ammodytes* was found beneath a small pile of rubble and due to the uniformly enlarged body and lack of a distinct bulge it was considered to be an overweight individual. The specimen's movements seemed rigid and within 3 minutes of being placed on a white cloth for photography it had regurgitated an adult Mediterranean banded centipede *S. cingulata* (Fig. 1D). The snake had a snout-vent length of 174 mm. The centipede when measured from the anterior margin of the head shield to the end of the telson was 89 mm (body length), but with the antennae and posterior legs totalled 111 mm (total length). The mean body length of a *S. cingulata* in the Mediterranean was found to be 74 mm (*n*=474, Stylianos Simaiakis, pers. comm.), emphasising the magnitude of the successful predator-prey interaction recorded here (mean + 20 %).

Scolopendra cingulata is a large venomous species belonging to a family notorious for their aggressiveness and ability to subdue comparatively large prey, including mammals, birds, lizards, and snakes (e.g. Dugon & Arthur, 2012; Zimić & Jelić, 2014). There are three failed cases of predation and ingestion of adult *S. cingulata* by *V. ammodytes* in the literature (Clark, 1967; Tan & Kretzschmar, 2009; Arsovski et al., 2014) and at least one case of a *S. cingulata* eating a 170 mm *V. ammodytes* (observation by Sochurek in Biella (1983)). Clark (1967) and Arsovski et al. (2014) both reported cases of juvenile *V. ammodytes* that had ingested adult *S. cingulata*. The *V. ammodytes* caught by Clark (1967) died within an hour of capture. The dead individual found by Arsovski et al. (2014) had the centipede's head protruding from the lower abdomen suggesting that the gut had either burst due to the pressure or that the live centipede had broken out of the snake's body cavity. The report by Tan & Kretzschmar (2009) is of an adult *V. ammodytes* being "paralyzed" mid-ingestion of an adult *S. cingulata*, suggested to be a case of envenoming by the prey. After 10 minutes, the snake was prodded using a stick after which it expelled the dead prey; it is therefore unclear whether the snake would have continued to ingest the centipede if it had not been disturbed.

Scolopendra centipedes are robust and hard to kill (personal observation). The three recorded accounts of them being predated on by *V. ammodytes* (Clark, 1967; Tan & Kretzschmar, 2009; Arsovski et al., 2014) alongside the record herein, suggest that the venom of *V. ammodytes* is sufficiently potent to subdue and ingest *S. cingulata*. Some members of the closely related Pelias group of vipers are known to be entomophagous with greater venom toxicity towards crickets (e.g. *V. lotievi* and *V. ursinii*; Starkov et al., 2007). A study by Lang Balija et al. (2020) compared the venom of the entomophagous *V. ursinii* with that of *V. ammodytes*, and found *V. ammodytes* venom to be significantly more toxic to mice than crickets (114-fold higher mass-normalized LD50), when compared to the venom of *V. ursinii* (5-fold higher mass-normalised LD50). This suggests that *V. ammodytes* has no significant dietary-related specialised toxicity towards arthropods (Lang Balija et al., 2020) but the study did not take in to account the possibility that the venom of the juvenile vipers could be more toxic to insects than that of older vipers. The protein composition of *V. ammodytes* venom is known to change with age (Arikan et al., 2014) and in other Viperidae this change has indicated the presence of prey-specific toxins that parallel changes in dietary preferences (e.g. Zelanis et al., 2008). Further research is required to determine whether this is also the case in *V. ammodytes*.

The observations described here further support the general diet of the iconic nose-horned viper and confirm the ability for small individuals to successfully subdue and ingest arthropods of significant size.

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The impressed tortoise (*Manouria impressa*) in India - extended range and natural history notes

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The state of Arunachal Pradesh in north-east India lies within the Indo-Burma and Himalayan biodiversity hotspot and 'Turtle Priority Area' (Mittermeier et al., 2011; Buhlmann et al., 2009). Nevertheless, the chelonians of the region are still relatively poorly known (Ahmed & Das, 2010) as exemplified by the discovery in 2019 of a large land-dwelling species, the impressed tortoise *Manouria impressa* (Günther, 1882), in Arunachal Pradesh; an addition to India's chelonian checklist (Mital et al., 2019).

Land tortoises of the genus *Manouria* are considered primitive (Le et al., 2006) and are represented by the two species *Manouria emys* and *Manouria impressa*. The more familiar *M. emys* is distributed across south-east Asia, southern China, Bangladesh and north-east India (Das & Das, 2017). All the records of *M. emys* come from south of the Brahmaputra River which is considered as a significant biogeographic barrier in the region (Pawar et al., 2007). *Manouria impressa* is distributed across Cambodia, southern China, Laos, peninsular Malaysia, Myanmar, Thailand, Vietnam (Htun & Platt, 2016), and north-east India. The Indian records of *M. impressa* come from two adult specimens from Lower Subansiri district of Arunachal Pradesh, north of the Brahmpautra River (Fig.1). A third living specimen of *M. impressa*, a sub-adult male, was rescued from the adjoining Papum Pare district, and subsequently transferred to captivity in Itanagar Zoological park, where all the three live individuals are currently housed. The nearest known population of *M. impressa* is found ca. 244 km away to the east in Myanmar, in the Hukaung Valley Wildlife Sanctuary and eastern Rakhine Hills (Htun & Platt, 2016).

In this note we report on a record of a living specimen recently found in Papum Pare district and summarise all known records of *M. impressa* in Arunachal Pradesh, with additional findings of shells from specimens that had been consumed and notes on natural history. Since the initial discovery of *M. impressa* in Arunachal Pradesh, we

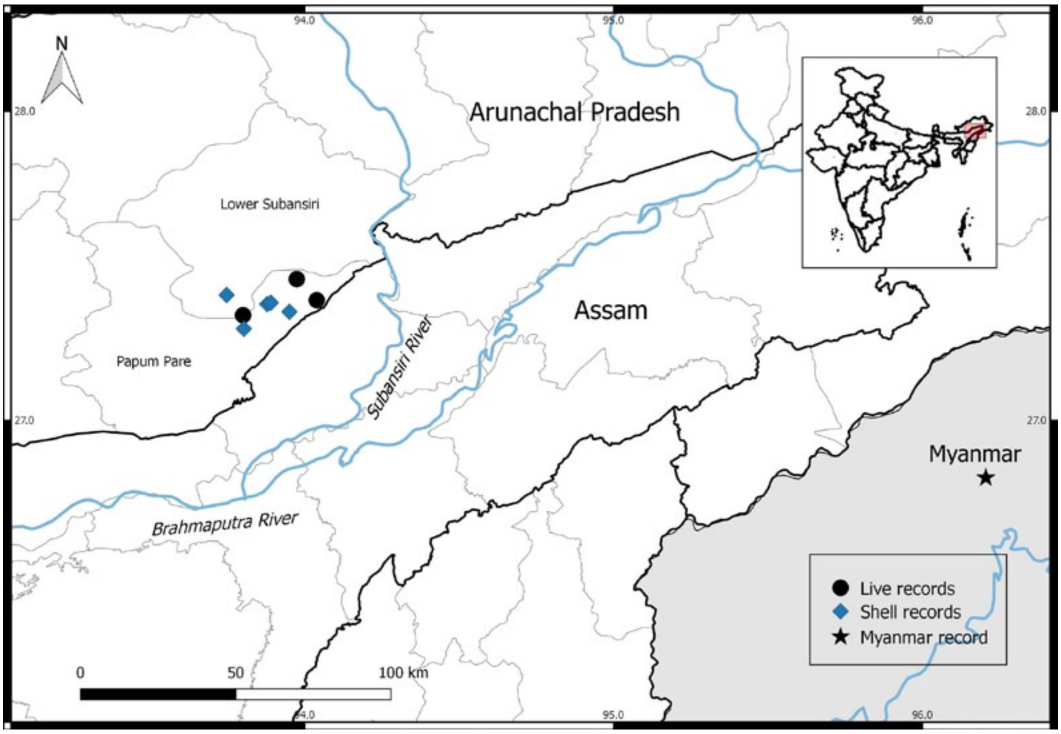

Figure 1. Map of Arunachal Pradesh showing locations where *M. impressa* is found in Lower Subansiri and Papum Pare districts, and locations of shell specimen encountered. Closest known record in Myanmar is also shown.

made two field visits to document additional records and to collect further information from researchers and local communities. The first indication of *M. impressa* in India was from the observation in 2012 of a shell in Cher village, Papum Pare district (Anwaruddin Choudhury, pers.comm.), but the occurrence of this species remained unreported until 2019 (Fig. 2). During field visits in September-October 2019 and June-July 2020, informal questionnaire surveys were completed with members of the local community (Nyishi tribe) who frequent the forests of Lower Subansiri and Papum Pare districts. During these interactions, four shells of *M. impressa* that had been consumed were recovered from local houses. These were photographed (Fig. 3) and measured on the spot (Table 1). The tortoises were sexed based on the concave nature of the plastron observed in adult males. The sex could not be assessed for two shells as their plastrons were missing.

On 26 June 2020 at 10:30 h an active adult female *M. impressa* (Fig. 4) was found in the forests of Papum Pare district in a swampy patch of grassland (locally known as phumdi). The area where the tortoise was found lies in the Panior forest about 4 km from Bumchi Bumte village (for co-ordinates see Table 1) on the left bank of Panior River. The head of the female was creamish-yellow with a pink snout. On 3 July 2020, this tortoise laid two spherical leathery eggs that were translucent white in colour. One of the eggs was destroyed by the female's movements and the second egg although damaged was sufficiently intact that it could be measured; it had a diameter of 38 mm. The two eggs may represent only part of the actual clutch as this species is known to lay 10-21 eggs (Brock, 2009). In the presence of local people, the tortoise was released back into the habitat from which it had been collected. Photographic vouchers of this tortoise have been deposited in the Lee Kong Chian Natural History Museum, National University of Singapore (ZRC[IMG] 2.543a-g).

Figure 2. Shell specimen of *M. impressa* tortoise that had been consumed, found in December 2012 in Cher village, Papum Pare district, Arunachal Pradesh

Table 1. Summary of all *M. impressa* and shell specimen records known from Lower Subansiri and Papum Pare districts, Arunachal Pradesh

	Date	Nearest location	Condition	Carapace length (mm)[a]	Age class / sex
1	26/06/19	Potin, Lower Subansiri (27° 19' N, 93° 48' E)	Live	238	Adult / male
2	26/06/19	Potin, Lower Subansiri (27° 19' N, 93° 48' E)	Live	292	Adult / female
3	23/08/19	Kakoi, Papum Pare (27° 23' N, 94° 02' E)	Live	-[b]	Sub-adult / male
4	26/06/20	Bumchi Bumte, Papum Pare (27° 26' N, 93° 58' E)	Live	305	Adult / female
5	22/12/12	Cher, Papum Pare (27° 21' N, 93° 57' E)	Shell	359	Adult[c]
6	29/06/19	Yazali, Lower Subansiri (27° 24' N, 93° 44' E)	Shell	265	Adult / female
7	18/10/19	Lichi, Papum Pare (27° 22' N, 93° 52' E)	Shell	290	Adult / male
8	3/07/20	Lichi, Papum Pare (27° 22' N, 93° 52' E)	Shell	293	Adult / male
9	14/07/20	Yabi, Papum Pare (27° 17' N, 93° 48' E)	Shell	296	Adult[c]

[a]Measured as straight carapace length [b]Measurement of individual not available, [c]Sexing of shell specimen was not possible

Figure 3. Shells of *M. impressa* tortoises that had been consumed, found during informal interviews in Lower Subansiri and Papum Pare district of Arunachal Pradesh

Figure 4. Carapace and plastron of the *M. impressa* adult female from Papum Pare district, Arunachal Pradesh

To date we have reported on four live *M. impressa* and the remains of five shells. These observations from Lower Subansiri and Papum Pare districts in Arunachal Pradesh represent the northern-most and western-most occurrences of *M. impressa* across its range. The natural vegetation in the area comprises tropical semi-evergreen and sub-tropical evergreen forests (2B/C1b, 8B/C1)(Champion & Seth, 1968) at altitudes of 45 m to 1200 m. The average annual rainfall is around 3200 mm with the peak monsoon period reported from April to September. All living specimens of *M. impressa* documented in 2019 and 2020 were found in the monsoon months which is consistent with previous observations of increased activity at the onset of heavy monsoon rains in Myanmar (Htun & Platt, 2016).

Our interview respondants revealed traditional folk knowledge of *M. impressa*, known locally as 'Chimin Rakhap'. They stated that the species is restricted to intact evergreen forests with closed canopies, the wild banana patches that intersperse forested hill slopes, and occassionally in large cardamom (Zingiberaceae) plantations. They are mainly encountered by local villagers during the monsoon months of June to August and are found during land and bamboo patch clearance, or while taking local cattle (*Bos frontalis*) to graze in the forest. They also opportunistically collect the keeled box turtle (*Cuora mouhotii*) from similar habitats, which due to its small size is often kept as a pet.

A characteristic of tortoises is that they have long lives and have a reproductive maturity proportional to their lifespan (Shine & Iverson, 1995). In the case of *M. emys*, sexual maturity has been reported to be as late as 10 to 15 years (Stanford et al., 2015) and this may similarly apply to *M. impressa*. *Manouria impressa* is currently classified as a vulnerable species by IUCN (2008), is known to be rare in the wild, and has low breeding and survival rates in captivity (Wanchai et al., 2012). In this situation, hunting of this apparently localised population of *M. impressa* could result in its extirpation from the forests of Arunachal Pradesh. A comprehensive study on the collection and hunting practices of *M. impressa* is vital to understand the actual scale of the threat. Currently, the four live records of *M. impressa* come from only a ~24.5 km^2 area across Papum Pare and Lower Subansiri districts. To develop conservation management strategies for this species in Arunachal Pradesh, further surveys in suitable habitats are needed to know the area that it occupies, the population size and genetic diversity, and its ecology and habitats. In India, tortoises are protected under the Schedule IV of the Indian Wildlife (Protection) Act, 1972, making it illegal to collect or kill them. Consequently, the State Forest Department, in collaboration with the local Nyishi community, should be encouraged to initiate awareness campaigns for the protection of this rare species.

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Macrovipera lebetinus in Pakistan

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Macrovipera lebetinus (L., 1758) is a medically important viper but information on its natural history is limited and its taxonomy is challenging. The species has been divided into several distinct subspecies or morphotypes. According to recent genetic data, four of these are valid; *lebetinus*, *obtusa*, *turanica* and *cernovi* (Stümpel & Joger, 2009; Stümpel, 2012). The species is currently believed to be distributed from Turkey, Cyprus, through the Middle East and Central Asia to Afghanistan, western Pakistan, and India (Kashmir) (Sindaco et al., 2013). While taxonomy and distribution are well known in the central and western parts of the species' range, the eastern part of the range is still poorly investigated. This is also true for the subspecific status of those populations.

During field investigations on 29 August 2018 in Pakistan, a subadult specimen attributed to the genus *Macrovipera* (Fig. 1A) was observed, in the Kaigah Community Game Reserve, Dasu (35.3656° N, 73.1357° E, 2,257 m a.s.l.), Upper Kohistan District, Khyber Pakhtunkhwa Province. The individual was found in a rocky habitat (Fig. 1B). This record represents a connection point of the currently known distribution of the species between Afghanistan and Kashmir. Although the specimen was not collected or better investigated in the field, the species identity is in no doubt and the dorsal colour pattern of a dark ground colour with a lighter, orange zig-zag pattern suggests that it is likely to be the subspecies *Macrovipera lebetinus turanica* (Szczerbak & Böhme 2005).

Although *M. lebetinus* has for a long time been known from the territory of current Pakistan, the data to its distribution are not consistent. Briefly, most of the records of this species are known from Pakistani provinces of Balochistan (Urak valley around Quetta) and Khyber Pakhtunkhwa (Waziristan; Ingoldby & Procter, 1923; Smith, 1943; Minton, 1966; Mertens, 1969; Khan, 2002, 2006; Sindaco et al., 2013). On the other hand, Khan (2006) mentioned a record from Chitral in northern Khyber Pakhtunkhwa province but without any details or distributional designation on the map. Sindaco et al. (2013) mentioned Pakistani Kashmir for this species without assigning any further details. Smith (1943) mentions *M. lebetinus* from Kashmir but there is no recent confirmation of this (but see the record of Sharma et al., 2008 from Indian Jammu and Kashmir State). Since there is a clear morphological difference between the specimen we have observed and known specimens from Balochistan (see the photo of the species in Khan, 2006, p. 240), we suspect that different taxa are present in Pakistan. To avoid further taxonomic confusions we do not speculate on the status of Pakistani populations. However, the subspecies *M. l. obtusa* is definitely not presented in Pakistan (e.g. Khan, 2006) as the range of this taxon is limited to western and north-western Iran (Moradi et al., 2014). Further morphological data and study of molecular relationships are required to clarify the distribution and taxonomy of eastern populations of *M. lebetinus* spp.

Figure 1. Subadult *M. lebetinus* from Dasu, Khyber Pakhtunkhwa Province, Pakistan observed in August 2018 - **A.** Detailed view of the individual which resembles the subspecies *M. l. turanica*, and **B.** generalised view on the same individual and its habitat

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Comments on 'Alien chelonians in north-eastern Spain: New distributional data' by Poch et al. - A possible novel *Mauremys mutica* x *Mauremys leprosa* hybrid

Poch et al. (2020) present a good review of records of chelonian alien species in north-eastern Spain, in the region of Catalonia, together with novel data supplied by the authors. In their article they depict two *Mauremys mutica*, one an old female specimen (their Fig. 4.4) the other an individual that appears to show a mixture of external characters diagnostic of both *Mauremys leprosa* and *M. mutica* (their Fig. 3.4). The authors indicate that at least one of the specimens was captured at "Riera de Santa Maria, Caldes de Malavella". This area is part of the Natura 2000 Network ES5120017 "Estany de Sils-Riera de Santa Coloma", where a population of the species *M. leprosa* is also known to be present (Generalitat de Catalunya, 2018). It would be helpful to know if the specimen in their Figure 3.4 was captured in this area as it could be a *M. leprosa* x *M. mutica* hybrid.

The proposed hybrid (Fig. 1B) shows an orange colored, elongated, but short postorbital blotch, that seems to be a combination of characteristics of both parental species. *Muremys mutica* (Fig. 1A) shows a prominent light yellow stripe running from the posterior border of the orbit over the tympanum to anterior neck (Ernst & Barbour, 1989; Yasukawa et al., 1996) while young *Mauremys leprosa* have a small, round orange blotch that lies between the tympanum and the orbit, that usually fades with age (Ernst & Barbour, 1989; Díaz-Paniaguaet al., 2015) (Fig. 1C). The proposed hybrid has two marked facial lines, under its jaw and in the commissure. *Mauremys leprosa* shows many other facial lines. For *M. mutica*, the throat and ventral surface of the neck is pale to light yellow, lighter than the dorsal surface of the head and neck. The neck and front limbs of both *M. leprosa* and the proposed hybrid show a series of marked orange stripes. *Mauremys mutica* does not show any pattern on its legs. The limbs and tail are dark olive dorsally and laterally, pale or greyish yellow ventrally. The neck shows a broad yellow stripe extending backward from the orbit over the tympanum to the neck. A second yellow stripe may extend diagonally downward and backward from the lower edge of the orbit or the corner of the mouth to below the tympanum. There is a dorsomedial yellow stripe on the neck. Head and neck are dorsally grey to olive (Ernst & Barbour, 1989). *Mauremys leprosa* is dark green colored (Diaz-Paniagua, 2015). The proposed hybrid is dark brown and apparently exhibits a mosaic of the external characters typical of both *M. leprosa* and *M. mutica*; the elongated postorbital blotch and the stripes on the forelimbs being diagnostic.

Such a hybrid has not been observed previously but its occurrence would not be surprising as Stuart and Parham (2007) state that among geoemydid species reproductive isolation mechanisms are not well established. Furthermore *M. mutica* is known to produce interspecific hybrids with *Mauremys reevesii* (Fujiiet al., 2014) and even intergeneric hybrids with *Cuoratri fasciata* (Parham, 2001). If possible, the specimen should be recaptured to make morphological measurements and to take samples for genetic analysis.

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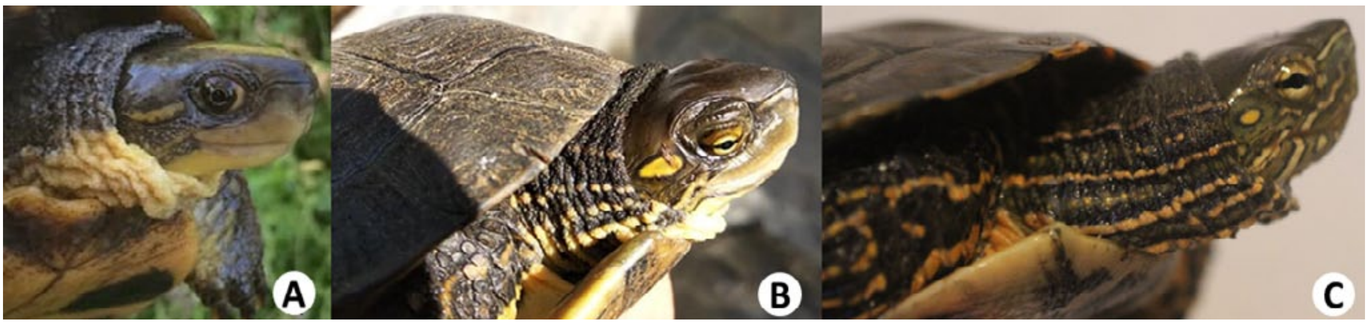

Figure 1. Comparison between **B.** Proposed hybrid *M. leprosa* x *M. mutica*, and its parent species **A.** *M. mutica* and **C.** *Mauremys leprosa*. **A. & B.** are from Poch et al., 2020

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Reply to 'Comments on 'Alien chelonians in north-eastern Spain: New distributional data' by Poch et al. - A possible novel *Mauremys leprosa* x *Mauremys mutica* hybrid'.

The two specimens of *Mauremys mutica* illustrated in Poch et al. (2020) showed several external characters that meet the description of this species, both in the plastral and cephalic coloration. Some of the characters proposed by López-Sánchez (above) to differentiate the potential hybrid from a non-hybrid specimen of *M. mutica* are of doubtful utility. For example, when describing the head coloration of *M. mutica*, this author omitted that it can also be blackish-brown (Bonin et al., 2006). Moreover, he noted that in a typical specimen of *M. mutica* the postorbital blotch is light yellow, whereas in the proposed hybrid it is orange, a trait inherited from a *M. leprosa* parent. However, on page 333 of Bonin et al. (2006) a specimen of *M. mutica* is shown with an orange-brown postorbital blotch, similar in coloration to the specimen found in Girona.

We believe that taking in account the wide intraspecific variability of *M. mutica* (Bonin et al., 2006), it would be impossible using a partial photograph alone to determine the parental species of the specimen proposed as a hybrid. Besides, the situation is complicated because a hybrid specimen could have been bred in captivity and then released. Therefore the parental species could be any other species of the genus *Mauremys* and cannot be restricted to *M. leprosa* not least because the presence of stripes on the forelimbs is not an exclusive character of this species (Bonin et al., 2006). Moreover when we captured the specimen for the first time, it had already reached adult size. If this specimen had been born in the wild, we would have probably detected it much earlier during our periodical surveys (Escoriza et al., 2020). Considering all the reasons above, we deem it bold for anyone to attempt the description of a novel hybrid based solely on partial photographs without any solid molecular evidence.

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BOOK REVIEW

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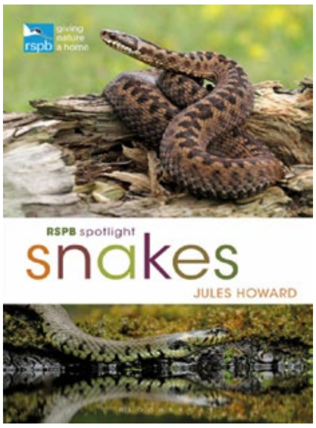
RSPB Spotlight Snakes

Howard, Jules

Bloomsbury Wildlife, 2020, ISBN 10: 1472971698 ISBN 13: 9781472971692, 128 pp.



The RSPB Spotlight series of books claims to introduce readers to the lives and behaviours of our favourite animals with eye catching colour photographs and informative expert text. Jules Howard's recent volume on snakes certainly achieves that claim and a lot more.

My inclination as a naturalist with an almost exclusive focus on British wildlife is to want, from the first page, to dive into the detail of the British species but Jules spends the first twenty pages or so taking us through the broader diverse world of snakes to build our understanding before we meet the residents. Our three native species are then described in expert detail, accompanied by some excellent representative photographs with a nice blend of wild and staged shots. The writing in this section is a great blend of essential description and personal anecdote and I was particularly jealous of Jules' account of his encounter with a one and a half metre grass snake. Of the many British snakes I've seen, I've only encountered a few that exceeded one metre and even then not by much. The species accounts also spend several pages on the slow worm and given the aim of the Spotlight series as an introduction to the topic of snakes, this is welcome as the slow worm so easily tempts less experienced observers to think it is a snake. Jules also provides some useful information and photographs of non-native species and the escapees which are increasingly encountered in urban settings.

On first read I was a little surprised to have got through the British species with so much of the book still to go. However, reading on, it is clear that this book is so much more than a field guide and the subsequent chapters started to add additional layers of information, all geared to our native species, about annual cycle, thermoregulation, feeding, mating and giving birth all brilliantly illustrated with some stunning photography. The topic of snakes then really broadens out further in a superbly entertaining way through the challenges they face, the historic cultural perspective, how snakes have been exploited and vilified including even the damaging way that snakes are presented in current films. This part of the book offers some great and engaging narrative again accompanied but some highly relevant illustrations.

A few things the book left me with.

First, it's one of the first publications I've read that's referred to the grass snake by its new name of barred grass snake since the elevation of *Natrix natrix helvitica* from sub-species to species (*Natrix helvetica*) (Fritz & Kindler, 2017). Whilst this felt a little unfamiliar, precision is important.

Secondly, seeing the estimate of 20,000 as the number of snakes that snake-catcher Brusher Mills was calculated as having removed from the New Forest in twenty years leading up to his death in 1905 was shocking. Coupled with the fact there were a further two generations of snake catchers after him it makes you realise just what an amazing place the New Forest must have been in late Victorian times especially if you couple those statistics with the Victorian reports of the dense and varied butterfly populations that existed there at that time.

Thirdly (and sadly) the term Jules uses of 'death by a thousand cuts' is such an appropriate way to describe the relentless combination of things, from climate change, roads, 'concrete-isation' right through to disturbance by over-zealous photographers that stand in the way of maintaining viable, wild populations of snakes in the Britain. Let's hope that the interest generated by this book and the advice given in the last few pages about how to get involved and help goes some way to mitigating some of those thousand cuts.

This is a brilliant and informative book written by a skilled communicator and well worth owning in its own right or as part of the rest of the Spotlight series.

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